University of the Western Cape

THESIS

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Proposed Degree: **PhD**

Department: **Medical Biosciences**

Title: **The Effect of Maternal Oral Health on Pregnancy Outcomes**

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Abstract

Adverse pregnancy outcomes such as preterm birth and low birth weight are major causes of maternal and neonatal morbidity and mortality. Increasing evidence points to an association between periodontal disease and adverse pregnancy outcomes and thus a better understanding of the nature of this association will assist in treatment planning to reduce adverse pregnancy outcomes. Among the Gram-negative anaerobic bacteria frequently associated with periodontal disease are *Treponema denticola*, *Tannerella forsythia* and *Porphyromonas gingivalis* which may be detected in plaque using the BANA test (N-benzoyl-DL-arginine-2-naphthylamide).

The aim of this study was to investigate the effect of periodontal disease on pregnancy outcomes and evaluate the use of BANA as a screening test for the risk of adverse pregnancy outcomes.

This study complied with the Declaration of Helsinki (2013) and included 443 pregnant women attending ante-natal clinics in KwaZulu Natal. At first visit, maternal oral health status was assessed by the measurement of periodontal indices and BANA testing of dental plaque from the same teeth. Patient demography and medical history were obtained by means of a questionnaire and all data compared with pregnancy outcomes.

While controlling for other factors, significant differences were found between the distributions of periodontal disease at BANA-negative and BANA-positive sites and between infant birth weight and maternal periodontal index scores such as plaque index and gingival index. The birth weight and gestational age at delivery of infants born of BANA-positive periodontally diseased mothers were significantly lower than those born of BANA-negative mothers with no periodontal disease. We may conclude that the presence of periodontal disease during pregnancy has a significant association with negative pregnancy outcomes and suggest that the risk for adverse pregnancy outcomes may be reduced by monitoring the oral health status of women during pregnancy.

Keywords: BANA, Periodontal disease, Low birth weight, Preterm birth.

Declaration

I, the undersigned, hereby declare that the work contained in this dissertation is my original work and that it has not been previously in its entirety or in part submitted at any university for a degree.

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Mervyn Turton Date

The work presented in this dissertation was undertaken in fulfilment for the requirements of the degree PhD. Department of Medical Biosciences, University of Western Cape, Bellville, Cape Town.

Acknowledgements

I would like to thank:

- Prof Charlene. W. J. Africa for her support, guidance and advice, her dedication and commitment was truly remarkable and exceptional.
- Prof Stefan Maritz for statistical assistance and advice.
- Prof Brenda Grant for advice and assistance.
- Nonhlanhla Mdunge for administrative assistance.
- The nursing personnel and staff at the various Maternity Obstetric Units that participated in the study.
- The participants in the study for their co-operation and permission to participate in this study.
- My parents, Afra and Kenneth and my brothers, Clive, Lionel and Russell for their unfailing, steadfast, encouragement and support.
- My partner, Genevieve for the remarkable encouragement and support.
- Finally, to all those who contributed to making this study possible that have not been mentioned.

Dedication

To my partner Genevieve, my son, Bryden and daughter, Elizabeth

For my parents, Kenneth and Afra Turton,
and my brothers,
Clive, Lionel and Russell,

For their unfailing and steadfast, love and support.

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CHAPTER 1: INTRODUCTION

The impact of oral health on life quality is in relation to socio-demographic factors, age group and social class background and in some cases it is in relation to housing, medication adherence and access to care and food (Kenagy et al., 2003).It is widely accepted that general health or disease status affects oral health. However the possibility that oral health or the oral cavity (as a portal of entry with access to the bloodstream and respiratory system) can cause alterations in general health seems less recognized (Offenbacher et al., 2001; Kinane and Bouchard, 2008).

Periodontal disease is one of the most common chronic disorders of infectious origin known in humans, with a reported prevalence varying between 10 and 60% in adults, depending on diagnostic criteria (Albandar and Rams, 2000). Periodontal disease refers to gingivitis (an inflammatory condition of the soft tissues surrounding a tooth or the gingivae) and periodontitis (involving the destruction of such supporting structures as the periodontal ligament, bone, cementum, or soft tissues) (Kinane and Bouchard, 2008). The two most prevalent and most investigated periodontal diseases are dental plaque-induced gingivitis and chronic periodontitis (Tatakis and Kumar, 2005; Preshaw, 2008). Commonly accepted clinical measures of periodontal disease are clinical attachment level (CAL): the distance between the cementoenamel junction and clinical pocket base, and probing depth (PD): the distance from the gingival margin to the apical part of the pocket (Preshaw, 2008; Tatakis and Kumar, 2005).

Periodontal disease is initiated by the overgrowth of certain bacterial species, with a majority of Gram-negative, anaerobic bacteria growing in subgingival sites. The host response to periodontal pathogens causes persistent inflammation and the destruction of periodontal tissues that support teeth leading to clinical manifestations of disease (Kornman et al., 2000). A limited number of cultivable species are associated with periodontal disease (Loesche, 1993) and may include *Aggregatibacter* (formally called *Actinobacillus*) *actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Campylobacter rectus, Eubacterium nodatum, Peptostreptococcus micros,*

Streptococcus intermedius and *Treponema* species. The bacterial numbers associated with disease are up to 10 times larger than those associated with health (Lovegrove, 2004).

These periodontopathogens share the ability to penetrate the gingival epithelium, such that their endotoxins, immunologically active compounds, and cytotoxic enzymes and molecules are presented directly to the host's inflammatory cells. This ability may be what distinguishes these Gram-negative species from the plethora of other Gram-negative species that inhabit the subgingival plaque (Loesche, 1993; Lovegrove, 2004). Criteria for defining periodontal pathogens have been developed and include association, elimination, host response, virulence factors, animal studies and risk assessment reasons (Lovegrove, 2004). Research has indicated that *A. actinomycetemcomitans* produces a leukotoxin, and the immunologic response of the host to this antigen may explain the unique pattern of tooth involvement in localized juvenile periodontitis. Both *P. gingivalis* and *T. denticola* have been identified to have a trypsin-like enzyme that could be a virulence factor (Loesche, 1993; Lovegrove, 2004). There has been an increase in research evidence suggesting associations between periodontal disease and increased risk of systemic diseases such as atherosclerosis, myocardial infarction, stroke, diabetes mellitus, and adverse pregnancy outcomes (Garcia, 2001; Offenbacher et al., 2001).

Periodontal disease may contribute to adverse pregnancy outcomes because of a chronic oral inflammatory bacterial infection. In a study by McCormick (2003) and Bobetsis (2006), pregnant hamsters infected with *P. gingivalis* at doses insufficient to induce either fever or wasting, produced litters with significantly reduced foetal weight, accompanied by a proportional rise in tumour necrosis factor alpha (McCormick, 2003; Bobetsis, 2006). In humans, adverse pregnancy outcomes that have been linked to periodontal disease include: preterm birth, low birth weight, miscarriage or early pregnancy loss, and pre-eclampsia (Appendix 1). Pre-eclampsia and preterm births are major causes of maternal and perinatal morbidity and mortality (McCormick 2003; Bobetsis, 2006).

The specific aetiologies and pathogeneses of these adverse pregnancy outcomes are still unclear, as few risk factors have been clearly identified as early predictors or modifiable risk

factors for purposes of determining intervention strategies (Kinane and Bouchard, 2008). Confirmation of periodontal disease as an independent risk factor for adverse pregnancy outcomes would be of great public health importance because periodontal disease is both preventable and curable (Offenbacher et al., 2006). Periodontal treatment during pregnancy is regarded as safe for both the mother and the child (Offenbacher et al., 2006; Jeffcoat et al., 2003), thereby preventing or reducing the occurrences of adverse pregnancy outcomes as well as maternal and perinatal morbidity and mortality (Offenbacher et al., 2006; Jeffcoat et al., 2003).

The "red complex" (*Porphyromonas gingivalis, Tannerella forsythia,* and *Treponema denticola),* has consistently been associated with periodontal infections (Kinane, 2001; Loesche et al., 1987; Offenbacher et al., 2009)**.** Bayingana, (2005), examined the prevalence of *T. denticola, T. forsythia*, and *P. gingivalis* in subginginval plaque samples from pregnant women to establish the prevalence of the red complex using N-benzoyl-DL-arginine-2-naphthylamide (BANA) and PCR and proposed the use of BANA as a screening test for mothers at risk of pre-term delivery or low birth weight. The present study, conducted under field conditions, is a more detailed study trying to correlate a treatment plan with BANA+ results in order to prevent/reduce the risk of adverse pregnancy outcomes. Further understanding of the nature of this association between periodontal disease and adverse pregnancy outcomes will assist in better maternal treatment planning in an attempt to reduce adverse pregnancy outcomes.

Therefore, the aim of this study was to investigate the effect of the presence of pregnancy-associated periodontal disease and a positive BANA test result on pregnancy outcomes.

CHAPTER 2: LITERATURE REVIEW

2.1. Introduction

This chapter critically reviews the literature on periodontal disease and adverse pregnancy outcomes in an attempt to update information and provide a current assessment of the state of the science in this area.

2.2. Definition and Classification of Periodontal disease

Periodontal diseases are a group of infectious diseases that cause inflammation and destruction to the tissues surrounding and supporting the teeth (Jared and Boggess, 2008) and may vary from inflammation of the gingivae alone (gingivitis) to severe inflammation of the periodontal ligament (periodontitis) as a result of a non-specific proliferation of the normal gingival crevice microbiota due to poor oral hygiene (Marsh and Martin, 1992). Plaque bacteria may produce disease directly, by invasion of the tissues, or indirectly via bacterial toxins (Marsh and Martin, 1992).

In the early stage of periodontal disease, the gingiva becomes red, swollen and bleed easily resulting in false pocket formation (Marsh and Martin, 1992; Mokeem et al., 2004). At this stage (gingivitis), the disease is still reversible and can usually be eliminated by improved oral hygiene practise (Mokeem et al., 2004; Jared and Boggess, 2008). If chronic gingivitis is not treated, bacterial poisons from the plaque penetrate the deeper tissues and destroy the periodontal ligament and alveolar bone (Mokeem et al., 2004). This advanced stage of periodontal disease is known as periodontitis. In periodontitis, the junctional epithelial tissue at the base of the gingival crevice migrates down the tooth resulting in deepening of the gingival pocket and true periodontal pocket formation (Levision, 1997). Serious damage to the gingiva and alveolar bone as well as destruction of the periodontal ligament and cementum results in tooth loss (Marsh and Martin, 1992; Mokeem et al., 2004; Jared and Boggess, 2008).

Periodontal diseases can be considered silent infections that have periods of exacerbation and remission that often go undiagnosed until irreparable damage occurs to the teeth and oral structures (Jared and Boggess, 2008). Gingivitis and mild periodontitis affect the majority of people and severe periodontitis affects approximately 10-15% of the population (Preshaw,

2004). Chronic destructive periodontal disease occurs in a small proportion of most populations, regardless of locality or socioeconomic status (Cuttress, 1986) but more than 23% of women between the ages of 30 and 54 years are affected with periodontal disease (Holmlund et al., 2006; Sparh et al., 2006). The classification of periodontal diseases is still far from being resolved. Disease may be classified according to age (e.g. pre pubertal, juvenile, and adult) rate of progress (chronic, acute, and rapid) and distribution of lesions (localised and generalised) (Holmlund et al., 2006; Sparh et al., 2006).

2.3. Risk factors for periodontal disease

The nature of the host response is determined primarily by genetic factors and environmental and acquired factors such as smoking (Preshaw, 2004; Baelum and Lopez, 2003). The host response is essentially protective in nature, but both under-activity (hypo-responsiveness) and over-activity (hyper-responsiveness) of aspects of the host response can result in enhanced tissue destruction (Preshaw, 2004).

Significant differences were recognised for race, gender, diabetes, education, smoking, and body mass index among categories of increasing severity of periodontal disease (Page and Kornman, 1997; Baelum and Lopez, 2003). Of all the subject-level factors, race, gender, and the presence of diabetes were the strongest contributors to disease expression with predisposition based on individual exposures being critical to how the disease is expressed (Page and Kornman, 1997; Armitage, 2004; Baelum and Lopez, 2003).

Offenbacher et al (2008) suggest that based on new data, the clinical characteristics of some complex diseases, such as periodontal disease, are influenced by the genetic and epigenetic contributions to clinical phenotype. Although the genetic basis for periodontal disease is considered imperative for setting an inflammatory capacity for an individual and thus, a threshold for severity, there is evidence to suggest an epigenetic component is involved as well (Page and Kornman 1997; Armitage 2004; Baelum and Lopez 2003). This is supported by factors long associated with periodontitis including bacterial accumulations, smoking, and diabetes and are known to produce strong epigenetic changes in tissue behaviour (Offenbacher et al., 2008; Al-Zahrani et al., 2003). As such, the clinical presentation of disease, or clinical phenotype, is typically based on clinical features of inflammation, such as redness, oedema, and bleeding on probing (BOP), and loss of supporting tissue, as evidenced

by probing depth (PD), attachment level, and alveolar bone loss (Page and Kornman, 1997; Armitage, 2004; Baelum and Lopez, 2003).

Baelum and Lopez (2003) state that "periodontal disease is a syndrome that comes in all sizes," suggesting that there are no clear demarcations between health and disease or between conditions. However, biologic data indicates that there are dramatic differences between individuals with very similar clinical presentations or patients with identical clinical presentations who may respond differently to the same therapy (Page and Kornman, 1997; Armitage, 2004; Baelum and Lopez, 2003). According to Page and Kornman, (1997) in addition to biologic phenotype, important subject-level variables that shape the host response to an organism may contribute to the clinical phenotype. These subject-level factors include smoking, obesity, and diabetes and increase the risk for progression of gingivitis to advanced stages of periodontal disease (Al-Zahrani et al., 2003; Page et al., 2003). A relatively small proportion of all bacterial species is consistently found within diseased tissue sites (Schenkein, 2002; Offenbacher et al., 2007). It is suggested that defining the multiple factors that contribute to the clinical presentation of the different types of periodontal disease, should make it possible to develop more consistent diagnostic categories and treatment modalities for the different types of periodontitis (Page and Kornman, 1997; Feinberg, 2007; Offenbacher et al., 2007). This allows for a more accurate prognosis, providing more insight into the ideal customized treatment for a given individual (Page and Kornman, 1997; Feinberg, 2007; Offenbacher et al., 2007).

2.4. Microbiology of periodontal disease

Periodontal disease can further be described as a chronic inflammatory response to the tooth-associated microbial biofilm which is plaque (Pihlstrom et al., 2005) that induces inflammation of the adjacent tissues and causes local tissue destruction and loss of the tooth attachment such as ligament and bone (Offenbacher et al., 2009 ; Jared and Boggess, 2008).Thorpe (2006) points out that although no evidence exists of an underlying relationship between gingivitis and calculus build up, many studies nevertheless report a high prevalence or increased severity of periodontal disease associated with poor oral hygiene or nutritional status. In the absence of adequate oral hygiene, periodontal bacteria accumulate in the gingival crevice of the teeth to form an organized complex structure known as a bacterial

biofilm (Jason et al., 2006; Sparh et al., 2006). The bacterial biofilms begin on the crown (supragingival), extend onto root surfaces under the gumline (subgingival) and penetrate into the root surfaces. They can become mineralized as hard deposits termed calculus (Offenbacher et al., 2009). The bacterial biofilm increases in virulence as it matures (Jason et al., 2006; Sparh et al., 2006) and manifests with the excretion of a protective and adhesive matrix (Thomas and Nakaishi, 2006). This bacterial matrix contains Gram-negative anaerobic and microaerophilic bacteria that colonize on the tooth structures (Philstrom et al., 2005), and initiates a systemic inflammatory and immune response (Slade et al., 2003). This can cause bone loss and the migration of the junctional epithelium, resulting in periodontal pocketing and periodontal disease (Thomas and Nakaishi, 2006; Sparh et al., 2006). Several bacteria, predominantly Gram-negative, anaerobic, and microaerophilic bacteria that colonize the subgingival area are responsible for initiating and sustaining the periodontal infection (Mokeem et al., 2004; Thomas and Nakaishi, 2006). Rosebury (1962) introduced the concept of an amphibiotic state to describe those infections attributed to the overgrowth of bacterial species that are normally present in the indigenous flora, but at low levels (Flemmig et al., 1996; Mombelli et al., 1996; Loesche, 1999). Certain changes in or on the mucous membranes allow these species to be selected for, and as a result of this overgrowth, they cause an endogenous infection[1], resulting in clinical disease (Flemmig et al., 1996; Mombelli et al., 1996; Loesche, 1999). An additional selection factor for periodontopathic bacteria would be their ability to utilize nutrients that are available because of tissue inflammation (Loesche, 1993b). Host products such as haemin, menadione, progesterone, estradiol, acetylmuramic acid, spermine, alpha-2 globulin, and ceruloplasmin seem to be essential growth factors for *P. gingivalis, T. forsythia (formally B. forsythus), T.denticola, and P. intermedia* (Loesche, 1993a; Loesche, 1999). Pathogenic bacteria though constantly present, are not the only cause of periodontitis (Thomas and Nakaishi, 2006; Philstrom et al., 2005) and the host defence mechanism plays an integral role in the pathogenesis of periodontal disease (Mokeem et al., 2004; Jared and Boggess, 2008).

[1]Medical examples of endogenous infections would be the several types of diarrhoea diseases that occur because of malnutrition, vaginal infections due to yeast, super infections that follow the usage of antibiotics, e.g., a Clostridium difficile infection after the use of Clindamycin (Flemmig *et al*., 1996; Mombelli *et al*., 1996; Loesche, 1999). Dental caries would be an endogenous infection due to the selection of the mutants' streptococci by frequent sucrose ingestion (Loesche, 1993a; Loesche, 1999).

Tissue destruction in periodontitis is mainly due to the activation of the host's immune cells by the microbial cell wall component, lipopolysaccharide. The lipopolysaccharide stimulates the production of host-derived enzymes, cytokines, and other pro-inflammatory mediators resulting in connective tissue destruction (Offenbacher et al., 1996; Lopez et al., 2002). This biologic process, including the characteristics of the biofilm and of the host inflammatory and immune responses, tend to vary among individuals, despite producing a similar clinical picture or diagnostic category (Page and Kornman, 1997; Offenbacher et al., 2007).The host response to the bacterial invasion regulates the severity of the disease by activating the immune system to mediate the disease process (Philstrom et al., 2005; Thomas and Nakaishi, 2006). The host's response to the pathogenic bacteria modulates how the disease is initiated and progresses as evidenced by the fact that gingivitis does not always progress into periodontitis (Philstrom et al., 2005; Thomas and Nakaishi, 2006).

Eighty per cent of individuals with periodontal disease have at least one risk factor that increases their susceptibility to the infectious process and subsequent tissue damage (Philstrom et al., 2005; Thomas and Nakaishi 2006). Often, multiple factors are present such as stress, poor dietary habits with high sugar intake, smoking and tobacco use, obesity, age, and poor dental hygiene have been identified as risk factors contributing to the development of periodontal disease (Philstrom et al., 2005; Holmlund et al., 2006; Thomas and Nakaishi 2006; Jared, and Boggess, 2008). Grinding teeth, genetic and other family factors, medical diseases such as diabetes, cancer, or AIDS, defective dental restorations, medication use, and conditions that change oestrogen and progesterone levels such as puberty, pregnancy and menopause have been identified as other major risk factors for periodontal disease (Philstrom et al., 2005; Jared and Boggess, 2008).

2.4.1. Non-specific Plaque Hypothesis

The Non-specific Plaque Hypothesis states that any plaque accumulation, regardless of its microbial make-up may cause disease (Loesche, 1976). For that reason, for adequate debridement of the tooth root surfaces with deep pockets, the pockets need to be eliminated surgically so that the patient can practice good oral hygiene on these newly accessible tooth root surfaces (Goodson, 1994; Loesche 1999; Offenbacher et al., 2009). Post-operative infections are prevented by the use of systemic antimicrobials and if the patient cannot maintain clean tooth root surfaces after the surgical approach, and the diseased pockets

return, then systemic antimicrobials are used in a rescue or salvage mode (Goodson, 1994; Loesche 1999; Offenbacher et al., 2009). Thus, in the Non-specific Plaque Paradigm, there would appear to be no contra-indications for the use of antimicrobial agents, if this use coincides with the surgical procedures, or is conducted if surgery fails (Goodson, 1994; Loesche 1999; Offenbacher et al., 2009). The established treatments are based on the non-specific plaque overgrowth paradigm, which, if therapy is maintained on a periodic basis for a lifetime, can provide satisfactory results for about 80% of periodontal patients (Loesche, 1999).

2.4.2. Specific Plaque Hypothesis

The Specific Plaque paradigm, in contrast, focuses on quality rather than quantity of plaque, by associating specific microbial species with disease and recommends that the short-term usage of antimicrobial agents, in combination with debridement, should precede any surgical intervention (Loesche, 1999; Offenbacher et al., 2009). This approach would require that one identifies the periodontopathic microbiota prior to any treatment and then use the appropriate antimicrobial agent (Loesche, 1999; Offenbacher et al., 2009). Numerous studies have implicated the overgrowth of anaerobic species as being statistically associated with advanced forms of periodontal disease. This would indicate the short-term usage of antimicrobial agents such as metronidazole and doxycycline directed against anaerobic members of bacteria (Offenbacher et al., 2009).

Both paradigms are antimicrobial, in that debridement is used to limit the numbers of bacteria that accumulate on the tooth surfaces. However, they differ in the treatment of patients with advanced disease (Loesche, 1999; Offenbacher et al., 2009). The two paradigms use the same modules of treatment but differ in the process of the sequencing of these modules. Studies on the microbial aetiology of most forms of periodontal disease have shown that advanced forms of periodontal disease can be successfully treated by short-term usage of metronidazole and doxycycline (Loesche, 1999; Offenbacher et al., 2009).

2.4.3. Bacterial species implicated in the aetiology of periodontal disease

Periodontal disease is an infectious disease caused mainly by anaerobic Gram-negative bacteria. Socransky and his colleagues (1998) divided these bacteria into microbial

complexes or clusters and assigned to each a colour for the convenience of discussion. The "blue," green," "yellow" and "purple" clusters include mainly bacteria that colonize the periodontal sulcus in the early stages of dental plaque formation. Organisms of the "orange" cluster (*Campylobacter rectus, Fusobacterium nucleatum, Peptostreptococcus micros, Prevotella intermedia* and *Prevotella nigrescence*) appear and provide the necessary habitat for the subsequent colonization and establishment of the more aggressive bacteria of the "red" cluster *(Porphyromonas gingivalis, Tannerella forsythia* and *Treponema denticola*). As the biofilm matures, it becomes more pathogenic (Madianos et al., 2001; Socransky et al., 1998; Loesche and Grossman, 2001). Although the exact role of each of these bacterial species in the progression of periodontal disease is not fully understood, it is clear that the presence of a large group of bacteria is necessary for the overall pathogenic effect (Madianos et al., 2001; Socransky et al., 1998; Offenbacher et al., 2006). Progression of the periodontal disease causes the host's immune system to respond by producing antibodies against the various bacterial species (Madianos et al., 2001; Offenbacher et al., 2006). Virulence factors include lipopolysaccharide (LPS) that may cause direct destruction to the periodontal tissues or stimulate the host local inflammatory response which even though intended to eliminate the infection, may lead to further loss of periodontal structures (Al-Shammari et al., 2006). Further consequences can be that bacteria and/or their shed virulence factors may enter the bloodstream, disseminate throughout the body and trigger the induction of systemic inflammatory responses with or without entopic infections (Thomas and Nakaishi ,2006; Al-Shammari et al., 2006).

2.4.4. The use of BANA as a diagnostic tool for periodontal disease.

DNA probes, cultural procedures, microscopic measures, and immunological techniques have been used to detect *P. gingivalis, T. forsythia,* and *T. denticola* in dental plaque (Loesche et al., 1987; Aimetti et al., 2007; Komiya et al., 2010; Andrade et al., 2010).

The "red complex" (*P. gingivalis, T. forsythia,* and *T. denticola),* has consistently been associated with periodontal infections (Kinane, 2001; Loesche et al., 1987; Offenbacher et al., 2009) and is known to possess a trypsin-like enzyme that can hydrolyse the synthetic trypsin substrate, BANA (Loesche, 1986). BANA is a rapid and effective diagnostic aid and has shown to correlate well with the clinical indices used to diagnose periodontal disease (Loeshe et al., 1997a, b; Grisi et al., 2001). BANA is able to detect between 10^3 - 10^5

targets/ sample (Loesche et al., 1992; Tran et al., 1999) which is the same range as immunoassays and nucleic acid probes. The commercially available BANA test (Oral B, Perioscan TM) is a modification of the BANA hydrolysis test adapted from the trypsin - like enzyme of the API - ZYM Kit (Laughon et al., 1982; Loeshe et al., 1990). SK-013 (carbobenzoxy -glycil -arginine -3.5 -dibromo -4-hydroxy arginine) has also been used for the detection of peptidase activity of T. *denticola, P. gingivalis* and *T. forsythia* in plaque samples (Ishihara., et al 1991). However, BANA appears to be more frequently employed. Other species such as *Bacterionema matruchotti, Rothia dentocariosa, Rothia (Stomatococcus) mucilaginous* and some *Capnocytophaga* species are known to be BANA positive, but these species have not consistently been associated with periodontal disease (Scannapieco, 2005; Loesche et al., 1987).

According to Loesche et al (1992) in the detection of *P. gingivalis, T forsythia* and *T. denticola*, the BANA test had a 92% sensitivity and 70% specificity when compared with DNA probes and polyclonal immunological reagents. The BANA Test had a sensitivity of 95%, when compared with checkerboard DNA-DNA hybridization using highly specific whole genomic DNA probes to *P. gingivalis, T. forsythia,* and *T. denticola*.Loesche et al., (1987), Andrade et al., (2010) and Africa et al., (2009) suggest that the BANA test could be used as a surrogate for DNA probes in the detection of these bacterial species in plaque samples. Smoking is a well-documented risk factor for periodontal disease (Hujoel et al., 2002) and is associated with significant proportional and absolute overgrowth of periodontopathic bacterial species in the subgingival plaque (Hujoel et al., 2002; Offenbacher et al., 2009). Plaques from smokers are 11 times more likely to be BANA positive compared to plaques from individuals who have never smoked and are less likely to respond to debridement/surgical procedures (Hujoel et al., 2002; Offenbacher et al., 2009). Andrade et al., (2010) reported that the BANA test was most effective for the detection of these organisms when their levels in the subgingival plaque were high during the initial diagnosis of chronic periodontitis. Teeth that do not respond to scaling and rootplaning remain colonized by the BANA positive species, *P gingivalis, T. forsythia and T. denticola* (Nishihara and Koneke, 2004; Offenbacher et al., 2009; Scannapieco, 2005). Elderly veterans who were dentate and whose plaques were BANA-positive, were twice as likely to have a diagnosis of coronary heart disease, as were elderly dentate veterans whose plaques were BANA-negative (Loesche et al., 1998a; Loesche, 1999).

2.5. Periodontal Disease and Systemic Conditions

Periodontal pathogens and their virulence factors have the ability to disseminate and induce both local and systemic inflammatory responses in the host. This has led to the hypothesis that periodontal disease may have effects beyond the periodontal tissues themselves (Azarpazhooh et al., 2006). Miller first reported this concept in 1891 when he published the theory of "focal infection" (Miller, 1891). According to Miller's theory, oral foci of infection were considered responsible for a number of regional and systemic diseases, such as tonsillitis, pneumonia, endocarditic and septicaemia however, the lack of scientific evidence condemned this theory to dormancy (Azarpazhooh et al., 2006). The "Focal Infection Theory" proposed by Hunter (1910) concurred that bacteria and their products from local infections could be disseminated throughout the body and cause diseases in other organs (Billings, 1912). This was supported by other studies which suggested that systemic spread of endotoxins in periodontal disease may be associated with adverse pregnancy outcomes (Dasanayake et al., 2001; Jeffcoat et al., 2001; Offenbacher et al., 2001). In contrast, Davenport and co-workers (2002) reported no association, and a systematic review reported limited evidence that periodontitis is associated with increased risk for preterm low birth weight (Madianos et al., 2002).

Despite these discrepancies, oral health and its relationship to systemic health is important to society because up to 90% of the worldwide population is affected by either gingivitis or periodontitis (Philstrom et al., 2005), with reports indicating that up to 30% of the general population has a genetic predisposition to periodontitis (Albandar et al., 1999). The systemic importance of periodontal disease has been highlighted by the recent progress in identification and characterization of periodontal pathogens, as well as the characterisation of potential systemic mechanisms of action of bacterial products and inflammatory cytokines (Mokeem et al., 2004), thus pointing to the association periodontal disease with an increased risk for cardiovascular disease, (Spahr et al., 2006; Holmlund et al., 2006) diabetes, (Jason et al., 2006; Al-Shammari et al., 2006) community and hospital acquired respiratory infections, (Azarpazhooh et al., 2006) and adverse pregnancy outcomes (Beck et al., 2005; Bogies et al., 2006; Lopez et al., 2002). According to Dasanayake et al (2003), individuals with periodontal disease have approximately 1.5 – 1.9 increased odds for developing cardiovascular disease and after adjusting for many known risk factors for heart disease, this association was found to be statistically significant (Beck et al., 1996; Loesche and Lopatin, 1998; Loesche, 1999).There also appears to be a bidirectional relationship between periodontal disease and

diabetes, with a 2-3 fold-increased risk for diabetes among individuals with tooth loss (Azarpazhooh and Leake, 2006; Dasanayake et al., 2003).

Teeth and periodontium may serve as a reservoir and may also contribute to respiratory infections with poor oral hygiene such as dental decay increasing the odds for pneumonia 2- to 9-fold (Azarpazhooh and Leake, 2006; Dasanayake et al., 2003).

Treatment of periodontal infection may reduce the risk of other systemic conditions. In a randomized clinical trial to estimate the effect of periodontal therapy on traditional and novel risk factors for cardiovascular disease and on markers of inflammation, D'Aiuto et al (2006), found that therapy reduced inflammatory cytokines, blood pressure, and cardiovascular risk scores (D'Aiuto et al., 2006). Faria-Almeida et al (2006) found that periodontal treatment of type 2 diabetic patients resulted in improved diabetic control (lower HbA1c levels) and treatment of mechanically ventilated patients with a daily oral hygiene regime consisting of a 0.12% chlorhexidine gluconate mouthwash reduced the risk for nosocomial pneumonia (Genuit et al.,2001; Koeman et al.,2006).

2.6. Pregnancy-associated periodontal disease

During pregnancy, changes in hormone levels promote an inflammatory response that increases the risk of developing gingivitis and periodontitis (Jared and Boggess, 2008). Because of varying hormone levels without any changes in the plaque levels, 50%-70% of all women will develop gingivitis during their pregnancy, commonly referred to as pregnancy gingivitis (Hasegawa et al., 2003; Leon et al., 2007; Barak et al., 2007). This type of gingivitis is typically seen between the second and eighth month of pregnancy (Hasegawa et al., 2003; Barak et al., 2007).

Increased levels of the hormones progesterone and oestrogen can have an effect on the small blood vessels of the gingiva, making it more permeable (Jensen et al., 1981; Barak et al., 2003). This increases the mother's susceptibility to oral infections, allowing pathogenic bacteria to proliferate and contribute to inflammation in the gingiva (Offenbacher et al., 1998; Hasegawa et al., 2003). This hyper-inflammatory state increases the sensitivity of the gingiva to the pathogenic bacteria found in dental biofilm. Females often see these changes during other periods of their life when hormones are fluctuating, such as puberty, menstruation, pregnancy, and again at menopause (Jensen et al., 1981; Barak et al., 2003). During

pregnancy, the ratio of bacterial anaerobes to aerobes and the proportions of *Bacteroides melaninogenicus, Prevotella intermedia (Bacteroides intermedius) and Porphyromonas gingivalis (Bacteroides gingivalis)* were found to increase (Kornman and Loesche, 1980). Pregnant women were established to have a level of *Bacteroides* species 55 times higher than that of non-pregnant women (Jensen et al., 1981; Barak et al., 2007).

Maternal infection with periodontal pathogens has a deleterious effect on foetal growth and viability. According to (Offenbacher et al., 2006) a hypothetical model of the association between maternal periodontal inflammation and foetal development may be proposed. Periodontal bacteria and their virulence factors, found in the periodontal pockets, induce a local periodontal host immune response that includes mainly the production of inflammatory cytokines such as IL-1, PGE2, TNF-α and antibodies against the bacteria. If this immune response and the neutrophils are not capable of keeping the infection localized (such as low maternal IgG response to bacteria), then the bacteria and/or their virulence factors and the inflammatory cytokines may gain access systemically via the blood circulation (Offenbacher et al., 2006). This would be evidenced clinically by signs of bleeding on probing and increased pocketing during pregnancy (Offenbacher et al., 2006). The presence of the bacteria in the blood circulation will trigger the host to elicit a second round of inflammatory response, systemic this time, mainly by the production of more inflammatory cytokines and acute-phase reactants such as C-reactive protein from the liver (Offenbacher et al., 2006).

Eventually, bacteria and/or their virulence factors and inflammatory cytokines appear to reach the placenta, as about 40 % of all pregnancies are associated with some foetal IgM antibody response to organisms of maternal oral origin (Offenbacher et al., 2006). This will create another site of bacterial challenge and possibly placental infection, leading to a new inflammatory response, localized at the foetal-placental interface this time, with the production of more inflammatory cytokines (Offenbacher et al., 2006). As in periodontal tissues, these cytokines, although produced with the intention to combat the infection, may also cause tissue destruction (Offenbacher et al., 2006). Because the structural integrity of the placenta is vital for the normal exchange of nutrients between the mother and the foetus, this placental tissue damage may contribute to impaired foetal growth, which may lead to low birth weight (LBW) (Offenbacher et al., 2006). In addition, structural damage in the placenta may disrupt the normal blood flow between the mother and the foetus, affecting the maternal blood pressure and leading to preeclampsia. The increase in the production of inflammatory

cytokines such as IL-1β and PGE2 also may contribute to preterm rupture of the membranes and uterine contraction and lead to miscarriage or preterm delivery (Offenbacher et al., 2006).

Finally, periodontal bacteria and/or their virulence factors and inflammatory cytokines may cross the placenta and enter the foetal circulation. There, they may trigger a new foetal-host immune response, as evidenced by the observed elevated levels of foetal IgM to periodontal pathogens. If the foetus cannot control the infection, the bacteria and/or their virulence factors may gain access to various tissues and initiate local inflammatory responses and, consequently, structural damage to the foetal tissues and organ systems. Depending on the extent of this damage, the newborn may or may not survive the perinatal period. However, survivors may possess deficiencies that may compromise their quality of life, even throughout adulthood (Offenbacher et al., 2006).

2.7. Adverse pregnancy outcomes defined

This study uses terminology from the World Health Organization International Statistical Classification of Diseases and Related Health Problems to ensure consistency with universally accepted definitions (WHO, 2005). A list of terms and their definitions are in appendix 6.

2.7. 1. Neonatal Low birth weight (LBW)

Low birth weight (LBW), is a significant public health issue in both developed and developing countries (Kramer et al., 1998; Barros et al., 2001). This obstetric complication is usually a direct result of pre-term labour, in which case it is referred to as pre-term delivery (less than 37 weeks) of low birth weight infants (PLBW) (WHO, 1984) . The prevalence of LBW in the United States is about 7.3% and in the United Kingdom, 6%. In Africa, the average LBW is around 12% and around 15% in Asia (Williams et al., 2000).

Various factors have been associated with the delivery of PLBW infants and maternal risk factors including age, height, weight, socioeconomic status, ethnicity, smoking, nutritional status and stress (Nordstrom et al., 1996). In addition, birth intervals, previous complications, pre- and ante-natal care, maternal hypertension, generalized infections, localized infections of the genital and urinary system, and cervical incompetence may also be important (Lamont, 1998; Walker et al., 1998). However, a significant proportion of LBW is

of unknown aetiology and infection is considered a major factor in these cases (Barros et al., 1992).

Infant mortality has fallen in many parts of the world (Lee et al., 1995) but there has been little or no success in preventing LBW and in some developing countries with reliable national or regional birth registration, preterm birth appears to be on the rise (Silva et al., 1998; Barros et al., 2001), as has also been reported from most developed countries (Joseph et al., 1998; Kramer et al., 1998).

2.7.2. Pre-term labour and pre-term premature rupture of membranes

Pre-term labour and premature rupture of foetal membranes (PPROM) are frequently associated with pre-term birth (Goldenberg et al., 2008). PPROM is associated with 30–40% of pre-term deliveries, complicates 2–5% of all pregnancies, and has been found to be the most common identifiable cause of preterm delivery (Menon and Fortunato, 2007). Although the exact cause(s) of pre-term labour are unclear, the following conditions have been associated with pre-term labour, cervical incompetence, or pre-term membrane rupture: premature activation of the foetal and maternal hypothalamus–pituitary–adrenal axis (e.g. mediated by maternal or foetal stress), systemic or ascending bacterial vaginal infections, decidual haemorrhage, uterine distortion, and uteroplacental insufficiency (Moutquin, 2003; Schneider et al., 2006). Early PPROM appears to be associated with underlying pathologic processes, most likely due to inflammation and/or foetal membrane infection (Simhan and Canavan, 2005) and clinical factors such as low socioeconomic status, low body mass index, tobacco use, pre-term labour history, urinary tract infection, vaginal bleeding at any time in pregnancy, and amniocentesis (Schneider et al.,2006).

2.7.3. Preterm birth

Preterm birth has a severe socioeconomic impact and is a major problem for modern obstetrics due to its increasing frequency and the accompanying expense (Pararas et al., 2006). Several maternal characteristics related to preterm birth have been identified (Pararas et al., 2006; Katz et al., 2009). The aetiology (see section 2.9 below) in most cases, however, remains inadequately understood (Pararas et al., 2006) but infection is considered as a risk factor for preterm delivery (Pararas et al., 2006; Katz et al., 2009). Various microorganisms

have been linked to the pathogenesis of preterm birth (Pararas et al., 2006 ; Katz et al., 2009) and these microbes may reach the amniotic cavity and foetus by ascending from the vagina and cervix, by haematogenous distribution through the placenta, migration from the abdominal cavity through the fallopian tubes, or through invasive medical procedures (Pararas et al., 2006). Very pre-term birth occurs in only a small proportion of the infant population, its societal and personal health impact is considerable because of its disproportionately high perinatal morbidity, mortality, and need for costly medical care (Wimmer and Pihlstrom, 2008).

Preterm birth is a critical priority in healthcare throughout the world because of medical, social, and economic reasons (Shennan and Bewley, 2006) however, interventions to prevent pre-term birth have proven to be almost universally ineffective (Shennan and Bewley, 2006; Wimmer and Pihlstrom, 2008).

2.8. Cost of infant morbidity due to preterm birth

Maternal infections have long been recognized as increasing the risk for pregnancy complications such as preterm birth and preeclampsia and Jared and Boggess (2008) point out that adverse pregnancy statistics could have a major impact on health care policy because they often form the basis for international comparisons and assessment of health care funding needs. It is also important because it is the foremost cause of death in the first month, causing up to 70% of all perinatal deaths (Andrews et al., 2000; Raju, 2008). Prematurity is responsible for almost 50% of all neurological complications in newborns, and leads to lifelong complications in health, including but not limited to visual problems, developmental delays, gross and fine motor delays, deafness, and poor coping skills (Andrews et al., 2000; Raju, 2008). These complications increase the health care dollars spent on each child and on average, the medical cost alone for a preterm birth is 10 times greater than the medical costs for a full-term birth (Andrews et al., 2000; Raju, 2008).

In 2005, the nationwide cost of preterm birth in the United States was more than $26.2 billion for health care and lost productivity (Jared and Boggess, 2008). Although there have been advances in technology to help save the infants who are born premature or low birth weight, there are problems associated with these conditions which persist throughout life (Andrews et al., 2000; Raju, 2008; Jared and Boggess, 2008). Preterm infants are at an increased risk for a number of serious health complications, including chronic lung disease, severe brain injury,

motor and sensory impairment, learning difficulties and behavioural problems (Wimmer and Pihlstrom, 2008). First-year mortality rates are significantly higher for preterm infants and these children often require significantly greater family practitioner services, education services and social services than infants born at term or normal birth weight (Stavros et al., 2003), and thus the economic impact associated with the perinatal period, as well as throughout life, can be substantial (OECD, 2001). On average, a preterm infant costs $51,600 more than the average cost for full-term infants in the first year of life with additional long-term costs often continuing over the individual's lifetime (Behrman et al., 2006) .

Globally, over 4 million babies die within the first 4 weeks of life and a third of these are secondary to pre-term birth (Lawn et al., 2005). Infant death rates appear to be highest in Africa and South Asia (WHO, 2006), with Liberia and Mauritania having the highest known rate of infant death in the world (104 and 111 respectively, per 1000 live births). Among industrialized nations, Japan, the United Kingdom, and the United States have a rate of nearly seven deaths per 1000 infants within the first 4 weeks of life (WHO, 2006). In the United States, pre-term birth is the second leading cause of neonatal mortality (ACOG Practice Bulletin 2001, 2003, WHO 2006). The US preterm birth (under 37 weeks) rate rose to12.8% in 2006, an increase of 21% since 1990 and the rate of low birth weight (under 2500 g) rose to 8.3% in 2006, an increase of 19% since 1990 (Martinet et al., 2007). There are also indications that pre-term birth is increasing in other countries with the risks of infant morbidity and mortality being greater with increased prematurity, especially when birth occurs before 34 weeks (Strebel and Bucher, 1994; Tracy et al., 2007).

Tracy et al (2007) reported an increasing trend for live pre-term birth among low-risk Australian women (5.9% in 1994 to 6.6% in 2003) and Langhoff-Roos et al., (2006) reported that pre-term deliveries in Denmark increased by 22% (5.2–6.3%) from 1995 to 2005. The incidence of spontaneous pre-term deliveries increased mostly among precipitous women at low risk (Tracy et al., 2007).

Complicated pregnancies impose a risk not only to the mother, but also, and primarily, to the offspring. The majority of very preterm infants (born at less than 32 weeks' gestation) enter the neonatal intensive care unit (NICU) owing to an increased risk of perinatal mortality, especially due to respiratory problems (asthma, lower respiratory infections, broncho pulmonary dysplasia, impaired lung development and function, chronic lung disease) (Michalowicz et al., 2006; Offenbacher et al., 2006). Fortunately, new modalities in perinatal

care, such as the use of lung surfactant treatments and maternal receipt of steroid injections to hasten foetal lung development, have improved the survival rates of preterm infants (Michalowicz et al., 2006; Offenbacher et al., 2006). However, preterm and LBW infants who survive the neonatal period also face a higher risk of developing neuro developmental problems (cerebral palsy, blindness, and deafness), behavioural problems (attention deficit hyperactivity disorder), learning problems, cardiovascular disease and metabolic abnormalities (obesity, type 2 diabetes mellitus) (Michalowicz et al., 2006; Offenbacher et al.,2006). The resulting obstetric complications are not only a significant health care expense (estimated at more than $5.5 billion annually), but also affect the wellbeing of the affected infants throughout life (Offenbacher et al., 2006). However, not all of the contributing factors have been identified, and more than 25 per cent of all complicated pregnancies occur without any known reason. With periodontal diseases being both preventable and treatable, it would be of significant public health interest in pregnancy if a cause-effect relationship with preterm birth could be demonstrated (Michalowicz et al., 2006).

Neurological disorders, such as cerebral palsy, which is often found with mental disability, epilepsy, and cognitive impairment, are of special concern for survivors of pre-term birth (Hack et al., 1994). Up to 40% of surviving infants with birth weight to 750g have modest to severe disabilities (Hack et al., 1994), while pre-school and school-age children born pre-term, may have cognitive and behavioural disorders. School-age children with very low birth weight (VLBW, under 1500 g) have higher rates of grade repetition and need for special education than term-born infants (Taylor et al., 2000). Research on adolescent outcomes of VLBW infants suggests that its sequelae persist over time (Saigal et al.,2000b) with the impact and burden of extremely low birth weight (ELBW, under 1000 g) adversely affecting families (Taylor et al., 2001). Compared with parents of term infants, parents of ELBW infants reported more marital stress (Saigal et al., 2000a) while parents of young children with VLBW reported more financial, social, and family stress (Cronin et al., 1995).

2.9. Causes of pre-term birth

According to Schneider et al., (2006) the main reported causes of adverse pregnancy outcomes are maternal infection and placental, foetal, or uterine pathosis. Maternal infection and placental pathosis appear to be the most important causal factors, but each can cause pre-term labour, premature pre-term membrane rupture, or result in medically induced pregnancy

interruption. Increasingly, very pre-term birth occurs with multiple pregnancies (Blondel et al., 2006) and is often a result of assisted reproductive technology (Mukhopadhaya and Arulkumaran 2007; Reddy et al., 2007; Schieve et al., 2007). Prominent risk factors for pre-term birth include history of previous preterm birth, demographic characteristics, periodontal disease, and behavioural factors such as tobacco use (Goldenberg et al., 2008).

Demographic factors associated with pre-term birth include, ethnicity, extremes of maternal age, low socioeconomic status, and low pre-pregnancy weight (MacDorman et al., 2007). Preterm labour and pre-term birth have also been associated with stressful life situations such as domestic violence; close family death; insecurity over food, home or partner; work and home environment (Eskenazi et al., 2007, Precht et al., 2007). A higher risk of spontaneous pre-term delivery has been associated with genetically driven excessive amniotic fluid IL-Ib or with a disturbance of bioavailability of this cytokine, which is central to the pro-inflammatory reaction to infectious stimulants (Witkin et al., 2003; Genc et al., 2004). The foetus also has a role in pre-term birth by recognising a hostile intrauterine environment and precipitating labour by the premature activation of the foetal–placental parturition pathway (Annells et al., 2004; Boggess et al., 2005; Gardosi, 2005).

Uterine bacterial infections[2] can occur between the maternal tissues and the foetal membranes (i.e. in the choriodecidual space), in the foetal membranes (the amnion and chorion), the placenta, the amniotic fluid, and the foetus (Goldenberg et al.,2000 ; Romero et al., 2006; Goldenberg et al., 2008). Intrauterine infectious organisms are believed to originate primarily from ascending the vaginal tract, and secondarily, haematogenously from other parts of the body (Goldenberg et al., 2000). Therefore, it is plausible that microbes originating from the oral cavity may be transmitted to the uterus. It is also possible that other oral microorganism besides periodontal pathogens could cause intrauterine infections (Morency et al., 2006; Han et al., 2006). Ascending vaginal or systemic infections are considered frequent causes of pre-term birth with or without-term premature rupture of membranes (Hillier et al., 1995; Goldenberg et al., 1998; Hill, 1998; Flynn et al., 1999). Increased concentration of endotoxin in cervical mucus (Platz-Christensen et al., 1993; Mattsby-Baltzer et al., 1998), vaginal fluid (Sjoberg and Hakansson 1991; Platz-Christensen et al., 1993) and microbial invasion of a sterile compartment such as the amniotic cavity (Romero et al., 1987a, b) is an indication of an increased Gram-negative microbiota such has

[2] Infection of the foetal membranes is termed chorioamnionitis; infection of the umbilical cord is funisitis; and infection of the amniotic fluid is called amnionitis (Goldenberg et al., 2000).

been reported in women with bacterial vaginosis (Romero et al., 2004) which, if occurring early in pregnancy, appears to be a particularly strong risk factor for pre-term delivery and spontaneous abortion (Leitich et al., 2003a). However, recent evidence suggests that the majority of pre-term births are caused by bacterial infections of the chorioamnion, in which the infecting organisms originate in the vagina (Goldenberg et al., 2002). A significant association has been reported between the detection of microbial DNA (*Streptococcus* ssp. and *Fusobacterium nucleatum*) and previous pregnancy complications including pre-term delivery and premature rupture of membranes (Bearfield et al., 2002). According to Smith et al., (2007) maternal infection is more likely to be associated with spontaneous very pre-term births in women with socio-economic deprivation.

Whether or not the endometrial cavity is normally sterile remains unanswered, although the traditional view holds that it is sterile (Horowitz et al., 1995). However, several studies such as Cowling et al. (1992), Moller et al. (1995), Espinoza et al. (2006), have reported microbiological colonization in the uterine cavity and there is controversy regarding the most effective microbiological sampling procedures, which pathways of infection exist, and where the microorganisms are likely to be located. Ansbacher et al. (1967), Duff et al. (1983), Espinoza et al. (2006) point out that in addition to the presence or absence of microorganisms, it is important to characterise the host response that microorganisms elicit under physiologic and pathologic circumstances (Costerton et al., 2003; Romero et al., 2004; Swidsinski et al., 2005).

Bacteria activate cell-mediated immunological responses, that can induce host production of proinflammatory cytokines, including interleukin-1 (IL-1), IL-6, tumour necrosis factor-α (TNF-α), and prostaglandins. Bacterial endotoxins such as lipopolysaccharides are a component of the cell wall of Gram-negative bacteria (Narahara and Johnston, 1993; Smith et al., 2007) and may precipitate pre-term labour if they reach the foeto-placental unit (Gibbs et al., 1992; Smith et al., 2007).

In an attempt to test the hypothesis that the risk for pre-term birth is increased by oral microorganism colonization of the uterine environment and/or the initiation of a foetal or maternal immune response by these bacteria, Boggess et al., (2005) collected 640 umbilical cord blood specimens and assayed cord serum levels of C-reactive protein, IL-1b, IL-6, and TNF-α, prostaglandin E2 (PGE2), 8-isoprostane, and the presence of foetal IgM antibody against selected oral pathogens. The joint effects of foetal IgM seropositivity and detectable

C-reactive protein, high 8-isoprostane, PGE2 or TNF-α resulted in significantly increased risk for pre-term birth. They concluded that foetal exposure to oral pathogens evidenced by an IgM response is associated with preterm birth, and the risk for pre-term birth is greatest among foetuses that also demonstrate an inflammatory response (Boggess et al., 2005). The method of dissemination of oral pathogens is unclear in this investigation and foetal exposure may occur because of systemic dissemination or these organisms may ascend from the vagina possibly following oral-vaginal translocation (Boggess et al., 2005). Boggess et al., (2005) further state that the foetal immune response itself needs to be critically analysed: the response could be non-specific or it could be targeted towards other antigens and be unrelated to maternal periodontal disease or exposure to oral pathogens. In this regard, it is interesting that no differences in IgG, IgM, and IgA umbilical blood concentrations were found in a study by Mahulja-Stamenkovic et al., (1993) comparing infants with and without signs of sepsis.

Clearly, the role of the foetal immune response to oral and other pathogens is an important area for future study. When present in the amniotic fluid, chronic high levels of cytokines and prostaglandins may lead to intra-uterine growth restriction (IUGR), spontaneous pre-term labour, premature rupture of membranes, and pre-term birth (Sadowsky et al., 2006).

2.10. Periodontal disease and adverse pregnancy outcomes

In the early 1990s, Collins and colleagues hypothesized that oral infection, such as periodontitis could act as a source of bacteria and inflammatory mediators that could disseminate systemically to the foetal-8S placental unit, via the blood circulation inducing pregnancy complications (Beck et al., 2005). Determining whether periodontal disease is associated with pregnancy complications derives from the fact that despite the advances in prenatal care and increased public awareness, adverse pregnancy outcomes still present a major public health problem worldwide (Michalowicz et al., 2006; Offenbacher et al., 2006).

There is increasing evidence to suggest an association between periodontal disease and adverse pregnancy outcomes (Katz et al., 2009; Persson et al., 2009) with severe periodontitis seen to lower haemoglobin levels which, in turn, had an adverse effect on pregnancy and foetal development (Kothiwale et al., 2014).

The literature is controversial on the role of periodontitis and its influence on adverse pregnancy outcomes (Jared and Boggess, 2008). Recognition and understanding of the importance of oral health for systemic health has led to significant research into the role of maternal oral health and pregnancy outcomes (Jared and Boggess, 2008). Studies by authors such as Minkoff et al., (1983) Redman et al., (1999) Gibbs (2001) von Dadelszen et al., (2002) produce a large body of evidence pointing to infection as a key factor in adverse pregnancy outcomes. Oral mechanical manipulation (e.g. tooth brushing, dental procedures, and even routine mastication) can cause bacteraemia (Sconyers et al., 1973). Authors such as Garcia et al., (2001); Gibbs (2001) and von Dadelszen et al., (2002) highlight that chronic periodontal infections can produce local and systemic host responses leading to transient bacteraemia. Lipopolysaccharide (LPS) endotoxins and other bacterial substances can gain access to gingival tissue, initiate and perpetuate local inflammatory reactions, and consequently produce high levels of proinflammatory cytokines. Such activations of maternal inflammatory cell responses and cytokine cascades play important roles in the pathophysiological processes of preterm labour, low birth weight, and pre-eclampsia (Offenbacher et al., 1998; Champagne et al., 2000; Garcia et al., 2001; Gibbs 2001; Paquette, 2002; von Dadelszen et al., 2002).

Several studies report associations of adverse pregnancy outcome with higher gingival crevicular fluid levels of PGE2 and IL-1b (Offenbacher et al., 1998; Konopka et al., 2003; Carta et al., 2004) and elevated amniotic fluid concentrations of PGE2, IL-1b and IL-8 (Dörtbudak et al., 2005). Despite a tendency of higher mean gingival crevice fluid IL-1b levels, Noack et al., (2005) found no periodontitis-associated increased risk for pre-term birth and/or low birthweight and the varying results could be due to the effects of specific environmental or genetic risk factors, which result in variable maternal reactions. In this regard, it has been reported that a higher proportion of women who delivered preterm carried the polymorphic TNFa_308 gene (Moore et al., 2004). Importantly, the combination of periodontal disease and this allelic variant did not increase the risk of pre-term delivery. Moreover, there did not appear to be any interaction between the carriage of allelic variants of IL-1b13953 and TNF-a_308 gene and periodontal disease with pre-term delivery as has been reported for BV (Moore et al., 2004). It is possible that IL-6 produced in inflamed periodontal tissues can affect the foetal membranes and cause preterm uterine contractions (Offenbacher et al., 1998, Wang et al., 2001). There is evidence that the gingival crevicular fluid levels of IL-6 are higher in gingivitis and periodontitis compared with healthy controls

(Giannopoulou et al., 2003). In healthy Caucasians, the G/G homozygote of IL-6 gene polymorphism at has been associated with increased plasma levels of IL-6, whereas the C/C homozygote has been associated with decreased plasma IL-6 levels (Fishman et al., 1998).

2.10.1. Animal studies

In 1916, pregnant guinea pigs were inoculated with streptococci harvested from human stillborn foetuses and this inoculation resulted in a 100% abortion rate (Galloway et al., 1931), thus confirming that the focal infection found in teeth, tonsils, sinuses, and kidneys pose a risk to the developing foetus (Galloway et al., 1931).

Various researchers have since explored the possible association of periodontal disease with pregnancy complications in several experimental animal models (Lin et al., 2003; Beck et al., 2005; Boggess et al., 2005; Offenbacher et al., 2005; Yeo et al., 2005). In most models, periodontal bacteria (*P. Gingivalis* or *C. rectus*) are injected in a small chamber that previously had been implanted subcutaneously in the pregnant animals (hamsters, mice, rabbits) (Boggess et al., 2005; Yeo et al., 2005). A periodontal infection is simulated by creating a site of infection distant to the foetal-placental unit and the result of these studies reveal that maternal infection with periodontal pathogens has a deleterious effect on foetal growth and viability (Boggess et al., 2005; Yeo et al., 2005). Collins and co-workers reported a 25% reduction in birth weight in pregnant hamsters challenged subcutaneously in the dorsal region with periodontal pathogen *P gingivalis*, compared with normal healthy pregnant hamsters (Collins et al., 1994). This was also demonstrated in another study using animal models, where infection with Gram-negative periodontitis-associated microorganisms adversely affected pregnancy outcomes (Offenbacher et al., 1996).

In a series of animal studies in which pregnant hamsters were injected with the periodontal pathogen *P gingivalis,* Beck et al., (2005) found that infection led to smaller foetuses (approximately 20 % reduction in weight) and to an increase of inflammatory mediators (TNF-α and PGE2) at the site of infection and in the amniotic fluid. In subsequent experiments, in which periodontal disease was induced in pregnant hamsters, the investigators found similar results in terms of foetal growth (Lopez et al., 2005). These were the first proof-of-principle experiments to suggest a possible association of periodontal disease with adverse pregnancy outcomes (Beck et al., 2005; Lopez et al., 2005). Since then,

many investigators have tried to establish whether this association is also present in humans (Beck et al., 2005; Offenbacher et al., 2006).

2.10.2. Human studies

To show the impact on humans, Galloway et al., (1931) obtained a full mouth radiographic series on 242 women presenting for prenatal care. Fifteen % (n=57) had an apical abscess and the suggested treatment was extraction of the affected tooth. Of those who were treated, none resulted in a miscarriage or stillbirth (Galloway et al., 1931). This demonstrated that the removal of a known focal infection was more beneficial than allowing the infection to manifest throughout the pregnancy. It was therefore recommended that all foci of infection perceived to be a source of danger to any pregnant woman, should be removed early in pregnancy[3] to the advantage of both the mother and the foetus (Galloway et al., 1931).

Both gingivitis and bacterial vaginosis (BV) have been linked to adverse pregnancy outcomes (Persson et al., 2009) with typical vaginal colonisers such as *Ureaplasma urealyticum, Mycoplasma hominis, Bacteroides spp., Gardnerella vaginalis, Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis*, and group B haemolytic streptococci being commonly isolated from the amniotic cavity following preterm delivery (Pararas et al., 2006). Persson et al., (2009) associated bacterial counts in BV with gingivitis, since higher vaginal bacterial counts could be found in women with BV and gingivitis in comparison to women with BV and no gingivitis, thereby suggesting a systemic infectious susceptibility. Another study (Oittinen et al., 2005) of periodontal disease and BV in 252 women showed a strong association between periodontal disease and adverse pregnancy outcome in the first 130 pregnant women but a borderline association between BV and adverse pregnancy outcome. These authors suggested bacterial synergy and that both diseases might have additive effects on adverse pregnancy outcome.

[3] It is estimated that over 50% of pregnant women suffer from some form of gingival disease, either gingivitis or periodontitis (Jeffcoat et al 2001; Line; 2002). Many recent studies (Lopez et al., 2002; Dasanayake et al., 2003; Bogies et al., 2006; Offenbacher et al., 2006; Jared and Boggess, 2008) have reported that maternal periodontal disease may be an independent contributor to abnormal pregnancy outcomes including preterm birth, low birth weight, risk for preeclampsia, mortality, and growth restriction. However, the causality of how periodontitis influences pregnancy outcomes has not been established (Jared, and Boggess, 2008; Dasanayake et al., 2003; Offenbacher et al., 2006).

Preterm low birth weight is an important cause of morbidity and mortality in newborns (Barros et al., 1992). Low birth weight (LBW) has been associated with maternal oral hygiene status, smoking habits, ethnicity, systemic diseases, previous LBW babies, complications of pregnancy and type of delivery (López, 2008), while preterm low birth weight (PLBW) has been related to the mother's age, onset of prenatal care, systemic diseases, previous LBW babies, complications of pregnancy and type of delivery (López 2008). Despite intensive research on the aetiology of preterm low birth weight, in over 50% of clinical cases, the cause remains unknown (Barros et al., 1992). Epidemiological and microbiological studies have lent credence to the concept that periodontal disease may be a separate risk factor for cardiovascular disease, cerebrovascular disease, respiratory disease, as well as pre-term delivery of low birth weight infants (Barros et al., 1992; DeStefano, 1993; Offenbacher et al., 1996; Katz et al., 2009; Persson et al., 2009).

High oral levels of periodontal pathogens such as *P. gingivalis* during pregnancy may be associated with an increased risk for pre-term delivery (Lin et al., 2007). The presence of *P.gingivalis* antigens in placental tissues have been identified using immunocytochemistry (Katz et al., (2009). The antigens were detected in the placental sanction trophoblasts, chorionic trophoblasts, decidual cells, and amniotic epithelial cells, as well as the vascular cells indicating that *P.gingivalis* may commonly colonize placental tissue, and that the presence of the organism may contribute to preterm delivery (Leon et al., 2007; Katz et al., 2009). This translocation is accompanied by an increase in inflammatory mediators in the placenta inducing a significant alteration in the architecture of the placenta, especially in areas that are critical for the exchange of nutrients between the mother and the foetus, thus causing a decrease in the size of the foetus and preterm delivery (Madianos et al., 2001; Offenbacher et al., 2006). A greater mean attachment loss and a higher prevalence of periodontitis in pre-term birth mothers (cases n=583) compared with term mothers, with no differences in microbial or serum antibody levels detected between the groups (Jarjoura et al., 2005), support the concept that maternal periodontal infection in the absence of a protective maternal antibody response is associated with systemic dissemination of oral organisms that translocate to the foetus and result in preterm deliveries (Madianos et al., 2001; Offenbacher et al., 2006).

Various studies demonstrate that when there is both foetal exposure to maternal oral bacteria and an inflammatory response, the relative risk of preterm delivery is huge, with a risk ratio of 4:7 (Madianos et al., 2001; Offenbacher et al., 2006).

In this context, it should be noted that variations of female sex hormones during pregnancy may affect the subgingival microbiota (Kornman and Loesche, 1980) and that elevated salivary levels of estradiol may be associated with bacteria such as *Campylobacter rectus*, which may contribute to the progression of periodontal disease in pregnancy (Yokoyama et al.,2008).

2.11. Evidence favouring an association of Periodontal Disease with Pre-Term Birth

Early evidence has shown that women delivering preterm low birth weight babies were almost 8 times more likely to have periodontal disease (Offenbacher et al., 1996). Madianos et al., (2001) examined the prevalence of various periodontal bacteria along with the maternal and foetal antibody response against these organisms and tried to correlate the results with pregnancy outcomes in 400 pregnant women. They concluded that there was a higher rate of preterm deliveries among mothers without a protective immunoglobulin (IgG) response against the bacteria of the "red" cluster (Madianos et al., 2001). Moreover, the foetal IgM response against periodontal pathogens of the "orange" cluster was stronger in preterm neonates than in full-term neonates (19.9% versus 6.9%). Specifically, the prevalence of positive foetal IgM to *C. rectus* was significantly higher for preterm infants, which raises the possibility that this maternal oral pathogen may serve as a primary infectious agent causing prematurity. Subsequently it has been established that from the foetuses with a robust IgM response to periodontal pathogens, the risk of preterm birth is greatest among those that also demonstrate an inflammatory response, as indicated by the increase in cord serum levels of C-reactive protein, IL-1β, IL-6, TNF-α, PGE2 and 8-isoprostane (Boggess et al., 2005; Madianos et al., 2001).

Lin et al., (2007) examined the association between periodontal diseases and preterm birth and explored the underlying microbial and antibody responses associated with oral infection. The authors found that antepartum, the levels of periodontal pathogens tended to be higher in the preterm (case group) deliveries compared to the term deliveries (control group), with maternal anti-*P gingivalis* IgG significantly lower in the preterm group compared to the term group (Lin et al., 2007). Postpartum, levels of *P. gingivalis, T. forsythia, P. intermedia*, and *P. nigrescens* were statistically significantly higher in preterm births compared to term deliveries when adjusting for baseline levels, demonstrating increased synergy between the

red and orange microbial clusters in the preterm group compared to the term group (Lin et al., 2007). This study confirmed that high levels of periodontal pathogens and low maternal IgG antibody response to periodontal bacteria during pregnancy are associated with an increased risk for preterm delivery (Lin et al., 2007). Other factors significantly associated with preterm birth include a history of previous preterm birth and a low birth weight, frequency of prenatal visit, preterm uterine contraction, antepartum haemorrhage, placenta previa and preterm premature rupture of membranes (Chan et al; 2010). After controlling for other risk factors, Chan et al. (2010) also associated preterm birth with BANA-positive plaques in the 3rd trimester.

In another case-control study, women who delivered a full-term infant weighing <2500 grams were matched to women who delivered full term infants weighing >2500 grams. All women received a periodontal evaluation after delivery, and poor periodontal health was determined to be an independent risk factor for delivering a low-birth-weight infant (Dasanayake 1998; Jared and Boggess 2008). The same conclusion was drawn from a recent case-control study on the relationship between maternal periodontal disease and low birth weight babies (Haerian-Ardakani et al., 2013) where bleeding on probing, presence of supra-gingival calculus and CPITN scores were used for periodontal assessment.

Two prospective cohort studies (Jeffcoat et al., 2001; Offenbacher et al., 2006) found that moderate to severe periodontitis identified early in pregnancy could be associated with an increased risk for spontaneous preterm birth, independent of other traditional risk factors. In the first study, investigators from the University of Alabama conducted a prospective evaluation of over 1300 pregnant women. Complete medical, behavioural, and periodontal data were collected between 21 and24 weeks gestation. Generalized periodontal infection was defined as 90or more tooth sites with periodontal ligament attachment loss of ≥3 mm. The risk for preterm birth was increased among women with generalized periodontal infection; this risk was inversely related to gestational age. After adjusting for maternal age, race, tobacco use, and parity, this relationship remained (Jeffcoat et al., 2001). Offenbacher et al., (2006) conducted a prospective study of obstetric outcomes of over 1000 women who received an antepartum and postpartum periodontal examination. Moderate to severe periodontal infection was defined as 15 or more tooth sites with pockets depth ≥4 mm. The incidence of increased periodontal pocketing, defined as clinical disease progression, was determined by comparing site-specific probing measurements between the ante partum and postpartum examinations (Offenbacher et al., 2006; Jared and Boggess, 2008). Disease

progression was considered present if 4 or more tooth sites had an increase in pocket depths by ≥2 mm, with the postpartum probing depth being ≥4 mm. Compared to women with periodontal health, the relative risk for spontaneous preterm birth < 37 weeks gestation was significantly elevated for women with moderate severe periodontal infection, adjusting for maternal age, race, parity, previous preterm birth, tobacco use, markers of socioeconomic status, and presence of chorioamnionitis. Here too, periodontal disease progression was found to be an independent risk factor for delivery < 32 weeks gestation (Offenbacher et al., 2006; Jared and Boggess, 2008).

Santos-Pereira et al., (2007) studied 124 women between the ages of 15-40 to determine if chronic periodontitis increased the risk of experiencing preterm labour (PTL). In this cross-sectional trial, women who were admitted for preterm labour, with intravenous tocolysis, were enrolled into the PTL group. The control group consisted of term pregnancies that were admitted following the PTL mother. Periodontal examinations were performed within 36-48 hours after delivery and before discharge. Chronic periodontitis was described as one site with clinical attachment loss (CAL) > 1 mm with gingival bleeding. The severity of periodontitis was classified as early (CAL <3mm), moderate (CAL > 3 mm and < 5 mm), and severe (CAL >5mm). The extent of periodontitis was either localized, CAL < 30%, or generalized CAL > 30%. They, and others, confirmed that chronic periodontitis increased the risk of having preterm labour and a low-birth-weight infant (Goepfert et al., 2004; Santos-Pereira et al., 2007; Jared and Boggess, 2008).

Self-reported periodontitis was considered to be an independent risk factor for poor pregnancy outcomes in a cohort of women who completed a self-report questionnaire during their second trimester of pregnancy to assess their demographic, medical and reproductive history, smoking, pre pregnancy weight, and physical activity at the first prenatal visit (Pitiphat et al., 2008). There was no significant increased risk of having a preterm birth or small-for-gestational-age infant when adjusting for smoking, race/ethnicity, socioeconomic status, BMI, history of preterm delivery, presence of genitourinary infection, weekly weight gain, and history of dental check-ups in these mothers but a significant increase in risk for those who reported having periodontitis and poor pregnancy outcomes was noted . However, caution should be taken when interpreting these results due to the sample size and the indirect measurement (self-reported) of periodontitis (Pitiphat et al., 2008).

Another prospective cohort, enrolled over 1200 women to evaluate the association between periodontitis and preterm birth and/or low birth weight (Agueda et al.2008). All women were between the ages of 18- 40 and were enrolled between 20-24 weeks gestation. Demographic data, socioeconomic status, and medical and obstetric history were collected and full mouth periodontal examinations; (PD, CAL, and BOP) were performed by a single calibrated examiner and recorded at six sites per tooth. Periodontal disease was defined as four or more teeth with one or more sites with PD > 4mm and CAL > 3mm at the same site (Bogies et al., 2006). After adjusting for confounding variables, a significant association was found between preterm birth and periodontitis. However, no significant association was found between low birth weight and periodontitis (Agueda et al., 2008).

A case-control study of pregnant or postpartum mothers, in which cases (mothers who currently or previously delivered PLBW infants) had significantly worse periodontal disease than controls (mothers who delivered fullterm), the study, after controlling for other risk factors, concluded that periodontal disease is a statistically significant risk factor for PLBW (Mokeem et al., 2004). Similarly, in another case-control study, Dasanayake et al., (1998) studied 55 pairs of women and using logistic regression, demonstrated that mothers with healthy gingiva were at lower risk for LBW infants.

Based on the analysis of 12 identified studies that met their inclusion and exclusion criteria (six case–control, three cross-sectional and longitudinal, and three interventional) Scannapieco et al., (2003), while recognising an association between periodontal disease and adverse pregnancy outcomes, reported that they found no clear evidence that periodontal disease has a causal role. Another analysis of 25 studies (13 case–control, nine cohort, and three controlled trials) that focused on pre-term low birth weight, low birth weight, pre-term birth, birth weight by gestational age, miscarriage or pregnancy loss, and pre-eclampsia, found that 18 of the studies suggested an association between periodontal disease and increased risk of adverse pregnancy outcome while others found no evidence of such an association (Xiong et al., (2006). These authors agree that even though it appeared that periodontal diseases might be a risk factor for preterm and/or low birth weight, more methodologically rigorous studies are needed to confirm this (Scannapieco et al., 2003; Xiong et al., 2006).

2.12. Evidence against an association of Periodontal Disease with Pre-Term Birth

While there are data suggesting a relationship between maternal periodontal infection and preterm birth, several studies have failed to demonstrate such an association (Holbrook et al., 2004; Buduneli et al., 2005; Dörtbudak et al., 2005; Rajapakse et al., 2005; Xiong et al., 2006). In one of the largest studies to date, Moore et al., (2004) examined the relationship between multiple periodontal parameters, including mean probing depths, % of tooth sites with probing depths ≥4 mm, % of sites with bleeding on probing, and % of sites with clinical attachment loss ≥2 or ≥3 mm. No difference was found in the periodontal parameters between women with preterm birth and without preterm birth (Moore et al., 2004). However, a positive association have been demonstrated between maternal periodontal infection and spontaneous abortion between 12 and 24 weeks (Moore et al., 2004; Jared and Boggess, 2008).

A systematic review by Vettore et al., (2006) identified 36 studies that met their inclusion criteria, 26 of which reported associations between periodontal disease and adverse pregnancy outcomes. They noted clear differences between studies concerning measurement of periodontal disease and adverse pregnancy outcomes. Moreover, they reported that most studies did not control for confounders, thus rising serious doubts about conclusions that can be drawn from them. For that reason, it was proposed that the most that can be derived from these studies is the conflicting evidence to support a relationship between periodontal disease and pre-term birth (Konopka et al. 2003).

In a case-control study, Budeneli and colleagues found no differences in periodontal infection between women who delivered preterm versus those who delivered full term (Buduneli et al., 2005). However, women were at significantly increased risk for preterm birth if either *P. Gingivalis* or *C. rectus* were found in their subgingival plaque (Buduneli et al., 2005). In a more recent case-control study, Vettore et al., (2008) recruited 542 postpartum women who were over 30 years old and sought to explore the relationship between periodontal disease and preterm low birth weight. Cases were divided into 3 groups: low birth weight (n = 96), preterm (n = 110), and preterm and low birth weight (n = 63). Cases were compared to controls who were no preterm and non-low-birth-weight individuals (n = 393). Periodontal measurements were collected and later stratified into 15 definitions of periodontal disease for analysis. Other covariates were also recorded and used for analysis. The results of this study indicated that periodontal disease levels were higher in control individuals than in cases, and

that the extent of periodontal disease did not increase the risk of preterm low birth weight. They also showed that in the preterm low birth weight group, the mean pocket depth and the frequency of sites with CAL > 3 mm were lower than in the control group. It was concluded that periodontal disease was less severe in women with preterm low-birth-weight babies (Vettore et al., 2008).Xiong et al., 2006 performed a systematic review and meta-analysis of 44 studies (26 case control, 13 cohort, and 5 controlled trials) to examine the relationship between maternal periodontal disease and adverse pregnancy outcome. The meta-analysis showed that maternal treatment of periodontal disease reduced the rate of preterm low birth weight infants as a group but not preterm or low birth weight individually (Xiong et al., 2006).

In a more recent study, Srinivas et al. (2009) compared the risk of adverse pregnancy outcomes (preterm birth, preeclampsia, foetal growth restriction, or perinatal death) in women with and without periodontal disease in a multicenter prospective cohort study and failed to demonstrate an association (Srinivas et al., 2009). A study of postpartum women in Italy yielded similar results (Abati et al., 2013).

A case-control study on the relationship between oral health status (periodontal disease and carious pulpal exposure (CPE)) and preterm low-birth-weight (PTLBW) infant deliveries among Tanzanian-African mothers found no evidence to support the claim that periodontal disease or carious pulpal exposures are significant risk factors for preterm low-birth-weight infant delivery among Tanzanian-African mothers (Mumghamba and Manji, 2007). However they found young age, hypertension and being unmarried as significant risk factors and suggested a need for research that is not only prospective in nature, but also conducted on a larger scale incorporating both periodontal pathogens and mediators of inflammation (Mumghamba and Manji, 2007).

Farrell et al., (2006) found no association between poorer periodontal health and either pre-term birth or low birth weight in a cohort study of 1793 women who reported no history of smoking. However, they reported an association between higher mean probing depth and late miscarriage.

2.13. Problems with Studies of periodontal disease and adverse pregnancy

Many studies have reported a positive association between maternal periodontal disease and adverse pregnancy outcomes, especially the increased risk of pre-term birth and low birth weight. However, it is impossible to make any clear conclusions from these studies because of the many different study designs, sampling methods, definitions of periodontal disease and adverse pregnancy outcomes, confounding factors, and possible effect modification by known or unknown factors (Borrell and Papapanou, 2005; Hyman, 2006; Vettore et al., 2008).

2.13.1. Differences in diagnostic criteria

In previous studies concerning the association of periodontal disease and adverse pregnancy outcomes, various periodontal disease definitions have been used with the commonly accepted clinical measures of periodontal disease that were established >50 years ago (Ramfjord 1959), still being used as diagnostic criteria today (Xiong et al., 2006). Although various indices have been developed since then (Loë and Silness 1963; Silness and Loë, 1964; Ainamo et al., 1982), most have limited validity (Carlos et al., 1986; Baelum and Papapanou, 1996).Without a universally accepted standard for periodontal disease diagnosis, most of the researchers used their own case definitions that combined pocket depth (PD) and clinical loss of attachment (CAL), based mostly on disease distribution within the study population (Xiong et al., 2006). Some studies defined periodontal disease in terms of Decayed, Missing, and Filled Teeth (DMFT) and Community Periodontal Index of Treatment Needs (CPITN), the Russell Periodontal Index (RPI) and similar indices, all of which have limited sensitivity for disease detection (Ainamo et al., 1982; Dasanayake 1998; Carta et al., 2004; Mokeem et al., 2004; Xiong et al., 2006). However, it is clear that the CPITN is unsuitable for measuring the prevalence and severity of periodontal disease (Baelum & Papapanou, 1996).

A consensus on a definition is essential to optimize the interpretation, comparison, and validation of clinical data and with no universal consensus on definition, any prior definitions may prove to be obsolete as we gain further information regarding the pathophysiology of the associations reported (Borrell and Papapanou, 2005). Clinical markers of periodontal disease, such as gingival recession, clinical attachment loss, or bleeding on periodontal probing, may be late manifestations of the local infection, such that bacterial

exposure may have already occurred with subsequent downstream deleterious effects (Kramer 1987; Borrell and Papapanou, 2005; Xiong et al., 2006). Recognition of the variation in clinical criteria used to define periodontal infection is important when reviewing the literature. Traditional measurements for diagnosing PD have focused on clinical morbidity, and often result in inconsistent diagnosis and the inability to recognize active disease thus studying the anaerobic bacterial burden and the inflammatory response may be more critical than measuring PD (Manau et al., 2008).

2.13.2. Consideration of confounding risk factors

Periodontal disease and adverse pregnancy outcomes are associated with a variety of risk factors, many of which, such as smoking and low socioeconomic status, are common to both (Hyman 2006). In addition to the lack of a consistent clinical definition, several of the studies (Moore, et al., 2004; Holbrook et al., 2004; Buduneli et al., 2005) with no association between maternal periodontal disease and adverse pregnancy outcomes did not control for potential confounding variables, thus questioning whether in those studies that reported an association, the observed associations represent a causal relationship or are due to the confounding effects of other variables such as low socio-economic status and smoking (Paquette, 2000; Hujoel et al., 2002; Hyman , 2006 and Xiong et al., 2006). Several important confounding variables such as history of adverse pregnancy outcomes, infections (e.g. bacterial vaginosis and chorioamnionitis), antibiotic use during pregnancies, excessive body mass index, or maternal disorders (hypertension, diabetes) must be considered (Paquette 2002; Hujoel et al., 2002; Xiong et al., 2006). Moreover, psychosocial and demographic factors as well as previous history of adverse pregnancy outcome are almost completely neglected, even though they are considered to be among the most prominent risk factors for adverse pregnancy outcome (Vettore et al., 2008).

In a study of poor, rural, non-smoking Sri Lankan women, Rajapakse et al., (2005) found that periodontal disease was not significantly associated with an increased risk of preterm low birth weight. The authors suggested that previously reported associations might have been due to the residual confounding effects of smoking and other variables, while also accepting that they were not adequately powered to test the association.

2.13.3. Differences in populations studied

Another potential reason for the disparate findings among studies is the differences in populations studied. Most studies that showed an association between periodontal disease and adverse pregnancy outcomes have consistently been found in populations with a high incidence of preterm deliveries and within economically challenged families. Quite the opposite is true for those studies that did not show an association. They were usually conducted in countries with universal health care and a lower incidence of preterm birth or low-birth-weight infants. Differential access to health care insurance, dental care, and prenatal care, may confound the relationship between maternal periodontal disease and adverse pregnancy outcome. Disparities in oral health may also be partially explained by racial differences in inflammatory and immune responses (Dasanayake 1998, Carta et al., 2004, Mokeem et al., 2004). One of the important differences found in studies conducted in the USA or in developing countries tended to include African American women and women from economically disadvantaged families, and they consistently reported significant associations between periodontal disease and adverse pregnancy outcomes. In contrast, the studies conducted in European countries or Canada (all of which offer their citizens universal health care) did not find an association between periodontal disease and adverse pregnancy outcomes suggesting that the effects of periodontal disease on adverse pregnancy outcomes may be different according to the socio-economic status and access to dental care (Xiong et al., 2006).

2.13.4 .Differences in definitions of adverse pregnancy outcomes

Another issue that complicates that association of adverse pregnancy outcomes with periodontal disease is the different ways that studies have defined adverse pregnancy outcome (Hyman, 2006; Xiong et al., 2006; Vettore et al., 2008). A number of studies do not use the anticipated delivery date as a fundamental reference baseline. Each definition of adverse pregnancy outcomes may have a very different genesis. For example, many types of inflammation and infection may potentially influence IUGR, pre-term labour, premature rupture of membranes, pre-term birth, and eclampsia and only one of which may be periodontal disease (Hyman, 2006; Vettore et al., 2008). It is clear that reports that do not clearly distinguish between the many types of adverse pregnancy outcomes and simply categorize infants as PLBW, apparently excluding preterm infants with normal birth weights

and full-term infants with low birth weights (i.e. intrauterine growth restriction) thereby adding little to our understanding of the potential relationship between periodontal disease and adverse pregnancy outcomes. Of the clinical trials reported to date, only that reported by Michalowicz et al., (2006) meets generally accepted standards for clinical trials including multicentre design, establishment of adequate sample size based on a priori criteria of a clinically meaningful outcome, evidence of appropriate data and safety monitoring by an independent review board, intent-to-treat analysis, reporting of secondary periodontal outcomes as well as the primary outcome variable, and public registration before trial initiation (Hyman, 2006; Vettore et al.,2008).

Adverse pregnancy and pre-term birth statistics can have a major impact on health care policy because they often form the basis for international comparisons and assessment of health care funding needs. Pre-term birth rates are based on gestational age. Because gestational age can only be reliably identified in about 15% of cases (Schneider et al., 2006), birth weight is often used as a surrogate measure of pre-term birth. As result, incorrect conclusions regarding-term births are often made. For example, only two-third of infants born in Germany weighing 2500g are preterm; the remaining are term-born underweight due to genetic or developmental restriction (Schneider et al., 2006; Wimmer and Pihlstrom, 2008).

In order to estimate the impact of any disease, it is critical that the disease be well characterized and accurately assessed. While the definitions of preterm birth, very preterm birth, low birth weight, small for gestational age, and other obstetric findings are reasonably well defined, no consensus has yet been achieved on the definition of periodontitis in periodontal research (Kramer 1987; Xiong et al., 2006). Unless this is done, one cannot judge whether the periodontal therapy was successful in reducing signs of disease (Carta et al., 2004; Mokeem et al., 2004).

2.14. Treatment of Periodontal disease

Since periodontal disease is considered to be plaque induced, most treatment and prevention techniques have used non-specific debridement procedures as the primary means of controlling plaque deposits (Loesche, 1976; Loesche, 1999).

2.14.1. Mechanical debridement

Nonsurgical treatment of periodontal disease includes the mechanical removal of supragingival and subgingival microbial deposits and calculus by debridement. Debridement can be implemented using hand or ultrasonic instruments to remove bacteria and calculus and to plane the root surfaces to remove diseased and bacterially contaminated root surfaces (Loesche and Grossman 2001). Preshaw (2004) asserts that the foundation of periodontal treatment is root surface instrumentation which is necessary to disrupt the subgingival biofilm mechanically and reduce the bacterial bio burden. Although bacteria are necessary for periodontal disease to occur, a susceptible host is also required (Preshaw, 2004; Offenbacher et al., 2008). The immune-inflammatory response that develops in the gingival and periodontal tissues in response to the chronic presence of plaque bacteria results in destruction of structural components of the periodontium leading, ultimately, to the clinical signs of periodontitis(Preshaw, 2004).

The "biologic systems model" proposed by Loesche (1999), Harmse and Ehmke (2006) and Offenbacher, et al (2008), highlights that the clinical presentation of periodontal disease is intimately tied to the underlying biologic phenotype. The model also states that the integration of subject-level factors, microbial composition, systemic immune response, and gingival tissue inflammatory mediator responses will better reflect the biology of the biofilm-gingival interface in a specific patient (Loesche, 1999; Harmse and Ehmke, 2006; Offenbacher et al., 2008).

This may provide insights on clinical management and in the treatment of periodontitis. The value of microbiological testing is closely linked to the value of a specific, adjunctive antimicrobial therapy. However, there is only little evidence that knowledge of a patient's oral microbial colonisation results in improved clinical outcomes (Loesche, 1999; Harmse and Ehmke, 2006; Offenbacher et al., 2008). The management of periodontal disease by means of a treatment strategy based on the Specific Plaque Hypothesis is easier and less expensive to implement than one based on debridement/surgical intervention, as espoused by the Non-specific Plaque Hypothesis (Harmse and Ehmke, 2006; Offenbacher et al., 2009).

2.14.2. Antimicrobial therapy for periodontitis

The option of anti-microbial therapy for periodontitis depends upon the type of bacteria that are responsible for the periodontal pathology, as clinical symptoms do not indicate which antimicrobial agent to use (Loesche, 1999; Offenbacher et al., 2009). According to Foster and Kolenbrander, (2004) microbial communities within the human oral cavity are dynamic associations of more than 500 bacterial species that form biofilms on the soft and hard tissues of the mouth.

Highly selective interspecies recognition is evident as initial colonizers pair with early and middle colonizers to form multispecies communities that grow on saliva (Foster and Kolenbrander, 2004; Kolenbrander, 2011).[4] Considering the non-specific plaque hypothesis, then it would be best to use antimicrobial agents that kill as many bacterial species as possible (Loesche, 1999; Offenbacher et al., 2009). On the other hand, considering the specific plaque hypothesis, which implies that the host response is to a limited number of bacterial types, would advocate that an agent that is active against the specific periodontopathic species is indicated (Loesche, 1999; Offenbacher et al., 2009). The difficulty in eliminating spirochetes or *A. actinomycetemcomitans, P. gingivalis,* and *P. intermedia* from plaques suggests that the elimination of these species is not a realistic treatment outcome (Flemmig et al., 1996; Mombelli et al., 1996; Scannapieco, 2005). Many, if not all, of the periodontopathic bacteria are so prevalent in plaque samples that they could be considered as members of the normal microbiota (Flemmig et al., 1996; Mombelli et al., 1996; Scannapieco, 2005). This persistence of the periodontopathic species probably relates to the underlying microbial ecology of the plaque and the relationship of these species to each other and to the host[4] (Scannapieco, 2005; Loesche, 1999, Africa 2012).

2.14.3. Periodontal treatment and pregnancy

Prevention and treatment of periodontal infection is aimed at controlling the bacterial biofilm, arresting progressive infection, and restoring lost tooth support (Jeffcoat, 1994). Recent

[4]Human oral bacteria interact with their environment by attaching to surfaces and establishing mixed-species communities. As each bacterial cell attaches, it forms a new surface to which other cells can adhere. Adherence and community development are spatiotemporal; such order requires communication (Foster and Kolenbrander, 2004; Kolenbrander, 2011).

studies have assessed the value of periodontal therapy in decreasing the incidence of pregnancy complications and as an independent risk factor for obstetric complications. However, they have been inconclusive on the effects of periodontal therapy during pregnancy for preventing adverse pregnancy outcomes (Davenport et al., 2002 ; Lopez et al., 2002b; Dasanayake et al., 2003; Lopez et al., 2005; Michalowicz et al., 2006; Jared and Boggess, 2008).

2.14.3.1. Studies supporting the effectiveness of therapy in reducing adverse pregnancy outcomes.

In a pilot trial of periodontal treatment, Offenbacher et al., (2006), found a trend towards reduced preterm birth among women treated during pregnancy compared with those who delayed therapy until postpartum. In another study, a statistically significant reduction in preterm birth was observed when comparing the effect of mechanical, non-surgical therapy plus the use of a powered toothbrush with oral hygiene alone and the use of a hand toothbrush (Offenbacher et al., 2006b). The authors concluded that treatment was safe, improved periodontal health, prevented periodontal disease progression, and resulted in a 3.8-fold reduction in pre-term birth (after adjusting for baseline imbalance in periodontal disease).

These studies demonstrated that women who were treated during pregnancy had a significant improvement in oral health measures and a reduction in oral pathogen burden. The women treated during pregnancy showed an improvement in clinical markers of periodontal infection, with reduction in clinical attachment loss and reduction in bleeding on dental probing. In another randomized, intent to treat study, Tarannum and Faizuddin (2007) found that nonsurgical periodontal treatment during pregnancy reduced the risk of preterm births ($p<0.001$) and low birth weight ($p<0.002$). An inverse correlation existed between CAL and birth weight in the control group, which may suggest that higher CAL were associated with lower birth weights. There was also an inverse correlation between gestational age and periodontal characteristic in both groups. This may suggest that shorter gestational ages were associated with higher values among periodontal parameters (Tarannum and Faizudin, 2007).

Several earlier clinical trials have also concluded that periodontal treatment during pregnancy may reduce the rates of pre-term birth or the composite outcome of preterm birth

and/or low birth weight (Lopez et al., 2002b, Giannopoulou et al.,. 2003, Jeffcoat et al., 2003, Lopez et al., 2005, Sadatmansouri et al., 2006, Offenbacher et al., 2006a). Similar outcomes were observed in randomized intervention studies (Mitchell-Lewis et al., 2001; Lopez et al., 2002; Jeffcoat et al., 2003), consisting of scaling and root planning of all teeth with or without the use of a chlorhexidine mouth rinse or metronidazole.

Lopez et al., (2005), enrolled 870 pregnant Chilean women with gingivitis in a randomized trial of periodontal treatment during pregnancy versus delayed treatment, and found almost a 2-fold reduction in pre-term birth and/or low birthweight deliveries among women treated during pregnancy. The pregnant women were randomly assigned to receive periodontal therapy before 28 weeks of gestation (n=580) or after delivery (n=290). Periodontal therapy consisted of plaque control instructions, supra and subgingival scaling, once-daily 0.12% chlorhexidine mouth rinses and oral hygiene instruction. Lopez et al., (2005) reported that women with gingivitis who received periodontal therapy before 28 weeks of gestation had a significantly lower incidence of pre-term birth and/or low birthweight deliveries than women who did not receive periodontal therapy.

Periodontal treatment was found to contribute to decreased adverse pregnancy outcomes following the effects of non-surgical treatment of periodontal disease during the second trimester of gestation (Sant'Ana et al., 2011; Reddy et al., 2014).

2.14.3.2. Studies showing no effect of therapy in reducing adverse pregnancy outcomes

Randomized clinical trials (RCTs) of antibiotic administration to reduce the rate of pre-term birth have yielded conflicting results (Morales et al., 1994; Hauth et al., 1995; McDonald et al., 1997; Carey et al., 2000; Paavonen et al., 2000; Rosenstein et al., 2000; Kekki et al., 2001; Lamont 2003; Ugwumadu et al., 2003) and there is no uniform proof that antibiotic use reduces the risk of pre-term birth.

A meta-analysis comprising thirteen trials and comparing 3,576 women in intervention groups with 3,412 women receiving usual care, demonstrated no significant reduction in preterm births and low birth weights. It was found that primary periodontal care during pregnancy could not be considered an efficient way of reducing the incidence of preterm birth (Sadatmansouri et al., 2006). Jeffcoat et al., (2003) reported the results of a single-centre, three-arm RCT conducted in the United States although a ‘‘trend’’ was reported, this study

found there was no statistically significant benefit of adjunctive metronidazole antibiotic to scaling and rootplaning in reducing adverse pregnancy outcomes (Jeffcoat et al., 2003). Indeed, consistent with other pre-term birth trials using metronidazole (Klebanoff et al., 2003); adjunctive use of this antibiotic to scaling and rootplaning actually increased the rate of pre-term birth compared with scaling and rootplaning plus placebo. Although periodontal treatment was reported to improve measures of periodontal disease, it did not alter the risk of adverse pregnancy outcome (Michalowicz et al., 2006), however, the study indicated that treatment of periodontitis in pregnant woman improves periodontal disease and is safe (Michalowicz et al., 2006).

2.15. The importance of oral health during pregnancy

Wilder et al., (2007) surveyed practicing obstetricians in five counties in North Carolina to assess their knowledge of periodontal disease and to determine their practice behaviours regarding oral disease and adverse pregnancy outcomes. They found that only 22% looked in a patient's mouth at an initial visit. In addition, while most (84%) considered periodontal disease a risk factor for adverse pregnancy outcomes, 49% rarely or never recommended a dental visit during pregnancy (Wilder et al., 2007).

Another North Carolina survey of 504 nurse practitioners, physician assistants and certified nurse midwives assessed the knowledge, behaviour, and opinions about periodontal disease and its relationship to adverse pregnancy outcomes. Of the 204 who responded, 63% reported looking in the patient's mouth to screen for oral problems at the initial visit, 20% felt that their knowledge of periodontal disease was current, and all agreed that their discipline should receive instruction regarding periodontal disease. A collaborative effort between the health care provider and the oral health care professionals was indicated by 95% in order to reduce the patient's risk of having an adverse pregnancy outcome (Thomas, 2008).

A later study, (Morgan et al., 2009) assessed how obstetrician-gynaecologists address oral health during pregnancy and found that most obstetrician-gynaecologists agree that routine dental care during pregnancy is important (84%), periodontal disease can have adverse effects on pregnancy outcome (84%), and treating periodontal disease positively affects pregnancy outcome (66%). This study found that the majority seldom ask pregnant patients whether they have recently seen a dentist (73%), ask about current oral health (54%), or provide information about oral care (69%). Over a third (38%) obstetrician-gynaecologists

reported not advising patients to see a dentist for routine prophylaxis, with 80% of these saying they had not previously thought about it. Most respondents (77%) reported having patients that declined dental services because of pregnancy (Morgan et al., 2009). It was concluded that obstetrician-gynaecologists recognize the importance of good oral health during pregnancy but largely do not address it. It was suggested that improved training in the importance of oral health including the recognition of oral health problems, and knowledge of procedure safety during pregnancy may make doctors more comfortable with assessing oral health and more likely to address it with patients (Morgan et al., 2009). A recent study (Chi et al., 2014) found that dentists who were knowledgeable about periodontal disease were more likely to counsel pregnant patients and proposed that future interventions should improve the oral health knowledge of dentists and other healthcare professionals regarding the importance of comprehensive dental care, including periodontal treatment when needed, for all pregnant patients.

The prophylactic programme for the pregnant woman, must entail a thorough interview include an informative session on the specific risks during this period, and the motivation of the patient towards oral-dental health and for implementing, the needed primary prophylactic measures (Iftime 2008).

Sunita and Kee (2013) and Avula et al. (2013) conducted knowledge, attitudes and practices (KAP) assessment of oral health and adverse pregnancy outcomes among pregnant women in Hyderabad, India and Brunei, Darussalam respectively. The authors found that most pregnant women need more information about oral health and disease prevention and concluded that it would be prudent to compile and consider their knowledge, attitudes and practices along with sociocultural characteristics, so that health education programmes are customised to different sectors of the population (Sunita and Kee 2013 ; Avula et al. 2013). More intense dental health education, including oral health promotion in maternal child health centers, can lead to improved oral and dental health and ultimately, favourable pregnancy outcomes (Sunita, 2013).Information and knowledge of the causes of the main oral-dental diseases during pregnancy will assist pregnant women to take corrective action and be able to minimize their effects. A well-defined, realistic and easily applicable programme during early pregnancy stages will ensure oral health personnel may achieve this goal (Iftime, 2008; Jeffcoat, 1994, Sunita, 2013).

Maintaining good oral hygiene before and during pregnancy is crucial for preventing gingivitis and periodontitis (Jeffcoat, 1994). Dental professionals can facilitate this level of oral health through assessment, education, and proper treatment planning. Verifying the hormonal status and other risk factors for periodontal diseases and poor pregnancy outcomes of women during the medical history process will enable the provider to customize the treatment plan and oral hygiene instructions. Behavioural interventions such as smoking cessation, exercise, healthy diet, and maintenance of optimal weight are also useful preventive measures against periodontal disease (Iftime, 2008; Jeffcoat, 1994). While the mechanisms of these interventions is unknown, they likely operate by reducing conditions that promote growth of pathologic bacteria, improving immune function, reducing inflammatory responses, and improving glucose control (Jeffcoat, 1994).

2.16. Dental procedure safety guidelines

Current guidelines and data suggest that dental care during pregnancy is safe. However, scaling and root planning is best accomplished between 14-20 weeks gestational age. Providing dental care for pregnant women will help remove potentially harmful bacteria from dissemination and possibly leading to other complications (Offenbacher et al., 2006).

In 2004, the American Academy of Periodontology (AAP) issued a position statement regarding dental care for pregnant women. The AAP recommended that all women who were pregnant or planning a pregnancy should receive preventive dental care, including a periodontal examination, a prophylaxis, and restorative treatment (American Academy of Periodontology, 2004). The American Academy of Periodontology (2004) also proposed that scaling and root planning should be complete early in the second trimester and that any presence of acute infection or abscess should be treated immediately, irrespective of gestational age. Treating infection as early as possible will remove a potential source of infection that could be harmful to the mother and the baby (American Academy of Periodontology, 2004). AAP confirmed that treatment of periodontitis in pregnant women is safe and should be performed to improve the oral health of the woman. This has been supported by The Academy of General Dentistry (AGD) whose recommendations are similar to the AAP but they suggest that pregnant women have a tiered treatment plan to include an examination in the first trimester, a dental cleaning in the second trimester, and then, depending on the patient, another appointment early in the third trimester (National Institute

of Dental and Craniofacial Research. 2006). They also recommend communication between the dental provider and the obstetrician for any dental emergency that would require anaesthesia or other medication to be prescribed.

The American Dental Association (ADA) suggestions are similar to the AAP and the AGD; however, they also address the safety issues surrounding taking a dental radiograph during pregnancy. If a radiograph is needed for diagnosis or treatment, as they often are, then pregnant women should have the radiographs taken. Matteson et al., (1991) estimated that a full mouth series of radiographs, with 20 radiographs, exposes the mother to <1 m rem of radiation. The foetus is usually exposed to approximately 75 m rems of naturally occurring radiation during a pregnancy. Therefore, dental radiographs contribute to a negligible amount of radiation exposure (Matteson et al., 1991). Care and caution should be taken to prevent further exposure by using a leaded apron with a thyroid collar (Matteson et al., 1991).

In 2004, Bright Futures Practice in Oral Health published an oral health pocket guide designed to provide health care providers with an overview of preventative oral health supervision for 5 developmental periods, including pregnancy and postpartum. Bright Futures began in 1990 and was initiated by the Health Resources and Services Administration (HSRA) Maternal and Child Health Bureau (MCHB). The guidelines suggest that health care providers assess the risk of oral disease and provide general suggestion to prevent carious lesions in pregnant women (Bright Futures Practice in Oral Health 2004). Other suggestions or recommendations for the prevention of carious lesions included to expectorate and not rinse the mouth after brushing with fluoridated toothpaste to allow the fluoride additional time to protect the teeth (Bright Futures Practice in Oral Health 2004). They recommended that pregnant women use an alcohol free, over the counter-fluoridated mouth rinse at night. While carious lesions do not lead to periodontal diseases, the accumulation of bacterial plaque biofilm is a culprit in these diseases. Like many other initiatives, Bright Futures recommends that pregnant women visit an oral health care provider for an examination and restoration of all active carious lesions as soon as possible (Bright Futures Practice in Oral Health 2004).

According to the National Institute of Dental and Craniofacial Research (2006), early in the second trimester (14-20 weeks gestation) is the most favourable time to perform dental procedures. During this gestational age there is no threat of teratogenicity, nausea and vomiting have usually subsided, and the uterus is below the umbilicus, providing more

comfort to the mother (American Academy of Periodontology, 2004; National Institute of Dental and Craniofacial Research, 2006). Unrestored carious lesions should be restored as soon as possible as some pregnant women require general anaesthesia with intubations at delivery. Some physicians are hesitant to intubate due to the increased risk of airway obstruction due to the decreased integrity of decayed teeth that could break off (American Academy of Periodontology, 2004; National Institute of Dental and Craniofacial Research. 2006).

If treatment is provided in the last trimester, care should be taken to prevent suppression of the inferior vena cava by keeping the woman in an upright position (American Academy of Periodontology, 2004; National Institute of Dental and Craniofacial Research. 2006). All health care providers should advise women that maintaining good oral health during pregnancy is not only safe but necessary to reduce the risk of infection to the mother and possibly the foetus. While it remains inconclusive whether maternal periodontal treatment improves pregnancy outcome, it is clear that treatment of varying degrees of clinical periodontal disease during pregnancy is safe and improves maternal oral health (Offenbacher et al., 2006; Jeffcoat et al., 2003; Michalowicz et al., 2006).

All dental services should be available to pregnant women; however, despite the benefit of treatment, periodontal infection in women of childbearing age remains highly prevalent, particularly among low-income women and members of racial and ethnic minority groups (Jared and Boggess, 2008).

2.17. Summary and objectives

This chapter reviewed the literature investigating periodontal disease as a possible risk factor for adverse pregnancy outcomes. The rate of pre-term birth appears to be increasing worldwide and efforts to prevent or reduce its prevalence have been largely unsuccessful. Thus, while there are indications of an association between periodontal disease and increased risk of adverse pregnancy outcome in some populations, there is no conclusive evidence that treating periodontal disease improves birth outcome.

Available evidence from clinical trials indicates that, although non-surgical mechanical periodontal treatment in the second trimester of pregnancy is safe and effective in reducing

signs of maternal periodontal disease, its ability to reduce the rate of pre-term birth has not been conclusively established.

Results of the studies of the association between periodontal disease and deliveries of preterm, low-birth weight infants vary with some of the studies showing a correlation. The variability among studies in definitions of periodontal disease and adverse pregnancy outcomes as well as widespread inadequate control for confounding factors have resulted in widespread results which makes it difficult to validate conclusions .

Null hypothesis

Positive BANA tests during pregnancy have no bearing on pregnancy outcomes.

Objectives

- To determine the presence or absence of periodontal disease in pregnant women.
- To conduct a BANA test in pregnant women to establish the presence of selected BANA positive species.
- To ascertain the pregnancy outcomes of women who do not have periodontal disease and a negative BANA test result.
- To ascertain the pregnancy outcomes of women who have periodontal disease and a positive BANA test result.
- To compare the pregnancy outcomes of women who do not have periodontal disease and a negative BANA test result and women who have periodontal disease and a positive BANA test result.

CHAPTER 3: RESEARCH DESIGN AND METHDOLOGY

This chapter discusses the research design and methodology used in the study. It describes the development of the research instrument and data collection method. While keeping a focus on the objectives, consideration was given to the methodology employed by other researchers who carried out similar studies.

3.1. Study sample

3.1.1. Subject selection

The study population consisted of pregnant women attending maternal obstetric units (MOU) in KwaZulu- Natal. Nonprobability sampling was employed to select the sites where participants were enrolled using convenience sampling. However, when logistical problems were encountered (lack of room space or staff support) and in the event that these problems could not be circumvented, the target was foregone and more patients were recruited from another site. The provincial Department of Health and the Municipal health district managers respectively granted permission for the research to be conducted at various hospitals and clinics selected (Appendix 2). Individuals attending MOU clinics located at Community Health Centres (CHC's) were approached and offered the opportunity to anonymously participate in the study. The nature of the study was explained to them verbally and in writing and they were requested to sign or indicate with an X that they had understood the nature of the study and voluntarily consented to participate.

3.1.2. Sample Size

The sampling of the participants from the MOU`s was by means of convenience sampling and a sample size of 400 participants was targeted. Regarding the question of the sample size required, it was necessary to have a knowledge of estimates of the variance ($\sigma^2 = \pm 5$) which was available from similar studies. According to the calculation, it was discovered that 400 participants would be adequate given a 95% confidence interval and a standard deviation of 10 (derived from similar studies). However, more participants were enrolled based on availability during the first phase of the study and to cover for attrition.

3.1.3. Inclusion Criteria

The participant was pregnant.

The participant was 18 years or older.

The participant gave written informed consent to be part of this study.

3.1.4. Exclusion Criteria

The exclusion criteria was a history of medications or medical problems that may affect the study outcome such as, current use of systemic corticosteroids, antibiotics, congenital heart disease, and existing hypertension or diabetes before pregnancy, asthma, and chronic renal disease. Participants with urinary tract infections, vaginal infections, history of cervical intraepithelial lesions or neoplasia were excluded. Further exclusion criteria were the mothers, whose labour was induced or delivered by C- section, multiple pregnancies and those who used tobacco, thus avoiding the bias of confounding.

3.2. Data Collection

A cross sectional study structure was used with data collection in a standardized format using a structured questionnaire and data capture sheets (Appendix 3).

3.2.1. Questionnaire data

The questionnaire had several sections that contained closed-ended questions and was interviewer-administered (Williams, 1996; Marshall and Rossman, 1995); all participants, however, had access to the researcher to clarify any uncertainties they had about answering the questions. The questionnaire elicited demographic information about the participants and contained items designed to obtain information regarding medical history, factors predisposing for periodontal disease and oral hygiene habits (Appendix 3).

3.2.2. Clinical examination

The clinical data was collected by recording the results of a clinical examination of the participants to determine the presence or absence of periodontitis. The clinical data was recorded on a data capture sheet (Appendix 3). The examination was conducted with the subject supine in a dental chair or hospital bed or doctor's examination couch and where necessary an external headlamp was used to facilitate the calibrated periodontal examination. The clinical assessment included obtaining the patient history which included administration of a questionnaire containing items designed to obtain information regarding medical history, factors predisposing for periodontal disease and oral hygiene habits (Appendix 3).

The calibrated periodontal examination entailed recording of clinical indices and recording the presence of any oral lesions. Measurements of the clinical indices and periodontal recordings were taken at the mesial or distal locations of the Ramfjord teeth (Table 1).

Table 1 Description of Ramfjord teeth

Maxillary right first molar 16	Maxillary left central incisor 21	Maxillary left first bicuspid 24
Mandibular right first bicuspid 44	Mandibular right central incisor 41	Mandibular left first molar 36

(Ramfjord, 1959)

If the teeth selected were missing, teeth substitution was as follows (Ramfjord, 1959; Gettinger et al., 1982):

16: by the mesial of 15, if not by the distal of 17

21: by the distal of 22

24: by the distal of 25

36: by the mesial of 35, if not by the distal of 37

41: by the distal of 42

44: by the distal of 45

The following clinical indices were recorded:

- Decayed, missing, and filled (DMF) teeth.
- Plaque Index (P1, Table 2) was scored from 0-3 using the plaque index criteria of Silness and Loë, (1964).
- Gingival Index (GI) was scored (Table 3) from 0-3 using the gingival index criteria of Silness and Loë, (1964).
- Periodontal pocket depth (PD) and clinical attachment level (CAL) measures were recorded to the nearest higher millimetre by means of the North Carolina periodontal probe (Hu-Friedy®, Chicago, IL, USA), 15 mm in length and 0.35 mm in diameter. Pocket depth was measured from the base of the pocket to the gingival margin.
- Clinical loss of attachment was measured from the cemento-enamel junction to the base of the pocket.
- Oral plain mirrors (Hu-Friedy®, Chicago, IL, USA) and an external headlamp was used to facilitate periodontal examinations. The presence or absence of calculus and oral lesions was also recorded.

The above data was used to classify the patient as either periodontally healthy or periodontally unhealthy (Loesche 1979; Watson et al. 1994). The clinical severity of the disease was determined in participants who have periodontitis by means of the following grading system adapted from Offenbacher et al. (2001).

- Mild Periodontitis ≥ PD 3mm or Clinical Attachment Level (CAL>2mm)
- Moderate (≥2 sites with PD ≥5 mm and ≥2 sites with CAL ≥2 mm)
- Severe Periodontitis(≥4 sites with PD ≥5 mm and ≥4 sites with CAL ≥2 mm)

Table 2 Measurement of Plaque Index (PI) scores:

0	No plaque in the gingival area.
1	A film of plaque adhering to the free gingival margin and the adjacent area of the tooth. Plaque may only be recognized, by running a probe across the tooth surface.
2	Moderate accumulation of soft deposits within the gingival pocket, on the gingival margin and /or adjacent tooth surface, which can be seen by naked eye.
3	Abundance of soft matter within the gingival pocket and /or on the gingival margin and adjacent tooth surface.

(Silness & Loë, 1964)

Table 3 Measurement of Gingival Index (GI) scores:

0	Normal gingival.
1	Mild inflammation – slight change in colour, slight oedema. No bleeding on probing.
2	Moderate inflammation – redness, oedema and glazing. Bleeding on probing.
3	Severe inflammation – marked redness, oedema and ulceration. Tendency to spontaneous bleeding.

(Silness & Loë, 1964)

3.3. Intra-examiner reliability

A single investigator was calibrated prior to the study and carried out all periodontal examinations. Intra-examiner reliability in using the dental examination criteria was tested (pilot test) by performing duplicate examination on 10 randomly selected mothers on two consecutive days. Intra examiner reliability was determined using Kappa statistic. Ninety-five percent agreement on criteria for pocket depth was obtained.

3.4. Plaque sample collection

The plaque sample collection similar to the method employed by Watson et al., (1994) was used which entailed using the tip of a sterile periodontal probe to obtain the subgingival plaque samples as follows. Supragingival plaque was removed from the site by means of a curette and discarded. The periodontal probe was then introduced subgingivally between the first molar and second premolar of each quadrant, or, if one of these teeth was missing, then between either the first and second molars, or between the premolars (Watson et al., 1994).

3.5. BANA assay

The BANA reagent card (Perioscan®, Oral-B Laboratories Inc., Redwood City, CA) was used according to the manufacturer's instructions (Perioscan, Oral B). Briefly, the principle of the test is as follows. The BANA hydrolysis test is a plastic card with two separate reagent matrices (strips). The lower strip is impregnated with BANA reagent and the upper strip contains a chromogenic diazo reagent, Fast Black K.B-naphthylamide. One of the hydrolytic products of the BANA reaction reacts with the Fast Black K, producing a permanent blue colour (Loesche et al., 1990b; Watson et al., 1994).

Following plaque collection, the tip of the periodontal probe containing the plaque was wiped onto the BANA impregnated lower strip, located on the bottom of the BANA reagent card. The reaction was immediately activated by water applied to the upper strip containing the fast black dye. The lower strip was folded onto the upper, and both strips were held in place with a metallic clip. The card was placed in an incubator at 55^{o}C for 15 min. Site information was recorded on the BANA Test card in the marked space (Loesche et al., 1990b; Watson et al., 1994).

Positive results appeared as blue spots that reflect weak or strong BANA reactions, whereas negative results did not show any colour change (Loesche et al., 1992) (Watson et al., 1994). The result of the BANA analysis was recorded on the data capture sheet (Appendix1).

3.6. Recording of Pregnancy Outcomes

The pregnancy outcomes data (whether the pregnancy was successful, stillborn or miscarriage) was obtained from the maternal notes of the participants along with and maternal age, weight, sex of infants, and method of delivery. In the event of a successful pregnancy the gestation period and the infant birth weights were recorded.

In the case of successful pregnancies, a live infant whose birth weight was less than 2.500 kg was considered to have low birth weight (LBW) and those delivered before 37 weeks gestation were considered as preterm births (PLBW). In the case of successful pregnancies, a live infant who weighed 2.500 kg or more and delivered at 37 weeks gestation or later was considered as normal birth (NB). The pregnancy outcomes information was recorded on a data capture sheet (Appendix 2).

Data was collected through structured interviews and medical records. Anthropometric and socio demographic characteristics' including height, Body Mass Index (BMI), ethnicity, education level, marital status, and information on work and income were ascertained. The women were questioned about smoking and alcohol intake, illicit drug use, physical violence, contraceptive methods, and parental desire for and satisfaction with pregnancy.

Pregnancy information - including gestational age, method of delivery, baby weight at birth, type of birth, sex of neonate, baby length, and proportion weight/gestational age were transcribed from medical records. Occurrences of hypertension, pre-eclampsia, hepatitis B, anaemia, gestational diabetes, urinary infection, and infections during pregnancy were also recorded (Leal et al., 2004; Vettore et al., 2008).

3.7. Ethical Considerations

- This research proposal was submitted to the University of the Western Cape research committee for ethical clearance and was registered accordingly.
- Permission in principle to conduct the study at the various hospitals and clinics was obtained from the KwaZulu-Natal Health Department.
- Specific permission was obtained from the participating hospitals and clinics to conduct the study at their facilities.
- The nature of the study was explained and informed consent was obtained in writing from the participants.
- Participation in this study was entirely voluntarily and the participants were allowed to withdraw from the study at any time if they wished to do so.
- Anonymity and confidentiality were achieved by not using the participant's names on the questionnaire, the participant was given a serial number and the hospital or clinic was recorded as a serial number.
- A letter of thanks and a small token of appreciation were sent to the participating hospitals and clinics staff who assisted in making the research possible.
- The study complied with the declaration of Helsinki (2013).

3.8. Pilot Survey

A pilot study was conducted on 20 participants to determine the validity and reliability of the data collection process. Conducting a pilot study gave the researcher the opportunity to ensure that the questions in the questionnaire were clear and unambiguous, and determined the length of time for executing the clinical examination the BANA test Conducting a pilot study created an opportunity to check if the data collection methods were viable, clear and unambiguous, and to make the necessary adjustments where necessary**.**

3.9. Data Analysis

Questionnaire data were categorized, coded and then captured in Excel for basic descriptive analysis. The database was imported into SPSS to perform complex statistical analyses. Statistical significance was defined as $p<0.05$.

Sensitivity (Sens), specificity (Spec), positive predictive value (PPV) and negative predictive value (NPV) for BANA and PD (periodontal disease) to predict adverse pregnancy outcomes were calculated as follows:

Sens = TP/ TP+ FN X 100

Spec = TN/FN+ FP X 100

PPV = TP/TP+FP X 100

NPV = TN/TN+FN X 100

Where: TP= True Positive, TN = True Negative, FP = False Positive
and FN = False Negative.

CHAPTER 4: MATERNAL PRENATAL ANALYSES

4.1. Study population and sample

There were 488 individuals who participated in the study and completed the questionnaires. However, 45 questionnaires were excluded from the analysis due to the exclusion criteria, incomplete data and/or loss to follow up. The final sample consisted of 443 pregnant female adults.

4.1.1. Racial distribution

Figure 4.1 demonstrates the racial distribution of the entire study population. Using the racial classification system commonly used to address national and regional inequities in South Africa, 360 (81.26%) of the group were Black, 56 (12.64 %) Coloured, 23 (5.19%) Indian and 4 (<1%) were White.

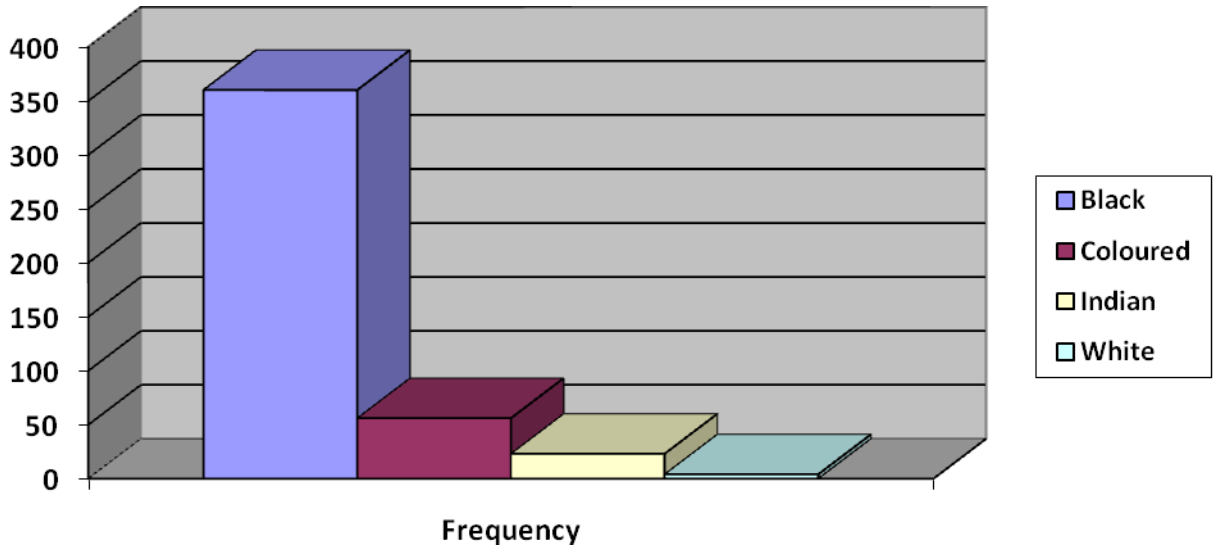

Figure 4.1: Racial distribution of the study population

4.1.2 Age distribution

The mean (SD) age of the study population was 24.13 (±5.30) years. The median age of participants was 23 years (range 18-42). The first quartile was 20 years and the third quartile was 27 years. The age distribution is reflected in Figure 4.2.

Figure 4.2 Age distribution of the study population

4.1.3. Regional distribution

It should be borne in mind that individuals often travel from their home towns to obtain care. Even though convenience sampling was utilised to select recruitment, it is erroneous to assume that participants live in the town where the MOU is situated (where the participant was recruited) and therefore participants were asked to disclose where they lived. These areas were classified as urban versus rural areas according to municipal regional classification. The majority of the participants lived in urban areas (Table 4.1).

4.1.4. Educational level

All the participants had some formal education. The majority had attained at least secondary level education (70.88%). Tertiary education was attained by 22.35 % and primary level education was attained by 6.77% (Table 4.1).

Table 4.1 Frequency distribution of the regional distribution and educational level of mothers who participated in the study

Variable	Frequency (%)
Regional distribution	
Urban	403 (90.97)
Rural	40 (9.03)
Education	
Primary	30 (6.77)
Secondary	314 (70.88)
Tertiary	99 (22.35)

4.1.5. Stage of pregnancy at time of sampling

The majority of the participants were in their second trimester of pregnancy (4-7 months) with 71 (16.03%) in their 4th month, 108 (24.4%) in their 5th month, 97 (21.9%) in their 6th month and 86 (19.4%) in their 7th month of pregnancy. Fifty-four (12.19%) and 8 (1.8%) were in the third trimester (8-9 months) and a few, 1(<0.5%) and 18 (4.06%) were in the first trimester (2nd and 3rd month respectively). Figure 4.3 demonstrates the pregnancy stage distribution of the study population.

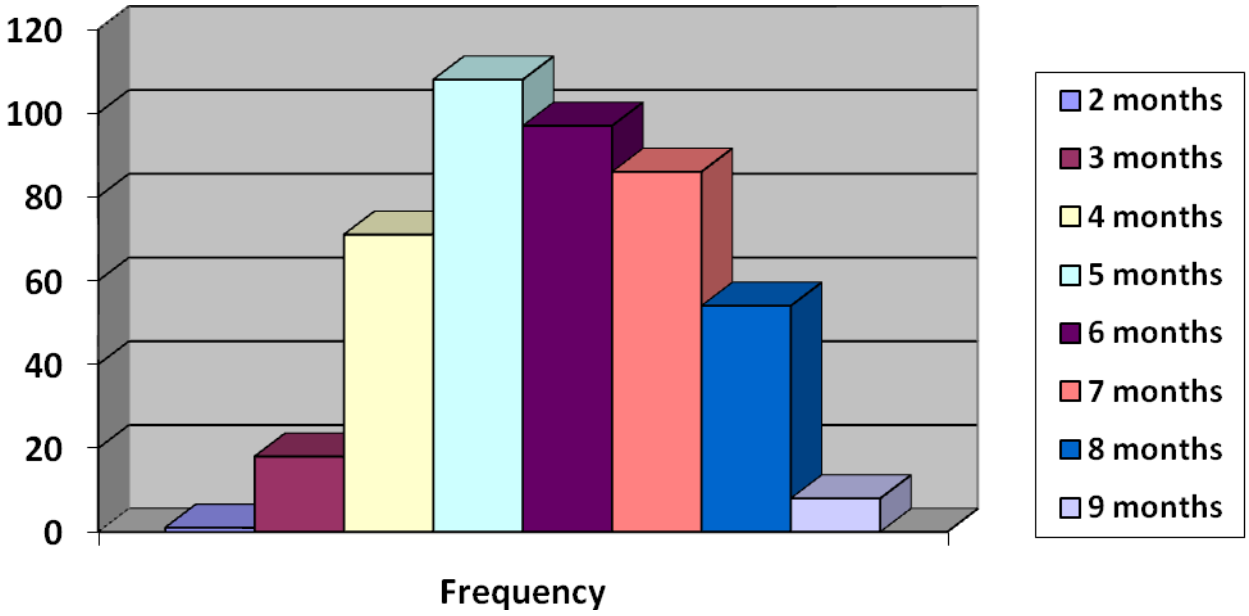

Figure 4.3 Pregnancy stage distribution of the study population

4.2. Maternal oral health status

4.2.1. Decayed Missing Filled Teeth (DMFT) scores

Figure 4.4 illustrates the DMFT scores of the participants. The mean (SD) DMFT was 7.187 (± 4.223).

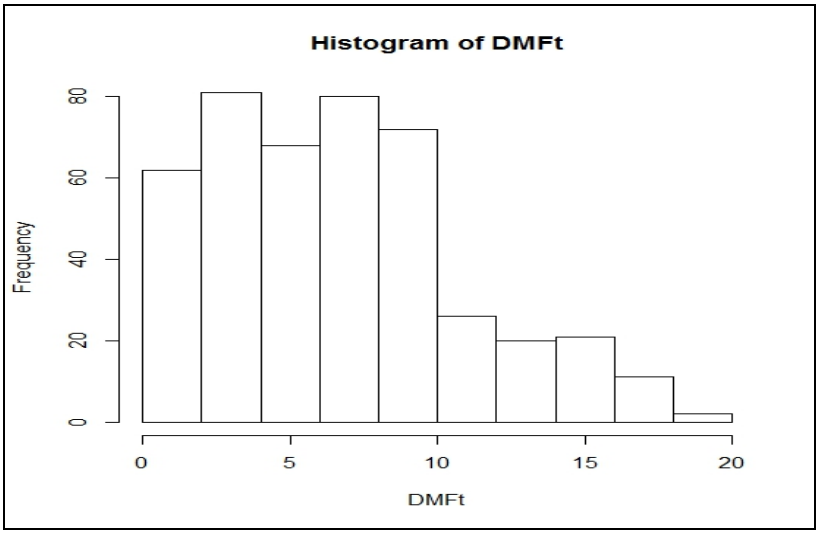

Figure 4.4 Distribution of DMFT scores

4.2.2. Plaque index (PI)

The mean of the plaque index was 2.172 (±0.647). Frequency distribution of the plaque index scores shows that most of the mothers had a plaque index of 2 or 3 (Figure 4.5).

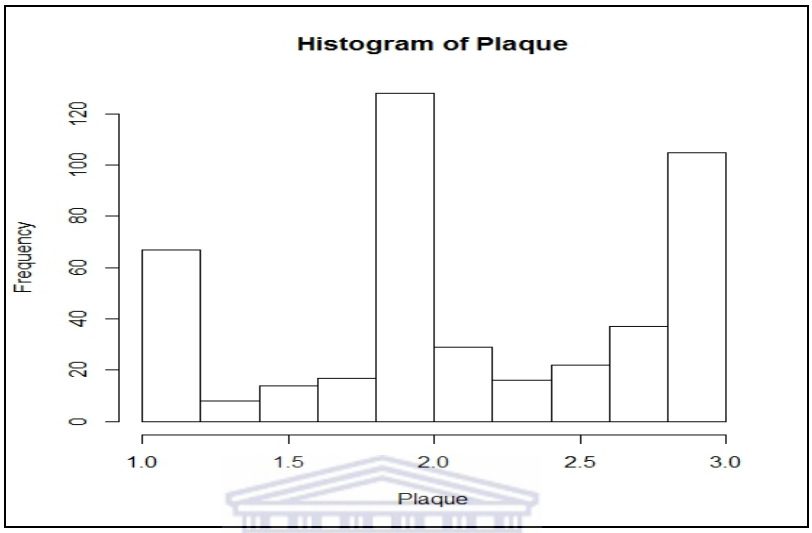

Figure 4.5 Distribution of plaque index scores

4.2.3. Gingival Index (GI)

The gingival index frequency is illustrated in Figure 4.10 and the mean (SD) of the gingival index was 2.439 (±0.581). The gingival index scores are indicative of severe gingivitis in more than half of the mothers examined (Figure 4.6).

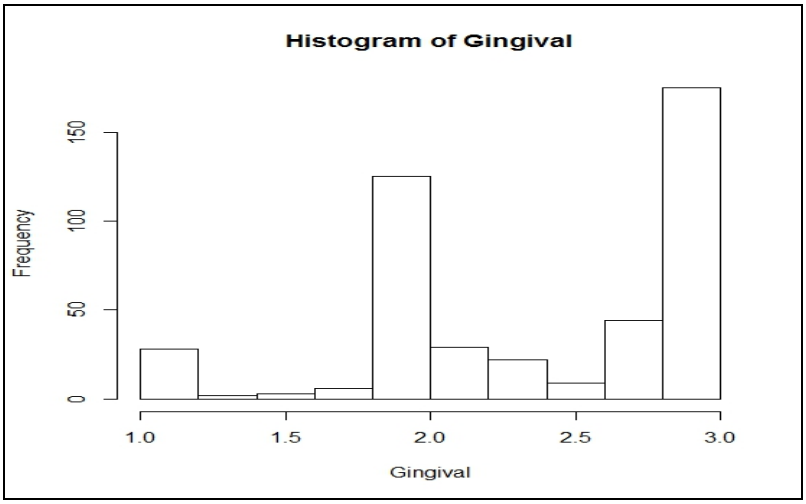

Figure 4.6 Distribution of Gingival Index scores

4.2.4. Maternal periodontal disease status (PD)

Maternal periodontal disease status was classified according to Offenbacher et al. (2001), using PPD and CAL measurements (Table 4.2). Of the 443 mothers who participated in the study, 72% had periodontal disease classified as either mild (44%), moderate (19%) or severe (10%). The remaining 27% showed no signs of periodontal disease (Table 4.2).

Table 4.2 Periodontal disease status in sampled population

	Frequency (%)
Periodontal disease classification	
None	123 (27)
Mild (PPD>3mm or CAL >2mm)	193 (44)
Moderate (≥ 2 sites with PPD ≥5mm and 2 sites with CAL ≥2mm)	82 (19)
Severe (≥ 4 sites with PPD ≥5mm and ≥4 sites with CAL ≥2mm)	45 (10)
BANA test	
Positive	282 (64)
Negative	161 (36)

4.2.5. BANA

BANA results were recorded as positive (indicating periodontal pathogens associated with periodontal disease) if a blue colour was produced or negative if there was no colour production. In line with the periodontal disease diagnosis above, 282 of the mothers tested produced a positive BANA test result while BANA-negative mothers constituted 161 of the 443 mothers recruited to this study, (Table 4.2).

4.3. Association of maternal demographics with clinical indices

An association was sought between maternal oral health status and the demographic variables of age, race, location, level of education and pregnancy stage at time of sampling.

4.3.1. Decayed Missing Filled Teeth (DMFT)

4.3.1.1. DMFT and age

Although no linear correlation was observed (Figure 4.7), the mean DMFT scores were found to differ significantly with age (Table 4.3).

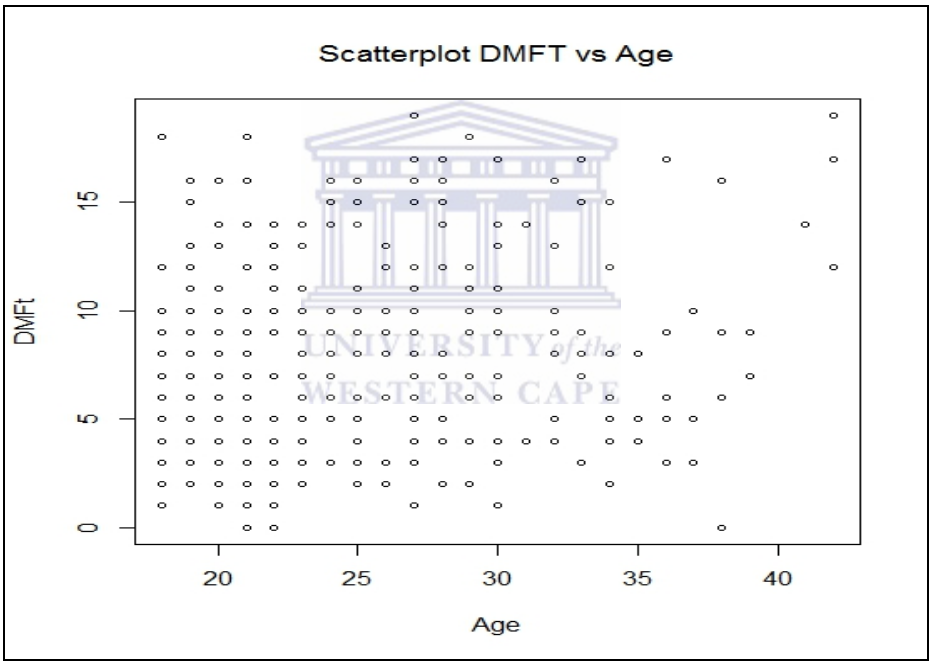

Figure 4.7 DMFT and age

The individual components of DMFT i.e. decayed, missing and filled teeth were also individually analysed and for ease of reading, will hereafter be referred to as D, M, or F, respectively. When looking at the DMFT components individually, significant correlations were observed between D and age and M and age, but no significant correlation was observed between F and age (Table 4.3).

Table 4.3 Correlation of DMFT scores with age

Score	*r*	*p*
DMFT	0.220	< **0.001**
D	0.107	**0.025**
M	0.200	<**0.001**
F	0.091	0.058

4.3.1.2. DMFT and race

The frequencies and means of DMFT for the four race groups are summarised in Table 4.4. Using one way ANOVA, significant differences were observed between races for mean DMFT scores (p= 0.012) as well as for F scores (p=0.0001).

Table 4.4 Comparison of mean DMFT, PI, GI scores with race

	African	Coloured	Indian	White	*F*	*df*	*p*
Frequency	360	56	23	4			
DMFT	6.889	8.661	7.783	10.000	3.681	3,399	**0.012**
D	2.938	3.125	2.956	2.000	0.263	3,438	0.852
M	2.825	3.482	2.695	3.000	1.186	3,439	0.314
F	1.155	2.160	2.043	5.000	11.59	3.439	**0.0001**
PI	2.167	2.100	2.404	2.225	1.235	3,439	0.297
GI	2.440	2.359	2.596	2.600	1.016	3,439	0.05

4.3.1.3. DMFT and education

The mean DMFT scores associated with different educational levels are listed in Table 4.5. Using one way ANOVA, a significant difference in DMFT was observed between those with secondary and tertiary education ($F_{2,440} = 5.591$, $p = 0.004$) but not between those with primary and tertiary education ($p = 0.088$). According to a one way ANOVA the D and M means did not differ significantly according to educational levels, while the F mean of the participants who had tertiary education was significantly greater ($p = 0.0002$) than the means of the participants who had primary and secondary education (Table 4.5).

Table 4.5 Education levels and mean DMFT, PI and GI scores

	Primary	**Secondary**	**Tertiary**	***F***	***df***	***p***
DMFT	6.933	6.822	8.424	5.591	2,440	**0.004**
D	3.200	2.907	3.030	0.226	2,439	0.797
M	2.733	2.780	3.343	2.012	2,440	0.135
F	1.033	1.178	2.050	8.901	2,440	**0.0002**
PI	2.350	2.146	2.198	1.460	2,440	0.233
GI	2.353	2.460	2.400	0.752	2,440	0.472

4.3.1.4. DMFT and urban/rural distribution

Figure 4.8 shows the frequency distribution of DMFT in urban/rural settings. Since this variable has exactly two levels, the ANOVA and Welch t-tests were used to compare the DMFT means of the urban and rural participants. No significant differences in DMFT, D and M scores were found between the two groups (Table 4.6). However, a significant difference was noted for F scores (Table 4.6).

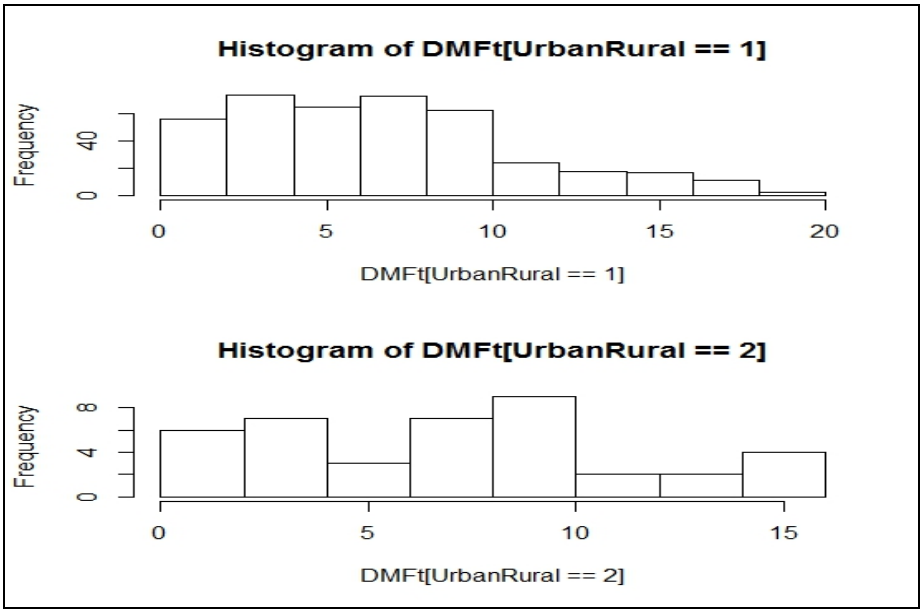

Figure 4.8 Frequency distribution of Urban (1) and Rural (2) DMFT scores

Table 4.6 Comparison Urban/Rural Distribution and DMFT

	Urban	**Rural**	***F***	***t***	***df***	***p***
Frequency	403	40				
DMFT	7.156	7.500	5.591		2,440	0.633
D	2.920	3.300		0.898	47.52	0.373
M	2.851	3.425	1.94		1,441	0.164
F	1.419	0.800	3.975		1.441	**0.046**

4.3.1.5. DMFT and pregnancy stage

No significant correlation was observed between DMFT and pregnancy stage (Table 4.7). The same applies to correlations between pregnancy stage and D or M. Significant differences were observed for F in the different stages of pregnancy at the time of sampling (Table 4.7).

Table 4.7 Correlation of pregnancy stage with DMFT scores

Score	**Pregnancy stage**	
	r	***p***
DMFT	0.057	0.232
D	-0.039	0.410
M	0.012	0.800
F	0	**0.0005**

4.3.2. Plaque index (PI)

4.3.2.1. PI and age

Age and PI were not significantly associated (r = 0.079, p = 0.05). The distribution is demonstrated in Figure 4.9.

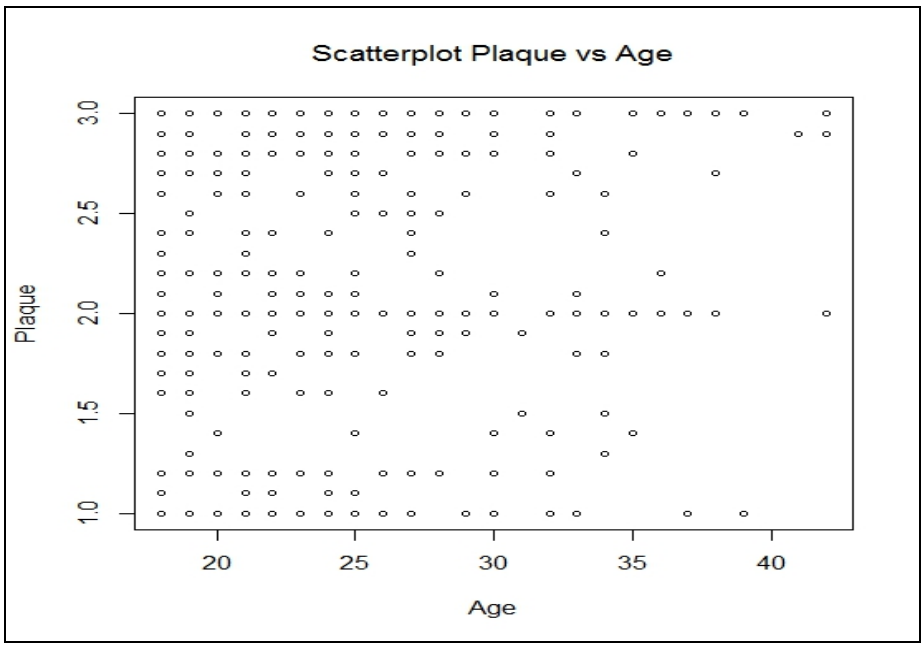

Figure 4.9 PI and age

4.3.2.2. PI and race

One way ANOVA confirms that plaque index scores did not differ significantly between the different race groups ($F_{3,439}$ =1.235, p = 0.297) as illustrated in Table 4.4.

4.3.2.3. PI and education

According to a one way ANOVA ($F_{2, 440}$ = 1.460, p = 0.233) the plaque index means of the participants who received primary, secondary and tertiary education did not differ significantly (Table 4.5).

4.3.2.4. PI and urban/rural distribution

The Welch Two Sample t-test showed that the difference between the plaque index means of urban and rural participants (data not shown) was not significant (p = 0.082).

4.3.2.5. PI and pregnancy stage at time of sampling

Table 4.8 lists the mean PI for each pregnancy stage. The correlation between plaque index and pregnancy stage at the time of sampling is illustrated in Figure 4.10. The red dots are the means and the line is a least squares regression line whose slope is -0.07155, demonstrating a significant correlation (p = 0.001).

Table 4.8 Mean PI and GI at pregnancy stage

Pregnancy stage (months)	2	3	4	5	6	7	8	9	***p*-value**
N	1	18	71	108	97	86	54	8	
Mean PI	2.100	2.528	2.225	2.291	2.125	2.051	2.030	2.125	**0.001**
Mean GI	2.000	2.478	2.530	2.528	2.436	2.331	2.341	2.275	**0.008**

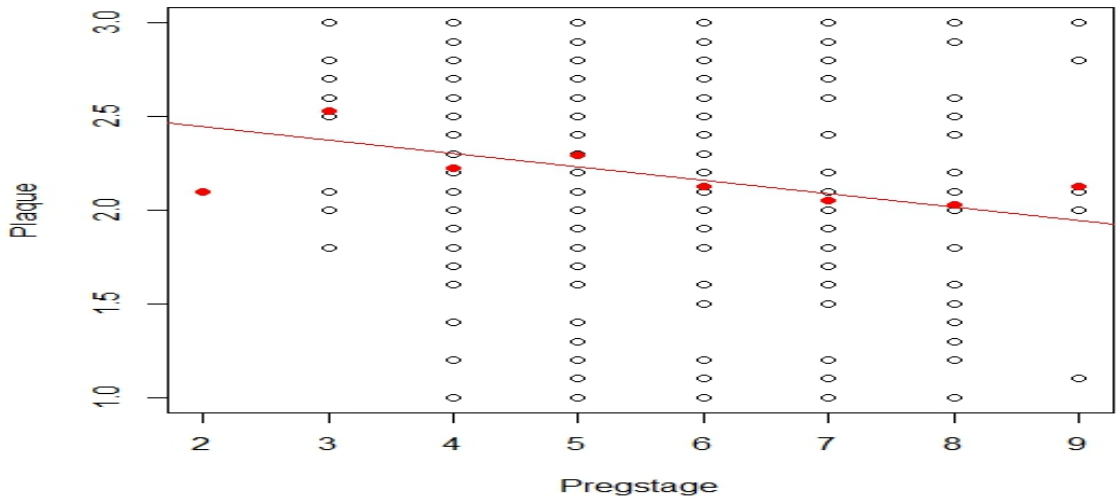

Figure 4.10 Correlation between PI and pregnancy stage at the time of sampling

4.3.3. Gingival Index (GI)

4.3.3.1. GI and age

No significant correlation was found between age and gingival index (*r*-0.015, $p = 0.05$). The distribution of gingival index scores according to age is demonstrated in Figure 4.11.

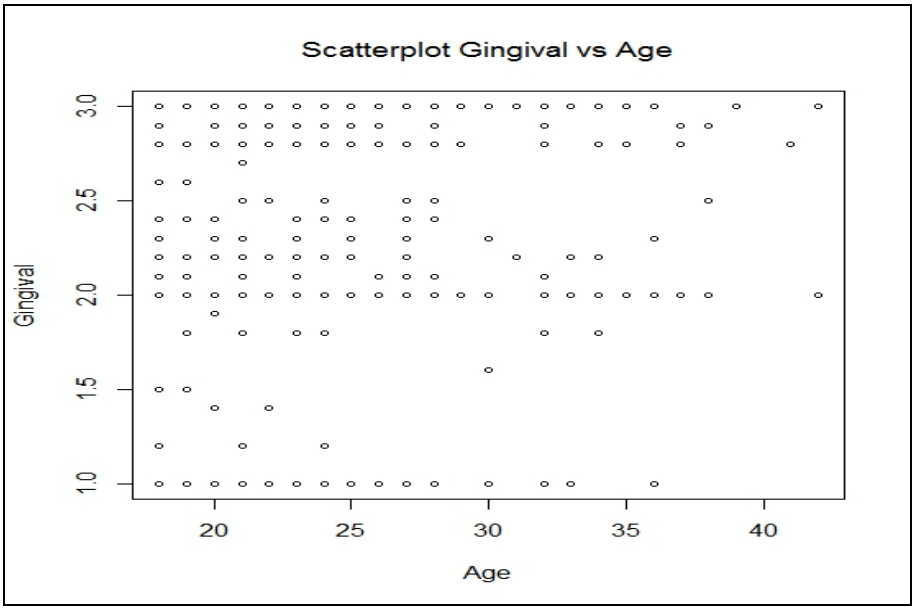

Figure 4.11 GI scores according to age.

4.3.3.2. GI and race

Although the mean gingival index scores differed between the race groups (Table 4.4), these differences were not found to be significant ($F_{3,439}$ =1.016, p = 0.05).

4.3.3.3. GI and education

One way ANOVA (Table 4.5) also revealed no significant difference between educational levels and mean gingival index scores ($F_{2, 440}$ = 0.752, p = 0.472).

4.3.3.4. GI and Urban/Rural distribution

The distribution of GI scores across the urban and rural populations is shown in Figure 4.12. According to the Welch Two Sample t-test the difference between the gingival index means of the urban and rural participants was not significant (p=0.729).

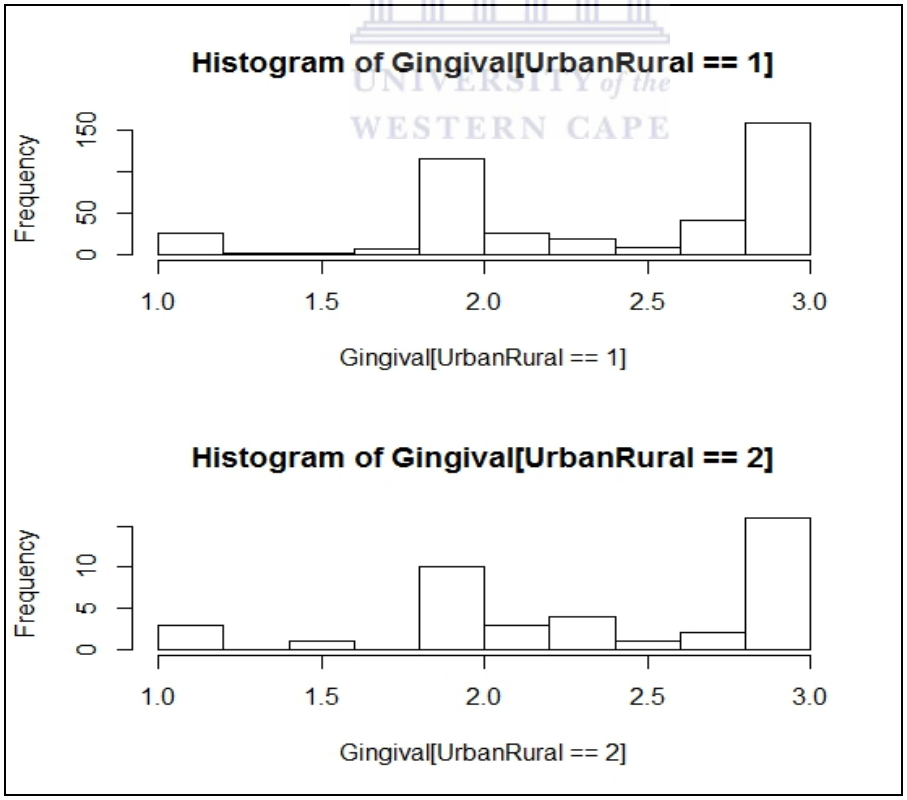

Figure 4.12 Urban/rural distribution of GI scores

4.3.3.5. GI and pregnancy stage at time of sampling

The frequency and means of the gingival index and pregnancy stage are illustrated in Table 4.9, while Figure 4.13 is a plot of gingival index and pregnancy stage. The red dots are the means; the line is a least squares regression line whose slope is -0.05030. It is significantly different from zero (p = 0.008).

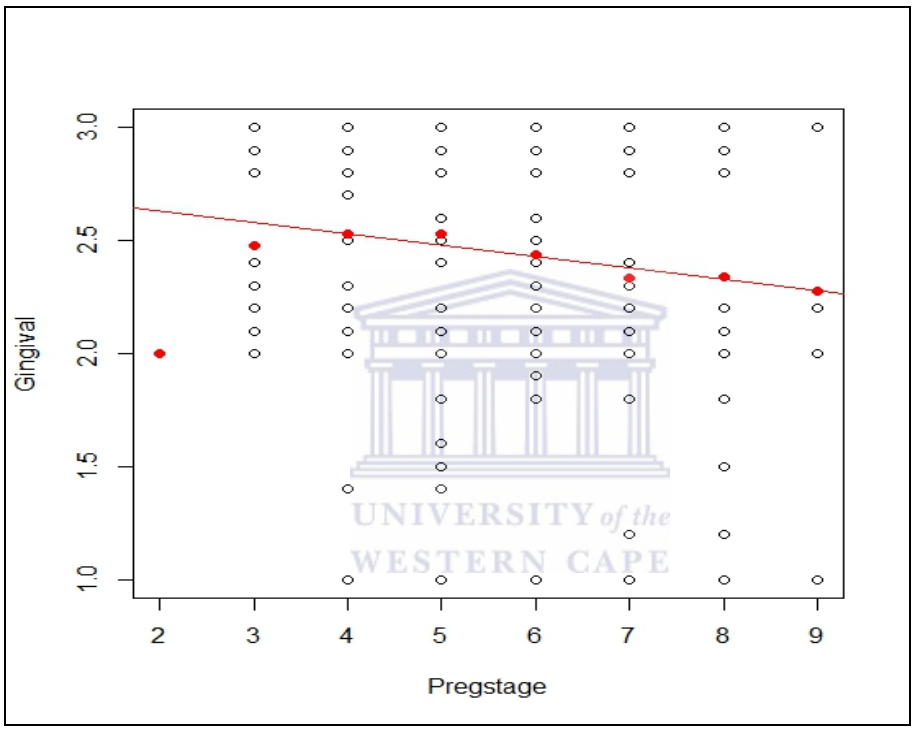

Figure 4.13 Correlation between Gingival index and pregnancy stage at the time of sampling

4.3.4. Probing Pocket Depth (PPD) and Clinical Attachment Loss (CAL)

The probing pocket depth and clinical attachment loss distribution is illustrated in Table 4.9.

Table 4.9 Frequency distribution of PPD and CAL

Variable	Frequency (%)
PPD	
1(< 3mm)	106(24)
2(≥ 3-5 mm)	285(64)
3 (>5 -7 mm)	51(12)
4 (>7 mm)	0(0)
CAL	
None	175 (39.5)
Mild (1-3 mm)	232 (52)
Moderate (3-6 mm)	34 (8)
Severe (≥ 6 mm)	2 (0.5)

4.3.5. Periodontal Pocket Depth (PPD)

4.3.5.1. Age and PPD

Table 4.10 illustrates that the one way ANOVA showed no significant association between age and probing pocket depths in this study ($F_{3,439} = 0.835$, p = 0.570).

Table 4.10 Probing Pocket depth and mean age distribution

	Pocket Probing Depth			
	1(< 3mm)	**2**(≥ 3-5 mm)	**3**(>5 -7 mm)	**4**(>7 mm)
Mean Age (yrs)	24.09	23.96	25.15	24.41
$F_{3,438}$	0.671			
***p*-value**	**0.570**			

4.3.5.2. PPD and race

Chi-squared analysis demonstrated no significant association between race and probing pocket depth measurements (Table 4.11).

Table 4.11 Pocket Probing Depth and race

Race	**Pocket Probing Depth**			
	1(< 3mm)	**2** (≥ 3-5 mm)	**3**(>5 -7 mm)	**4**(>7 mm)
African	84	221	42	13
Coloured	16	36	0	3
Indian	3	16	3	1
White	0	4	0	0
X^2	11.551			
Df	9			
***p*-value**	**0.2398**			

4.3.5.3. PPD and education

Table 4.12 shows the distribution of probing pocket depths according to education levels of the participants. The observed chi-squared shows no significant difference (X^2 = 5.6556, df = 6, p = 0.4629).

Table 4.12 Frequency distribution of PPD, CAL and education

	Primary	Secondary	Tertiary	X^2	df	*p*
PPD (mm)				5.6556	6	0.4629
1	23	73	7			
2	58	202	17			
3	11	29	5			
4	7	9	1			
CAL				5.218	6	0.516
None	11	119	45			
Mild	15	167	50			
Moderate	4	26	4			
Severe	0	2	0			

4.3.5.4. PPD and Urban/Rural distribution

This Chi-squared test showed a significant difference in the distribution of probing pocket depth between urban and rural participants (Table 4.13)

Table 4.13 PPD and Urban/Rural distribution

	Probing Pocket Depth			
	1	**2**	**3**	**4**
Urban	99 (24.6)	255 (63.4)	33 (8.2)	15 (3.7)
Rural	4 (10)	22 (55)	12 (30)	2 (5)
X^2	20.873			
Df	3			
***p*-value**	**<0.0001**			

4.3.5.5. PPD and pregnancy stage

All the PPD and pregnancy stage data are summarised in Table 4.14. A chi-squared test applied to the reduced table shows no significant association (X^2 = 25.892, *df* = 21, *p* = 0.2106).

Table 4.14 Probing Pocket Depth and Pregnancy Stage

	Probing Pocket Depth			
Pregnancy Stage	**1**	**2**	**3**	**4**
2	1	0	0	0
3	5	8	2	2
4	16	41	9	5
5	27	65	10	6
6	28	60	9	0
7	17	61	5	3
8	9	36	8	1
9	0	6	2	0
X^2	25.892			
Df	21			
***p*-value**	**0.2106**			

4.3.5.6. PPD and PD status

According to a chi-squared test (Chi-squared = 378.6152, df =9, p-value < 2.2e-16) there is a highly significant association between periodontal disease status and probing pocket depth (Table 4.15).Inspection of the table indicates that at Periodontal disease =1 the Probing depth is predominantly 1 or 2. At Periodontal disease =4 the Probing depth is predominantly 3 or 4.

Table 4.15 Probing Pocket Depth and Periodontal Disease Status

	PPD			
PD	**1**	**2**	**3**	**4**
1	76	46	0	0
2	20	163	10	0
3	7	60	15	0
4	0	8	20	17
Chi-squared	378.6152			
Df	9			
***p*-value**	**< 0.0001**			

4.3.6. Clinical Attachment Loss (CAL)

4.3.6.1. CAL and age

One way ANOVA revealed no significant association between age and clinical attachment loss ($F_{3,439} = 0.835$, p=0.475). The distribution of clinical attachment loss according to age is captured in Figure 4.14.

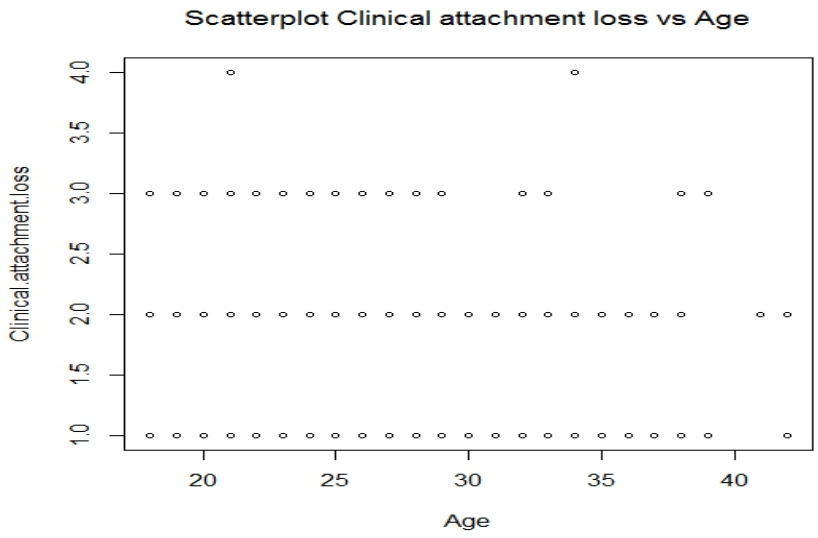

Figure 4.14 CAL and age

4.3.6.2. CAL and race

The following is a frequency table of clinical attachment loss and race. Chi-squared observation revealed a significant difference in clinical attachment loss according to race (Table 4.16)

Table 4.16 Frequency Table of CAL and race

Race	**CAL mm**			
	1	**2**	**3**	**4**
African	119	206	33	2
Coloured	36	19	1	0
Indian	16	7	0	0
White	4	0	0	0
X^2	37.03			
Df	9			
***p*-value**	**<0.0001**			

4.3.6.3. CAL and education

The chi-squared observation of clinical attachment loss distribution and the education level of the participants (Table 4.12) did not reveal a significant difference either (X^2 = 5.218, *df* = 6, *p* =0.516).

4.3.6.4. CAL and Urban/rural distribution

The clinical attachment loss distribution and the location of the participants are summarised in Table 4.17. According to the Fisher exact test there is no significant association (*p* =0.111).

Table 4.17 Urban/rural distribution of clinical attachment loss

CAL	Urban	Rural
None	162	13
Mild	212	20
Moderate	27	7
Severe	2	0
Frequency	403	40
Mean	2.442	2.408
***p*- value**	**0.111**	

4.3.6.5. CAL and pregnancy stage

All the clinical attachment loss and pregnancy stage data are summarised in Table 4.18. A chi-squared test applied to the table shows no significant association between pregnancy stage and clinical loss of attachment (X^2=11.015, df=12, p = 0.528).

Table 4.18 Clinical attachment loss and pregnancy stage

Pregnancy stage	**Clinical attachment loss**			
Months	**1**	**2**	**3**	**4**
2	1	0	0	0
3	5	10	3	0
4	25	40	6	0
5	43	56	9	0
6	45	46	6	0
7	34	46	6	0
8	21	29	2	2
9	1	5	2	0
X^2	11.015			
Df	12			
***p*-value**	**0.528**			

4.3.6.6. CAL and PPD

According to a chi-squared test (Chi-squared =209.1284, df =9, p-value < 2.2e-16) there is a highly significant association between clinical attachment level and probing pocket depth (Table 4.19). Inspection of the Table indicates that as clinical attachment loss increases, so does probing pocket depth e.g. at CAL=1 the PPD is predominantly 1 or 2, while at CAL=3 the PPD is predominantly 3.

Table 4.19 Clinical attachment loss and Probing Pocket Depth

	Probing Pocket depth			
Clinical attachment loss	**1**	**2**	**3**	**4**
1	72	90	6	6
2	29	182	15	6
3	2	5	23	4
4	0	0	1	1
X^2	209.1284			
Df	9			
***p*-value**	**< 0.0001**			

4.3.6.7. Clinical attachment loss and periodontal disease status

Comparison of clinical attachment loss with periodontal disease status (Chi-squared =156.7557, df = 9, p-value < 2.2e-16) revealed that there is a highly significant association between the two (Table 4.20). Increased severity of periodontal disease occurs at increased values of clinical attachment loss (Table 4.20).

Table 4.20 Clinical attachment loss and Periodontal Disease Status

	Periodontal disease			
Clinical attachment loss	**1**	**2**	**3**	**4**
1	89	58	19	9
2	33	130	51	18
3	1	5	12	16
4	0	0	0	2
X^2	156.7557			
Df	9			
***p*-value**	**<0.0001**			

4.3.7. BANA

4.3.7.1. BANA and DMFT

Table 4.20 shows that the DMFT means of the BANA- negative and BANA-positive groups were not significantly different.

4.3.7.2. BANA and PI

Table 4.20 also shows that the PI means of the BANA-negative and BANA-positive groups were significantly different (F-statistic: 66.17 on 1 and 439 DF, p-value: 4.279e-15).

4.3.7.3. BANA and GI

A significant difference was also observed (Table 4.21) between BANA- negative and BANA-positive groups for GI (F-statistic: 57.21 on 1 and 439 DF, p-value: 2.312e-13).

Table 4.21 Association of BANA with DMFT, PI and GI

	BANA		**F**	**df**	**p**
	Negative	**Positive**			
DMFT	7.503106	7.021429	1.327	1,439	0.25
PI	1.860248	2.349286	66.17	1, 439	**<0.0001**
GI	2.177019	2.586071	57.21	1,439	**<0.0001**

4.3.7.4. BANA and PPD

Table 4.22 shows that the distributions of probing pocket depths at BANA-negative and BANA-positive are significantly different (Chi-squared = 102.3855, df = 3, p-value < 2.2e-16).

Table 4.22 Association of BANA with PPD

	Probing Pocket Depth			
	1	**2**	**3**	**4**
BANA negative	78	78	3	1
BANA positive	24	199	41	16
X^2	**102.3855**			
Df	3			
***p*-value**	**<0.0001**			

4.3.7.5. BANA and CAL

The distributions of clinical attachment loss at BANA negative and BANA positive are considerably different. According to a chi-squared test (Chi-squared = 73.5438, df = 2, p-value < 2.2e-16) there is a highly significant association between BANA and clinical attachment loss (Table 4.23). The frequency of BANA-negative samples decreased with increased clinical attachment loss.

Table 4.23 Association of BANA with CAL

	Clinical Attachment Loss			
BANA	**1**	**2**	**3**	**4**
Negative	105	55	3	0
Positive	70	176	32	2
Chi-squared	73.5438			
Df	2			
***p*-value**	**< 0.0001**			

4.3.7.6. BANA and PD

The distributions of BANA-negative and BANA-positive samples are considerably different according to periodontal disease status. Using chi-squared analysis, the difference was found to be highly significant (Table 4.24).

Table 4.24 Association of BANA with Periodontal Disease Status

	Periodontal Disease			
BANA.	**Absent**	**Mild**	**Moderate**	**Severe**
Negative	117	38	5	1
Positive	6	155	76	45
Chi-squared	260.3			
Df	3			
***p*-value**	**< 0.0001**			

Figure 4.15 illustrates this association of periodontal disease with BANA. As the severity of periodontal disease increased, the number of BANA-negative plaques decreased. On the other hand, the number of BANA-positive plaques was very low for subjects with no periodontal disease, reaching a high in subjects with mild periodontal disease, then decreasing for moderate and severe periodontal disease respectively.

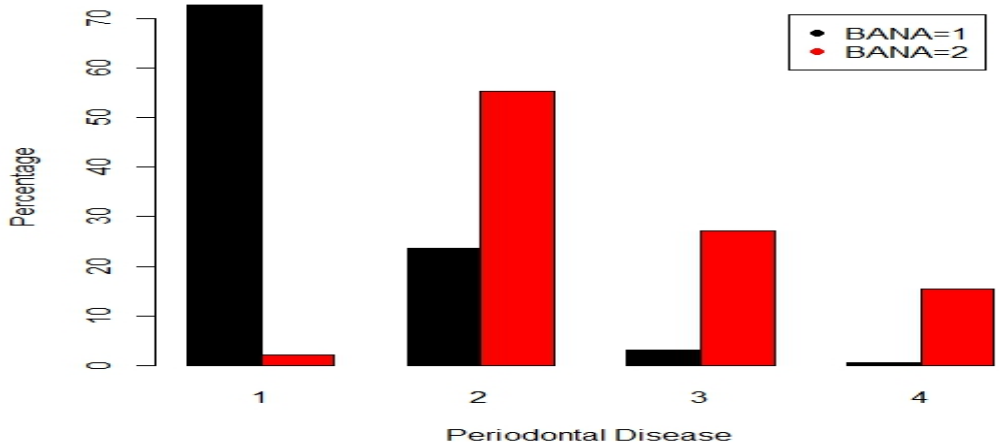

Figure 4.15 Decrease of BANA-negative samples with increased severity of Periodontal Disease

1= No PD, 2= Mild PD, 3=Moderate PD, 4=Severe PD

BANA=1 (black): BANA-negative; BANA=2 (red): BANA-positive

CHAPTER 5: PREGNANCY OUTCOMES

The preceding chapter focused on the assessment of the mothers when visiting the antenatal clinic in order to establish risk factors which may threaten their pregnancies. This chapter examines the outcomes of those pregnancies and through association, identifies risk factors for adverse pregnancy outcomes. The pregnancy outcomes of 442/443 mothers follow.

5.1. Gestational age at delivery (GA)

The mean (SD) for gestational age at delivery are indicated in Table 5.1.One hundred and forty-two infants were born preterm (PTB, <37 weeks gestation) and 300 were born full term (FTB, ≥ 37 weeks gestation).

Table 5.1 Gestational age at delivery

Gestational age		**Total**	**Mean**	**SD**	**Min**	**Max**
PTB (< 37 weeks)	FTB (≥ 37 weeks)					
n=142	n=300	442	37.317	2.502	22	40

5.1.1. GA and maternal demography

ANOVA showed that there was no correlation between gestational age and race, location or level of education (Table 5.2).

Table 5.2 Association between GA and race, location and education

Variable	N	Mean GA (weeks)	*F*	df	*p*
Race			1.332	3.438	**0.263**
African	360	37.21			
Coloured	56	37.83			
Indian	23	37.43			
White	4	38.50			
Location			0.820	1,440	**0.366**
Urban	403	37.35			
Rural	40	36.97			
Education			1.226	2,439	**0.294**
Primary	30	37.43			
Secondary	314	37.20			
Tertiary	99	37.64			

5.1.2. GA and maternal oral health

Likewise, correlation coefficients showed no significant correlation between GA and maternal age, DMFT, PI or GI when statistically evaluating the entire study group (Table 5.3). These results did not change significantly when the group was divided into preterm (PTB) and full term (FTB) as shown in Table 5.4.

Table 5.3 Correlation coefficients for maternal age, DMFT, PI and GI

Variable	***R***	***p***
Maternal age	0.0016	0.739
DMFT	0.037	0.434
PI	-0.003	0.958
GI	-0.079	0.097

Table 5.4 Comparison of PTB and FTB for DMFT, PI and GI

Variable	**Gestational term**		***t***	***df***	***p***
	PT	**FT**			
DMFT Mean (SE)	6.782 (0.310)	7.397 (0.256)	-1529	328	**0.127**
PI Mean (SE)	2.207 (0.053)	2.159 (0.038)	0.736	291	**0.462**
GI Mean (SE)	2.496 (0.044)	2.411 (0.035)	1.509	312	**0.132**

5.1.3. Gestational Age (GA)

5.1.3.1. GA and PPD

The mean gestational age at delivery showed a significant trend of decreasing with an increase in probing pocket depth severity (Table 5.5). By ANOVA, the differences between the means are highly significant (p =2.547e-05).

Table 5.5 Gestational age at delivery and Probing Pocket Depth

	Probing Pocket Depth				*F*	df	*p*
	1	**2**	**3**	**4**			
N	103	276	45	17			
Mean GA	37.534	37.493	36.689	34.706	8.2	3,437	**<0.0001**

This significant difference remained with Chi-squared comparison of preterm birth and full term birth (Table 5.6). FTB was more likely to occur when PPD was ≤ 2 and decreased with increased PPD (Table 5.6).

Table 5.6 Comparison of PTD and FTD with Probing Pocket Depth

	Gestational term		X^2	df	*p*
Probing Pocket Depth	**PTB**	**FTB**	16.5362	3	**0.0008**
1	28	75			
2	82	194			
3	20	25			
4	12	5			

5.1.3.2 GA and CAL

As observed with PPD, the mean gestational age at delivery showed a trend of decreasing with an increase in CAL (Table 5.7). Using ANOVA, this difference between means was found to be significant (p = 0.0139).

Table 5.7 Gestational age at delivery and Clinical attachment loss

	CAL				*F*	df	*p*
	1	**2**	**3**	**4**			
n	175	231	34	2			
Mean GA	37.611	37.272	36.235	35.000	3.583	3,438	**0.0139**

However, when gestational age was classified as PTB and FTB, Chi-squared analysis revealed no significant differences between the two for CAL (Table 5.8).

Table 5.8 Comparison of PTB and FTB for Clinical Attachment Loss

	GA	
CAL	**< 37weeks (PTB)**	**≥ 37weeks (FTB)**
1	51	124
2	74	157
3	15	19
X^2	2.9532	
Df	2	
***p*-value**	**0.2284**	

5.1.2.3. GA and PD severity

Most of the mothers were found to have mild or no periodontal disease (Table 5.9). Further analysis of data compared the periodontal disease classification (none, mild, moderate and severe) with mean gestational age. One way ANOVA ($F_{3, 438}$ =11.54, p<0.0001) showed that mean gestational age decreased significantly with an increase in periodontal disease severity (Table 5.9). This trend continued when divided into PTB and FTB (Table 5.10).

Table 5.9 Correlation with gestational age and periodontal disease severity

Variable	**n**	**Mean gestational age (weeks)**	***p***
Periodontal disease (PD)			**<0.0001**
None	123	38.09	
Mild	193	37.39	
Moderate	82	36.79	
Severe	45	35.80	
BANA			**<0.0001**
Positive	282	37.97	
Negative	161	36.92	

Of the 45 mothers with severe periodontal disease, 26 (58%) delivered preterm, while of the 123 with no periodontal disease, 99 (80.5%) delivered full term (Table 5.10).

Table 5.10 Comparison of PTB and FTB for periodontal disease status

GA	Periodontal Disease Status				Total
	Absent	**Mild**	**Moderate**	**Severe**	
PTB	24	56	36	26	142 (32.1)
%	5.4	12.7	8.1	5.9	
Row %	16.9	39.4	25.4	18.3	
Column %	19.5	29.2	43.9	57.8	
FTB	99	136	46	19	300 (67.9)
%	22.4	30.8	10.4	4.3	
Row %	33	45.3	15.3	6.3	
Column %	80.5	70.8	56.1	42.2	
Total	123 (27.8)	192 (43.4)	82 (18.6)	45 (10.2)	442 (100)
Missing					1
X^2	28.540				
Df	3				
***p*-value**	**< 0.0001**				

5.1.3.4. GA and BANA

The mean gestational age of the BANA-negative and BANA-positive deliveries differed significantly ($p< 0.0001$) with a lower gestational age associated with a BANA-positive test. When categorised into preterm and full-term, BANA was significantly associated with gestational term as evidenced by 107 of 142 (75%) PTD having a BANA-positive test (Table 5.11). In the light of the very large observed X^2 one can take every difference in percentage between PTB and FTB as statistically significant.

Table 5.11 Contingency Table for PTB/ FTB and BANA

Gestational term	**BANA**		**Total**
	Negative	**Positive**	
< 37weeks (PTB)			
Frequency	35	107	142
%	7.9	24.3	
Row %	24.6	75.4	
Column %	21.7	38.4	
≥ 37weeks (FTB)			
Frequency	126	172	298
%	28.8	39.0	
Row %	42.3	57.7	
Column %	78.3	61.6	
Total (effective sample size)	161(36.6)	279 (63.4)	440 (100)
Frequency missing			3
X^2	12.1405		
Df	1		
***p*-value**	**0.0005**		

5.2. Infant Birth Weight (BW)

The mean infant birth weight of the 442 mothers was 2.986 kg (Table 5.12).

Table 5.12 Descriptive statistics of infant birth weight

N	Mean	SD	Min	Max
442	2.986	0.531	1.5	4.2

5.2.1. BW and maternal demography

As with GA, no significant correlation was observed between infant birth weight and race, location, and education levels (Table 5.13), nor was a correlation found with age (Table 5.13).

Table 5.13 Association of infant birth weight with race, location and education

Variable	N	mean	*F*	df	*p*
Race			0.146	3,439	0.932
African	360	2.98			
Coloured	56	3.02			
Indian	23	2.99			
White	4	2.87			
Location			0.122	1,441	0.727
Urban	403	2.98			
Rural	40	2.95			
Education			0.697	2,440	0.499
Primary	30	2.93			
Secondary	314	2.98			
Tertiary	99	3.04			

5.2.2. BW association with oral health

5.2.2.1. BW association with PI and GI

A significant correlation was observed between infant birth weight and maternal PI and GI (Table 5.14). As the PI and GI increased, the birth weight of the infant dropped (Figures 5.1 and 5.2).

Table 5.14 Correlation of infant birth weight with maternal PI and GI

Variable	**R**	**p**
Age	0. 008	0.872
Plaque index	0.217	**<0.001**
Gingival index	0.257	**<0.001**

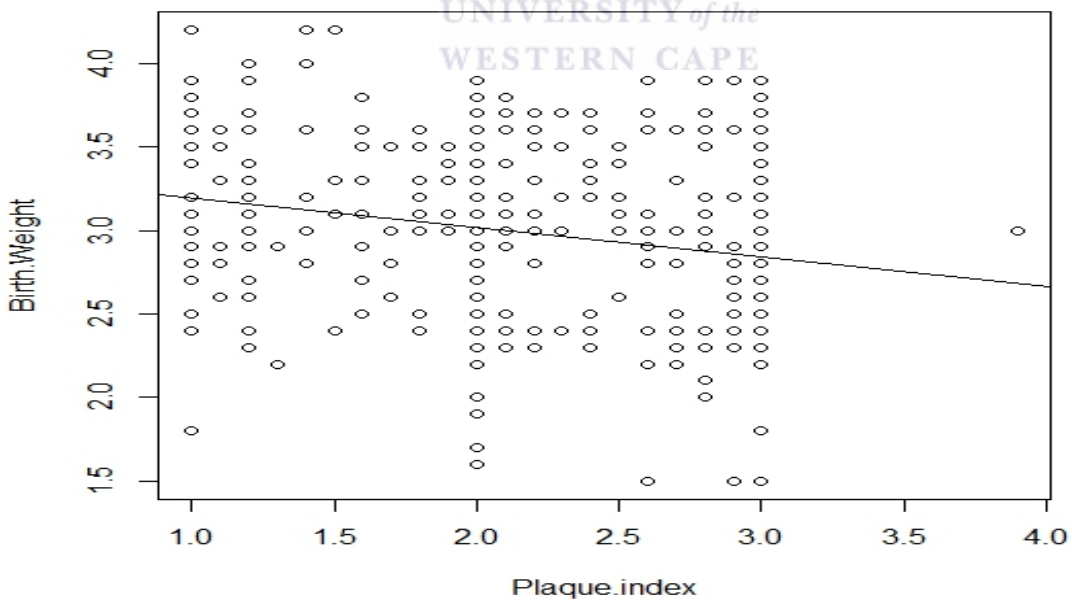

Figure 5.1 Infant Birth Weight and Maternal PI

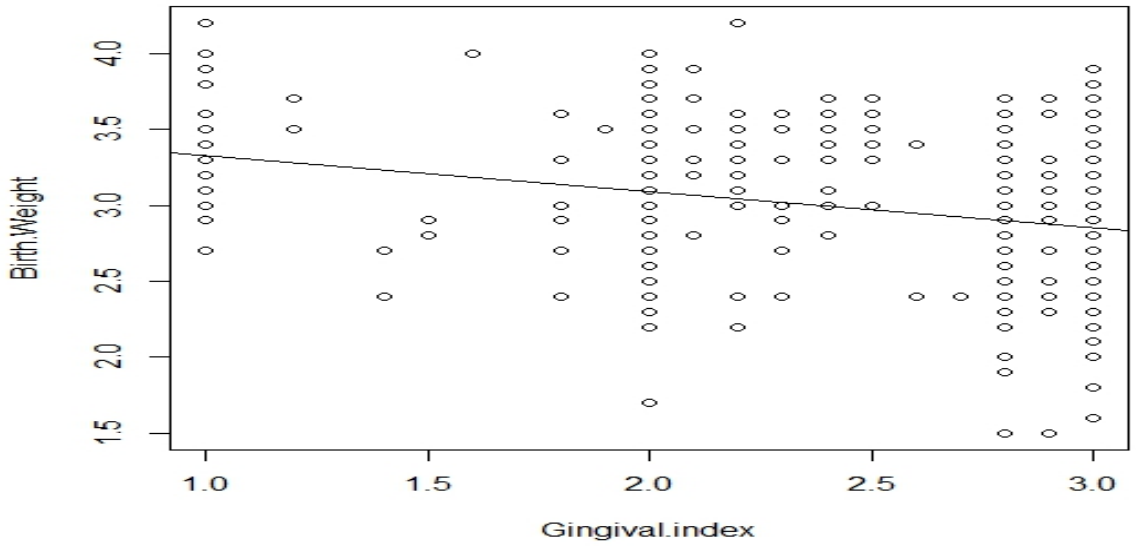

Figure 5.2 Infant Birth Weight and Maternal GI

Low birth weight (LBW, < 2500 grams) was compared with normal birth weight (NBW, ≥ 2500 grams) for DMFT, PI and GI. No significant difference was observed between DMFT scores in mothers who delivered LBW infants compared to mothers who delivered NBW infants (Table 5.15). However, when comparing birth weight with PI and GI, highly significant differences were observed (Table 5.15).

Table 5.15 LBW compared to NBW for Maternal DMFT, PI and GI

Variable	**LBW Mean (SE)**	**NBW Mean (SE)**	**Welch *t***	***df***	***p value***
Maternal DMFT	6.818 (0.406)	7.309(0.231)	-1.052	185	0.259
Plaque Index	2.422 (0.055)	2.092(0.036)	4.997	207	< **0.0001**
Gingival Index	2.647 (0.043)	2.371(0.033)	5.100	247	< **0.0001**

5.2.2.2. Birth weight and PPD

Even though BW was found to decrease with increasing PPD, the difference between the means was not significant as evident with ANOVA (Table 5.16).

Table 5.16 Mean birth weight and PPD

	Pocket Probing Depth			
	1	**2**	**3**	**4**
Mean BW	3.413592	3.228881	2.622222	2.376471
$F_{3,438}$	2.534			
***p*-value**	**0.05637**			

However, when birth weight was divided into low birth weight (LBW, <2500g) and normal birth weight (NBW, ≥ 2500g), Chi-squared showed the distributions of PPD at LBW and NBW (Table 5.17) to differ significantly (X^2= 70.8876, df = 3, p = 2.755e-15).

Table 5.17 Birth Weight and Probing Pocket Depth

	Birth Weight	
Probing Pocket Depth	**LBW<2500gm**	**NBW>=2500gm**
1	9	94
2	59	218
3	23	22
4	15	2
X^2	70.8876	
df	3	
***p*-value**	**<0.0001**	

5.2.2.3. Birth weight and CAL

One way ANOVA (Table 5.18) shows a significant trend in decreasing mean BW with increasing CAL. This is clearly demonstrated in Figure 5.3 where the red circles indicate the mean clinical attachment loss for birth weight.

Table 5.18 Birth Weight and Clinical Attachment Loss

Clinical Attachment Loss	**1 (None)**	**2 (Mild)**	**3 (Moderate)**	**4 (Severe)**
N	175	232	34	2
Mean BW	3.091	2.964	2.638	2.200
$F_{3,439}$	9.216			
P	**0.001**			

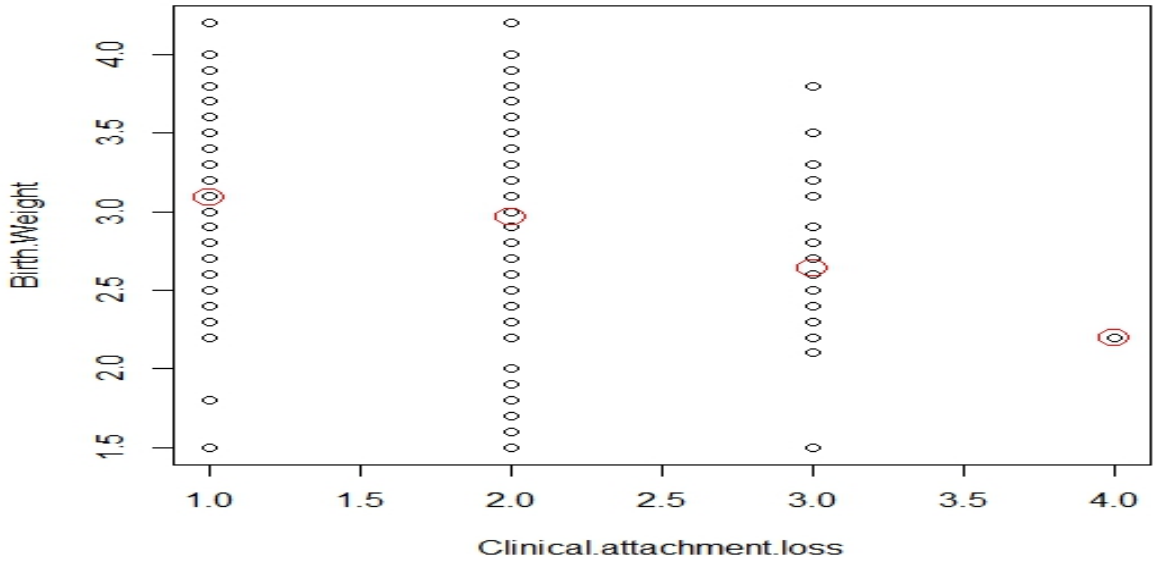

Figure 5.3 Birth Weight and Clinical Attachment Loss (1, 2, 3 and 4)

When divided into categories of LWB and NBW, the distributions of clinical attachment loss at LBW and NBW are notably different. According to a chi-squared test (X^2= 23.7121, df = 2, p = 7.096e-06), this difference is highly significant (Table 5.19).

Table 5.19 Birth Weight and CAL

	Birth Weight	
Clinical attachment loss	**LBW <2500gm**	**NBW ≥2500gm**
1	30	145
2	55	177
3	19	15
4	2	0
X ^{2}	23.7121	
Df	2	
***p*-value**	**<0.0001**	

5.2.3. Infant birth weight and maternal periodontal disease status

The mean BW of infants born of mothers with no, mild, moderate or severe periodontal disease is shown in Table 5.20. The association between BW and PD is reflected in Figure 5.4. A significant decrease in infant birth weight was observed with increased severity of periodontal disease (Table 5.20).

Table 5.20 Frequency distribution of Infant birth weight, maternal periodontal disease and BANA

Variable	n	Mean BW/g	*F*	Df	*p*
Periodontal disease			2.940	3,439	**<0.0001**
None	123	3.28			
Mild	193	3.04			
Moderate	82	2.70			
Severe	45	2.41			
BANA			36.43	1.438	**<0.0001**
Positive	282	2.849			
Negative	161	3.224			

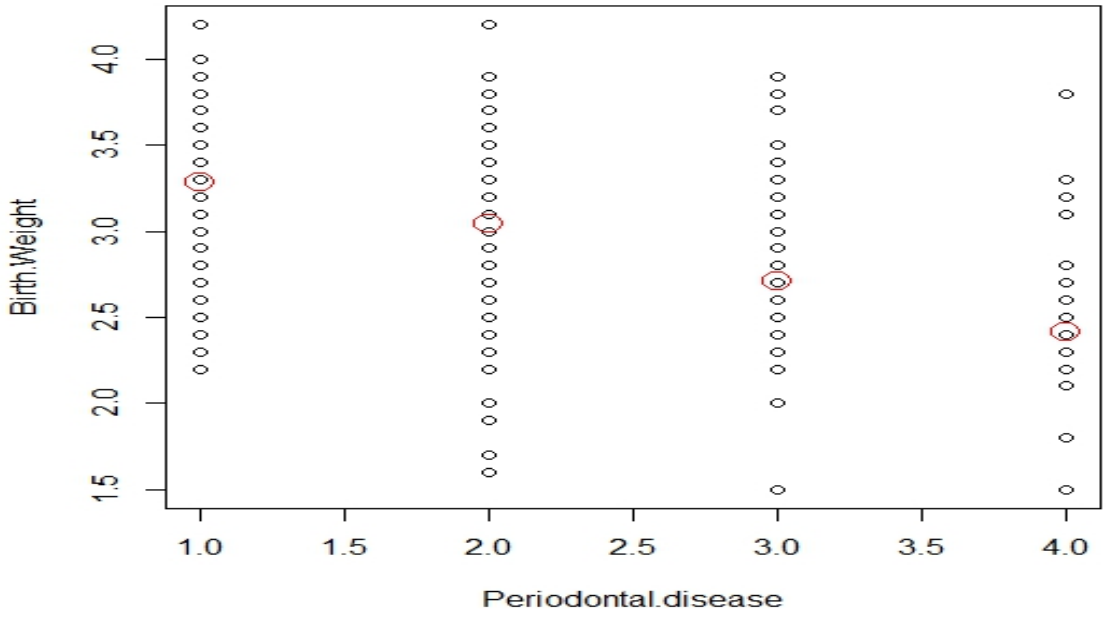

Figure 5.4 Infant birth weight and maternal periodontal disease

Categorising the above into LBW and NBW revealed a similar significant association (p<0.000001). The majority of mothers who delivered NBW infants had some form of periodontal disease. Although not all LBW may be attributed to periodontal disease severity, maternal periodontal disease severity showed a definite decrease in the delivery of infants with NBW (Table 5.21).

Table 5.21 Comparison of LBW and NBW for maternal periodontal disease

Birth weight	**Periodontal Disease Status**				**Total**
	Absent	**Mild**	**Moderate**	**Severe**	
< 2500g LBW					
Frequency	4	33	39	34	110
%	0.9	7.5	8.8	7.7	24.8
Row %	3.6	30	35.5	30.9	
Column %	2.7	17.0	47.5	75.5	
≥ 2500g NBW					
Frequency	119	160	43	11	333
%	26.9	36.11	9.7	2.5	75.2
Row %	35.7	48	12.9	3.3	
Column %	96.7	82.9	52.4	24.4	
Total	123 (27.8)	193 (43.6)	82 (18.5)	45 (10.2)	443 (100)
X^2	121.598				
Df	3				
***p*-value**	**<0.000001**				

5.2.4 Infant birth weight and BANA

BANA positive plaques are an indication of periodontal disease activity. Table 5.20 shows a highly significant correlation between infant birth weight and BANA ($F_{1, 438}$ = 36.43, p <0.0001). This significance remained after categorising the infants into LBW and NBW. The number of LBW infants increased significantly when BANA was positive (Table 5.22) i.e. 95/109 (87.1%).

Table 5.22 Frequency of LBW and NBW for BANA

Birth weight	**BANA**		**Total**
	Negative	**Positive**	
< 2500g (LBW)			
Frequency	14	95	109
%	3.2	21.5	24.7
Row %	12.8	87.2	
Column %	8.7	33.9	
≥ 2500g (NBW)			
Frequency	147	185	332
%	33.3	42.0	75.3
Row %	44.3	55.7	
Column %	91.3	66.1	
Total (effective sample size)	161 (36.5)	280 (63.5)	441 (100)
Frequency missing			2
X^2	33.63		
Df	1		
***p*-value**	**0.000001**		

Mean GA and BW were compared in mothers who were BANA-, PD- and BANA+, PD+ (Table 5.23). Both GA and BW decreased significantly when PD and BANA were positive, providing evidence of an association between PD/BANA and both GA and BW.

Table 5.23 Birth Weight, Gestational Age association with BANA and Periodontal disease status

Variable	**BANA-** **PD-** (n = 167)	**BANA+** **PD+** (n=274)	***p* -value**	**Frequency missing**
Mean birth weight (grams)	3222	2842	**< 0.0001**	2
Mean gestational age (weeks)	38.018	36.875	**< 0.0001**	2

5.3. Birth weight for gestational age (BWGA)

The mean BWGA for all the infants is indicated in Table 5.24.

Table 5.24 Mean Birth Weight for Gestational Age

N	**Mean BW/g**	**SD**	**Min**	**Max**
443	0.080	0.013	0.044	0.150

5.3.1. Birth Weight for Gestational Age and maternal demography

Regression analysis revealed that there was no significant correlation between BWGA and maternal age *(r* = 0.005, p = 0.912), race, location nor education (Table 5.25).

Table 5.25 Comparison of Birth Weight for Gestational Age and maternal demography

Variable	**N**	**Mean BW for gestational age/g**	***F***	**Df**	***p***
Race			0.240	3,438	0.869
African	360	0.08			
Coloured	56	0.08			
Indian	23	0.08			
White	4	0.07			
Location			0.005	1,440	0.943
Urban	403	0.08			
Rural	40	0.08			
Education			0.473	2,439	0.623
Primary	30	0.08			
Secondary	314	0.08			
Tertiary	99	0.08			

5.3.2. Birth Weight for Gestational Age and maternal oral hygiene status

5.3.2.1. Plaque Index

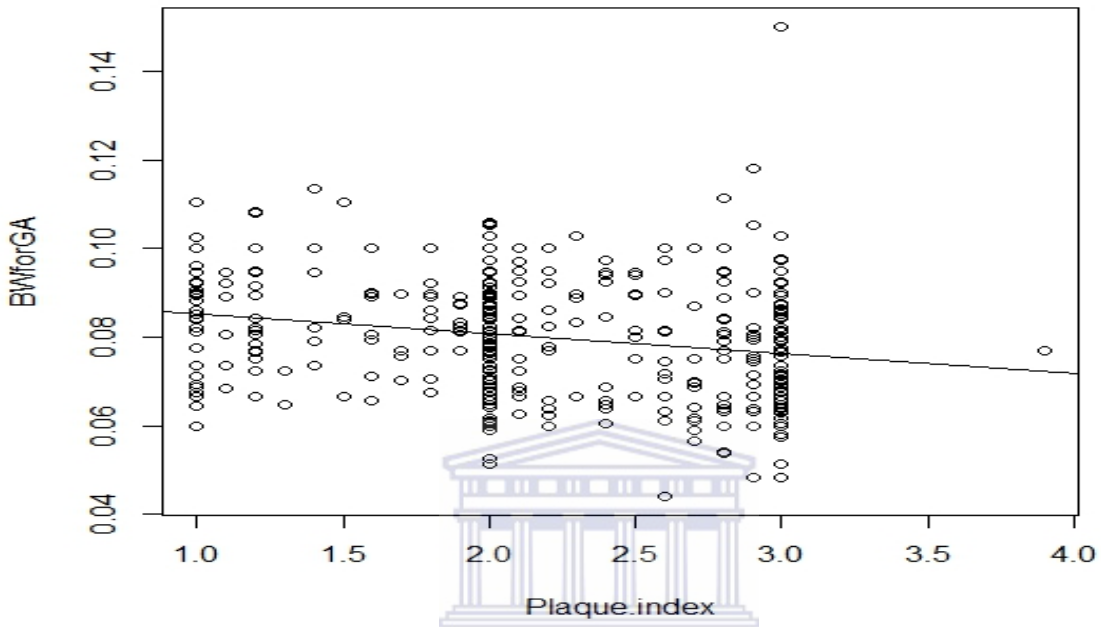

Figure 5.5 Birth Weight for Gestational Age and Plaque Index

Figure 5.5 demonstrates a significant correlation between birth weight for gestational age and plaque index (r = 0.220, p <0.001). As the PI increased, the BWGA decreased.

5.3.2.2. Birth Weight for Gestational Age and Gingival Index

A significant correlation between BWGA and GI was also demonstrated (r =0.230, p <0.001) (Figure 5.6). Increased GI resulted in lower BWGA.

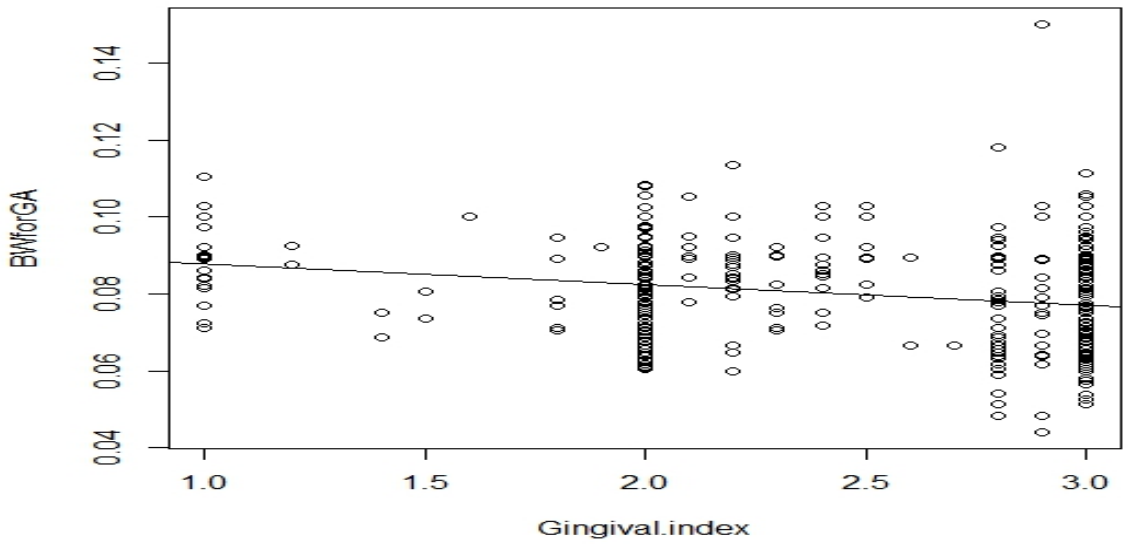

Figure 5.6 Birth Weight for Gestational Age at delivery and Gingival Index

5.3.2.3. Birth Weight for Gestational Age and Probing Pocket Depth

One way ANOVA ($F_{3,438}$ = 1.454, p = 0.2265) showed that although the mean BWGA decreased with increased PPD, this association was not significant (Table 5.26).

Table 5.26 Birth Weight for Gestational Age and pocket Probing Depth

	Probing Pocket Depth			
	1	**2**	**3**	**4**
Mean BW for GA	0.091	0.088	0.072	0.070
$F_{3,438}$	1.454			
***p*-value**	**0.2265**			

5.3.2.4. Birth Weight for Gestational Age and Clinical attachment loss

The BWGA means of the different clinical attachment loss groups differ significantly ($F_{3,438}$ =5.023, p = 0.002). The trend is decreasing mean with increasing clinical attachment loss as indicated in Table 5.27.

Table 5.27 Birth Weight for Gestational Age and Clinical attachment loss

	CAL			
BWGA	None	Mild (1-3mm)	Moderate(3-6mm)	Severe(6mm or more)
N	175	232	34	2
Mean	0.0821	0.0795	0.0740	0.0629
$F_{3,438}$	5.023			
***p*-value**	**0.002**			

Figure 5.7 illustrates the relation between BWGA and CAL. The red circles indicate the mean BWGA.

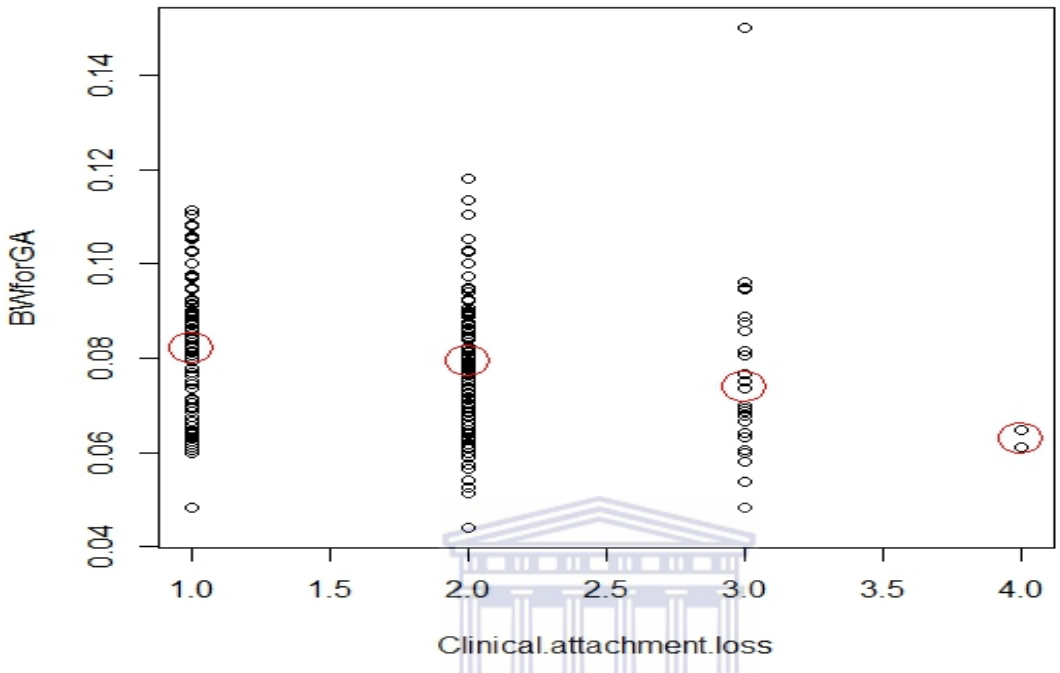

Figure 5.7 Birth Weight for Gestational Age and Clinical attachment loss

5.3.2.5. Birth Weight for Gestational Age and Periodontal disease

Regression analysis revealed that when controlling for PD, BWGA decreased significantly from 0.086g to 0.068g ($F_{3, 438} = 5.023$, $p =$ **0.002**). Results for ANOVA are clearly demonstrated in Figure 5.8. The red circles indicate the mean BWGA.

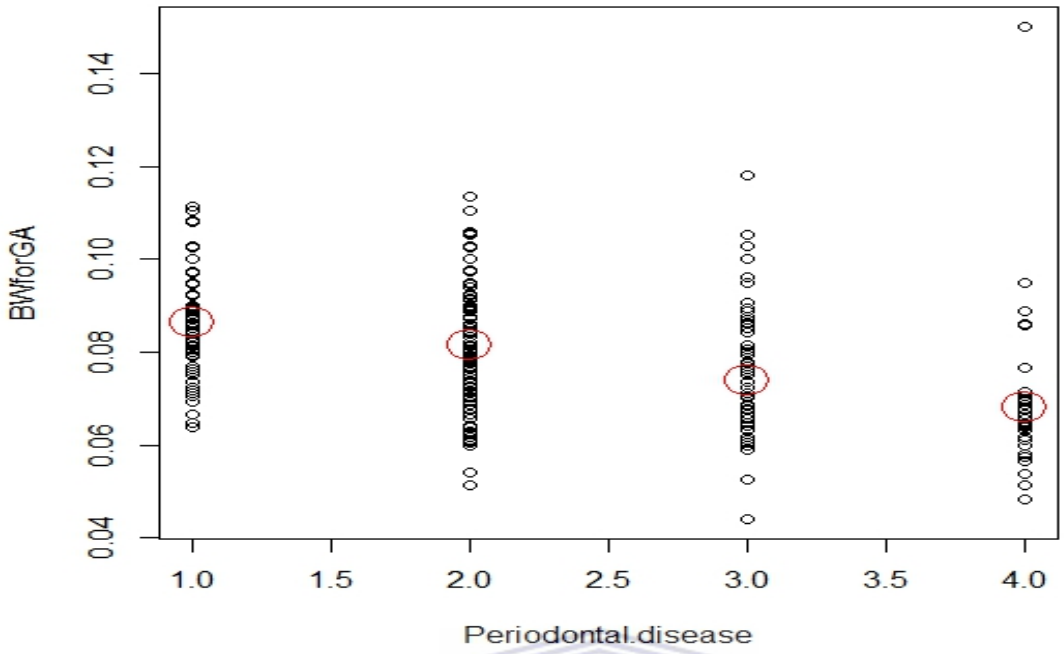

Figure 5.8 Birth Weight for Gestational Age at delivery and periodontal disease

5.3.2.6. Birth Weight for Gestational Age and BANA

The mean BWGA differed significantly between BANA- negative and BANA- positive subjects (Table 5.28). According to the Welch t-test these two means are significantly different at level $p<0.0001$.

Table 5.28 Birth Weight for Gestational Age and BANA

BANA	**Negative**	**Positive**
N	161	282
Mean BWGA/g	0.084	0.077
p-value	**<0.0001**	

5.4. PTLB and FTNB

Further analysis of BWGA grouped the pregnancy outcomes as preterm low birth weight (PTLB) and full term normal birth weight (FTNB). For this comparison, PTLB was defined as preterm birth (gestation that lasts less than <37 weeks) and Low Birth Weight (birth weight < 2500 grams). FTNB was defined as full term birth (gestation that lasts for ≥37 weeks) and normal birth weight (birth weight ≥2500 grams). No significant differences were observed between PTLB and FTNB for DMFT and GI, while a marginal significance was observed for PI (Table 5.29).

Table 5.29 Comparison of PTLB and FTNB for Maternal DMFT, PI and GI

Variable	**Gestational term/Birth Weight**		***t***	***Df***	***p***
	PTLB	**FTNB**			
DMFT Mean (SE)	6.676 (0.456)	7.299 (0.223)	-1.2271	106	0.2225
PI Mean (SE)	2.311 (0.073)	2.148 (0.034)	2.0341	104	0.0445
GI Mean (SE)	2.531 (0.057)	2.420 (0.031)	1.7002	115	0.0918

5.4.1. Comparison of PTLB and FTNB for PPD

The PTLB frequency for the different levels of PPD differed significantly from the FTNB (Table 5.30). The trend is decreasing FTNB frequency with increasing PPD.

Table 5.30 PTLB compared to FTNB for probing pocket depths

Gestational term	Probing pocket depths n (%)				Total
	1	**2**	**3**	**4**	
PTLB	10	44	17		71
FTNB	75	171	14		260
Total	85	215	31		331
X^2	25.367				
df	2				
***p*-value**	**<0.0001**				

5.4.2. Comparison of PTLB and FTNB for CAL

The PTLB frequency for the different levels of CAL differed significantly from the FTNB (Table 5.31). The trend is decreasing FTNB frequency with increasing CAL. Note the small frequency at CAL >3mm.

Table 5.31 Comparison of PTLB and FTNB for CAL

	Clinical attachment loss			
	None	**Mild (1-3mm)**	**Moderate (3-6mm)**	**Severe (≥6mm)**
PTLB	19	41	9	2
FTNB	156	190	25	0
Total	112	140	9	0
Chi-square	17.1867			
df	3			
***p*-value**	**0.0006**			

In the analysis of the preterm low birth weight and clinical attachment loss, where the standardized residual exceeds 2 in absolute value, a significant difference between row percentages, at level $p = <0.05$, is indicated.

5.4.3. Comparison of PTLB and FTNB for Periodontal Disease

However, when PTLB and FTNB were compared while controlling for the periodontal status of the mothers, Chi-squared revealed that the difference was highly significant (Table5.32)

Table 5.32 PTLB compared to FTNB for periodontal disease status

Gestational term	**Periodontal Disease Status n (%)**				**Total**
	Absent	**Mild**	**Moderate**	**Severe**	
PTLB	4 (5.6)	25 (35.2)	20 (28.2)	22 (31.0)	71 (16.1)
FTNB	119(32.1)	167 (45)	62 (16.7)	23 (6.2)	371 (83.9)
Total	123(27.8)	192 (43.4)	82 (18.6)	45 (10.2)	442 (100)
X^2	56.4703				
df	3				
***p*-value**	**<0.00001**				

5.4.4. Comparison of PTLB and FTNB for BANA

The BANA means of PTLB and FTNB means differ significantly (Table 5.33). In the light of the very large observed chi-squared one can take every difference in percentage between PTB and FTB as statistically significant.

Table 5.33 Contingency Table for PTB/ FTB and BANA

Gestational term	**BANA**		**Total**
	Negative	**Positive**	
< 37weeks/<2500g (PTLB)			
Frequency	9	62	71
%	12.7	87.3	
Row %	12.7	87.3	
Column %	5.6	22.2	
≥ 37weeks/ ≥ 2500g (FTNB)			
Frequency	152	217	369
%	41.2	58.8	
Row %	41.2	58.8	
Column %	94.4	77.8	
Total (effective sample size)	161(36.6)	279 (63.4)	440 (100)
Frequency missing			3
X^2	19.6577		
df	1		
***p*-value**	9.263e-06 i.e. **<0.0001**		

The sensitivity (Sens), specificity (Spec), positive predictive value (PPV) and negative predictive value (NPV) for periodontal disease and BANA to predict PTD confirmed this association regardless of which variable was used as the gold standard (Table 5.34). Periodontal disease (PD) could predict PTD with a sensitivity of 83% and a negative predictive value of 80.5% (Table 5.34). BANA showed a specificity of 78% and a positive predictive value of 75.3% for PTD. PTD was likely to occur in 75.3% of mothers with a positive BANA test and did not occur in 78.3% of mothers with a negative BANA test.

Table 5.34 Usefulness of BANA and PD diagnosis to predict preterm delivery (PTD)

Gold Standard	Sens	Spec	PPV	NPV
PD	83	33	36.9	80.5
BANA	38	78	75.3	42.3
PTD	75.3	42.3	38.35	78.3

In evaluating the likelihood of BANA and PD to detect LBW, BANA showed a sensitivity of 87% and a NPV of 91%, while PD showed a sensitivity of 96% and NPV of 97% (Table 5.35). LBW was showed a specificity of 91% for BANA.

Table 5.35 Usefulness of BANA and PD diagnosis to predict low birth weight (LBW)

Gold Standard	Sens	Spec	PPV	NPV
PD	96	36	33	97
BANA	87	74	34	91
LBW	34	91	29	44

CHAPTER 6: DISCUSSION

6.1 Introduction

This study investigated the effect of the presence of periodontal disease and a positive BANA test result during pregnancy on pregnancy outcomes. The objectives of this research were to compare the pregnancy outcomes of women who do not have periodontal disease and a negative BANA test result with pregnancy outcomes of women who have periodontal disease and a positive BANA test result. In this chapter, the findings of this study are discussed. It commences by highlighting the socio-demography of the study population. The discussion expands on the oral health status and the birth outcomes of the participants. Where appropriate, reference is made to the literature in order to consider this investigation in the context of its similarities and differences with similar studies locally and abroad.

6.2. Socio-demographic data

There were 488 women that participated in the study and completed the questionnaires. However, 45 questionnaires were excluded from the analysis due to medical exclusion and incomplete data. The final sample size was 443. The average age of the study population was 24.13 years (± 5.30) which is in the age group (20-29 years) that represents a substantial portion of the most economically active individuals (Swartz and Roux, 2004; Dorrington et al, 2004; Bachnann and Booysen, 2003).The median age of participants was 23 years (range 18-42). The first quartile was 20 years and the third quartile was 27 years.

In South Africa the black racial group constitutes the majority of the public health facility users (Swartz and Roux, 2004; Dorrington et al, 2004) and as was expected, the sample consisted predominantly of individuals of black ethnic origin, (81.26%) followed by Coloured (12.64 %) then Asian (5.19%) and White (>1%). reflecting the racial constitution of SA population (Dorrington et al, 2004). It should be borne in mind that individuals often travel from their dwelling areas to the MOU to obtain care. Even though convenience sampling was utilised to select recruitment, it is erroneous to assume that participants live in the town where the MOU is situated (where the participant was recruited) and therefore participants were asked to disclose where they lived. These areas were classified as urban

versus rural areas according to municipal regional classification. The majority of the participants lived in urban areas (90.97%).

All the participants had some formal education with more than half of the participants had attained at least a secondary education (70.88%) and Tertiary education was attained by 22.35 % and primary level education was attained (6.77%). The educational level determines the level of empowerment and employability of the individual though there are no guarantees as other market factors also contribute to determining employment status and income (Bachnann and Booysen, 2003; Swartz and Roux, 2004; Dorrington et al, 2004).

Participants in the survey were at different stages of pregnancy, the majority of the participants were 5 months pregnant (24 38%), followed by 6 months (21.90%), 7 months (19.41%) and 8 months (12.19%). A few of the participants were 2 months (0.22%) and 9 months (1.81%).

6.3. Oral health Status

6.3.1. Decayed Missing Filled Teeth (DMFT)

The mean DMFT of the participants recorded was 7.187 (± 4.223). The correlation coefficient between DMFT and age was 0.220, which was low but statistically significant ($p<0.001$). The means of the DMFT for the four race groups were Black (6.889), Coloured (7.783), Asian (8.661) and White (10.000), and statistically significant ($p = 0.012$). According to the Welch t-test the DMFT means of the urban (7.156) and rural participants (7.500) were not significantly different ($p = 0.633$). The DMFT means of primary (6.933) secondary (6.822) and tertiary (8.424) education were significantly different ($p = 0.004$). The correlation coefficient of DMFT and pregnancy stage was small (0.057) and not statistically significant (p-value =0.232).

There was a significant correlation between decayed teeth and age ($p = 0.025$). However no significant association was found between decayed teeth and location of the participants, race, education nor pregnancy stage at time of examination. The correlation coefficient of missing teeth and age was small (0.200) but statistically significant ($p <0.001$). No significant correlation was found between missing teeth and race, location, nor education.

A significant correlation was found between filled teeth and race ($p <0.0001$), with the mean F score of the White race group being significantly greater than the Black, Coloured and

Asian groups. Significant correlations were also found between filled teeth and location (p = 0.046), education (p = 0.0002), and pregnancy stage (p=0.0005). No correlation was found between filled teeth and age (p = 0.058). Race could probably dictate the socio-economic status of the participants. Whether the participants lived in urban or rural areas would influence their access to medical and dental care as well as access to educational schooling, which in turn would influence their affordability for dental fillings thus explaining these findings.

6.3.2 Plaque Index

This study investigated the association between the clinical parameters used to diagnose periodontal disease and maternal demography. The mean plaque index of this study was 2.172 (± 0.647) which differed from the mean plaque index of 0.69 reported by Tilakaratne et al. (2000) and that of 0.85 reported by Silness and Löe (1964). No significant association was observed between plaque index and age (p = 0.05), race (p = 0.297), location (p = 0.082) nor education (p = 0.233). These results agree with previous reports (Silness and Löe, 1964; Yalcin et al., 2002; Taani et al., 2003) but disagree with the one reported by Tilakaratne et al., (2000).

6.3.3 Gingival Index

The mean of the gingival index of this study was 2.439 (± 0.581)) which was slightly higher compared with the results found by Löe and Silness (1963) in their study of pregnant women (1.03 ± 0.03). However studies by (Yalcin et al., 2002; Family Gentle Dental Care, 2003; Lieff et al., 2004) showed the prevalence of pregnancy gingivitis ranging from 35% to 100%).In this study the correlation coefficient between age and gingival index was very low (-0.015) and it was not statistically significant (p= 0.05) similar to that of Yalcin et al., (2002) who reported no association between the increase of age and clinical parameters. These findings disagree with Taani et al., (2003) who reported that the increase of age was associated with significantly higher gingival scores.

The Gingival index for the Black (2.440), Coloured (2.359), Asian (2.596) and White (2.600) race groups did not differ significantly (p = 0.05). Nor could the gingival index be significantly associated with location (p = 0.729) or education (p =0.472). Gingival index

and pregnancy stage of this study was significantly different from zero at level 0.008. Gingival index scores were reported to increase 10 to 30 times more during the third trimester of pregnancy than during the menstrual cycle (Agbelusi et al., 2000; Tilakaratne et al., 2000; Yalci et al., 2002; Taani et al., 2003). This study yielded similar results. Progesterone and oestrogen levels are said to increase causing dilatation of gingival capillaries, gingival exudates and permeability which causes the redness and bleeding tendency during pregnancy (Zachariasen et al., 1993; Sooriyamoorthy, 1989).

6.3.4 Clinical Attachment Loss (CAL) and Probing Pocket Depth (PPD)

Clinical Attachment Loss (CAL) and/or Probing Pocket Depth (PPD) may well be the best indicators to use in epidemiology of periodontal diseases (Albandar *et al*., 1999; Kingman and Albandar, 2002), CAL gives an indication of past periodontal disease and PD may give better indication of current disease status. This study demonstrated a highly significant association between CAL and PPD ($p < 0.0001$) such that as CAL increases so does PPD. The high prevalence of score 3 may also reflect elevations in gingival inflammation leading to enlarged gingiva and, hence, an increase in PPD and CAL, though this may not always be true for CAL as enlarged gingiva may give rise to pseudo-pocketing as well.

This study found no significant association between clinical parameters of PPD ($p = 0.570$), and CAL ($p = 0.475$) with age, similar to results of Bayangani (2005) and Yalcin et al., (2002) who reported no association between the increase of age and clinical parameters of PPD or CAL . These findings disagree with Taani et al., (2003) who reported that the increase of age was associated with significantly higher CAL and PPD scores. There was no significant association between PPD measurements and race (p-value-0.2398), education levels ($p = 0.4629$) and pregnancy stage ($p = 0.2106$). However there was a significant difference in the distribution of PPD between urban and rural participants (p-value <0.0001).

A significant difference in CAL between the different race groups (*p*-value <0.0001) which is in agreement with the generalisation that periodontal disease profiles differ from one population group to another (Miyazaki et al., 1991; Williams et al ., 2000). However there was no significant association found in the CAL distribution and the location of the participants (p =0.111), pregnancy stage ($p = 0.528$) and the education level of the participants (p =0.516).

Periodontal disease progresses and CAL occurs through the destruction of the periodontal ligament and its adjacent alveolar bone subsequently leading to gingival recession and pathological probing depth (Kinane, 2001) therefore the degree of CAL reflects the severity of CAL and can be used as an indicator of the severity of periodontal disease (Baelum et al., 1995).This study found a highly significant association between CAL and PD status (p-value < 0.0001). Similarly there is a highly significant association between PD status and PPD (p-value < 0.0001). Increased severity of PD occurred at increased values of CAL and PPD.

The distributions of periodontal disease for BANA-negative and BANA-positive mothers differed significantly (p<0, 0001). Though one case of severe periodontal disease in this study tested negative for BANA, these findings confirm a correlation between BANA and the clinical indices used to indicate periodontal infection with the red complex (*T. denticola, T. forsythia, P.gingivalis*) (Loesche et al., 1990; Grisi et al., 1999; Grisi et al., 2001; Figueiredo et al., 2000; Africa, 2011).

6.4. The influence of maternal demography and oral health on pregnancy outcomes

6.4.1. Gestational Age (GA)

Various factors such as age, height, weight, socioeconomic status, ethnicity, smoking, nutritional status and stress have been associated with the delivery of PTLBW infants (Nordstrom et al., 1996; MacDorman et al., 2007). The mean gestational age in this study was 37.317 (± 2.502) with the minimum gestational age 22 and the maximum gestational age 40 weeks.

Gestational age and race were not significantly associated in the present study contrary to the findings of Nordstrom et al., (1996), who found an association between delivery of PLBW infants and ethnicity. Nor was a significant association found between gestational age and location (p=0.366), pregnancy stage (p=0.662), education (p=0.294), and maternal age (p = 0.739).When pregnancy outcomes were compared (i.e. PTB and FTB), DMFT, plaque index, and gingival index did not differ significantly either in the present study.

Periodontitis can induce a primary host response in the chorioamnion, leading to PTB (Dortbudak et al., 2005). This has been confirmed in other cases of adverse pregnancy outcomes as well (Lopez et al., 2002; Dasanayake et al., 2003; Bogies et al., 2006; Offenbacher et al., 2006; Jared and Boggess, 2008) with PPD and CAL scores found to be

significant risk indicators for PTB (Offenbacher et al. 2001; Radnai et al. 2004; Jarjoura et al. 2005). In this study, the mean gestational age at delivery showed a significant trend of decreasing with an increase in probing pocket depth severity (p <0, 0001) and this significant difference remained with comparison of PTB and FTB. FTB was more likely to occur when PPD was ≤ 2 and decreased with increased PPD. As observed with PPD, the mean gestational age at delivery showed a trend of decreasing with an increase in CAL and the difference between means was found to be significant (p = 0.013). Other studies found no difference in the periodontal parameters between women with PTB and FTB (Holbrook et al., 2004; Moore et al. 2005; Rajapakse et al. 2005; Abati et al. 2013).

Gestational age could be significantly associated with periodontal disease severity in this study (p<0.0001). The mean gestational age for no periodontal disease was 38.09 weeks, for mild periodontal disease it was 37.39 weeks, for moderate periodontal disease it was 36.79 weeks and for severe periodontal disease it was 35.80 weeks.

BANA could also be significantly associated with gestational age with the mean gestational age for the BANA-positive and periodontal disease group being significantly smaller than the gestational age mean of the BANA-negative and no periodontal disease group (p<0.0001).

Since BANA is an indicator of the presence of three of the most commonly detected periodontopathogens, this study concurs with that of Lin et al., (2007) who examined the association between periodontal diseases and preterm birth and explored the underlying microbial and antibody responses associated with oral infection. The authors found that ante partum, the levels of periodontal pathogens tended to be higher in the preterm (case group) deliveries compared to the term deliveries (control group).

Using periodontal disease (PD) as the gold standard in this study, PTD could be predicted with a sensitivity of 83% and a negative predictive value of 80.5%. With BANA as the gold standard, a specificity of 78% and a positive predictive value of 75.3% for PTD was achieved. PTD as the gold standard revealed that it was likely to occur in 75.3% of mothers with a positive BANA test and did not occur in 78.3% of mothers with a negative BANA test. The results of the present study indicate that BANA could be a useful screening method for PTD.

6.4.2. Birth Weight

The mean birth weight in this study was 2.986 (± 0.531) with the lowest weight being 1.5 kg and the highest weight 4.2 kg.

Young maternal age was previously reported to be one of the most significant risk factors in PTLBW infant delivery, with mothers <20 years and >35years of age being more likely to give birth to LBW babies (Nadar et al ,1995; Nordstrom et al., 1996; Mumghaba and Manji, 2006; López ,2008). However in this study there was no significant association between birth weight and age of the mother (p=0.872). Race did not significantly influence birth weight either in this study, nor did location, education levels and pregnancy stage.

Moderate to severe periodontitis identified early in pregnancy has been associated with an increased risk for spontaneous preterm birth, independent of other traditional risk factors (Offenbacher et al., 1996; Jeffcoat et al., 2001; Offenbacher et al., 2006 Haerian-Ardakani et al., 2013; Kothiwale et al., 2014; Reddy et al., 2014). Other studies have failed to demonstrate such an association (Davenport et al., 2002; Moore et al., 2004; Holbrook et al., 2004; Buduneli et al., 2005; Rajapakse et al., 2005 Mumghaba and Manji 2006; Agueda et al., 2008 ;Abati et al.,2013).

This study showed that the correlation coefficient of birth weight and plaque index and that of birth weight and gingival index were both statistically significant ($p<0.001$) which is in accordance with the findings of Persson et al., (2009) who linked gingivitis to adverse pregnancy outcome (APO). A significant association between birth weight and periodontal disease severity was established in the present study ($p<0.0001$) with a trend of decreasing birth weight with increasing periodontal disease severity.

When comparing the means of the plaque index and gingival index of LBW and NBW the Plaque Index mean for the LBW group was 2.422 (±0.055) and the gingival index was 2.647 (±0.043) while for the NBW group the plaque index and gingival index were 2.092 (± 0.036) and 2.371(± 0.231) respectively . The means for both PI and GI differ significantly at level $p<0.0001$ unlike the DMFT means which showed no significant difference.

Birth Weight was found to decrease with increasing PPD, and when birth weight was divided into LBW (<2500g) and NBW (≥ 2500g), the distributions of PPD at LBW and NBW differed significantly ($p<0.0001$). Similarly this study showed a significant trend in decreasing mean BW with increasing CAL ($p = 0.001$) and when divided into categories of

LWB and NBW, the distributions of clinical attachment loss at LBW and NBW were significantly different (p<0.0001). This is agreement with earlier studies which associated PPD and CAL with LBW (Dortbudak et al. 2005; Jarjoura et al. 2005). However, Moore et al. (2004) also looked at PPD and CAL and concluded that there is no association between LBW and periodontal disease nor was an interruption of periodontal therapy seen to increase the risk for LBW (Hujoel, 2006; Michalowicz, 2006).

The distributions of periodontal disease severity for LBW were 4 (3.6%) for no periodontal disease, 33 (30.0%) for mild periodontal disease, 39 (35.5%) for moderate periodontal disease, and 34 (30.9%) for severe periodontal disease in the present study. For NBW, the distributions were 119 (35.7%) for no periodontal disease, 160 (48.0%) for mild periodontal disease, 43 (12.9%) for moderate periodontal disease and 11(3.3%) for severe periodontal disease. The result of applying a chi-squared test of association yielded a highly significant result p-value < 2.2e-16 (i.e.<0.000001) lending support to other studies which identified periodontal disease severity as a risk for LBW (Sembene et al. 2000; Louro et al. 2001; Romero et al. 2002; Kothiwale et al. 2014).

This study showed a significant correlation (p <0.0001) between periodontal disease and LBW as demonstrated in previous studies (Jeffcoat et al. 2001; Romero et al. 2002; Goepfert et al. 2004; Manau et al. 2008). However the findings of the present study were contrary to reports from the UK which found no evidence of an association between the severity of periodontal disease and pregnancy outcome (Davenport et al. 2002; Moore et al. 2005).

In this study, the birth weight means of infants born of BANA-negative (3.224) and BANA-positive (2.849) mothers were significantly different (p<0.0001) as were their gestational ages (p<0.0001) and birth weight for gestational age (p<0.0001). Chan et al (2010) in their prospective study in Changhua, Taiwan reported similar findings and concluded that BANA-positive plaques in the 3rd trimester were associated with preterm births, after controlling for other risk factors. BANA-positive mothers with periodontal disease delivered infants with a mean birth weight of 2.842 Kg which was significantly smaller (p<0.0001) than the birth weight mean of BANA-negative mothers with no periodontal disease (3.222 kg) supporting the study of Mokeem et al., (2004) which found that periodontal disease may influence pregnancy outcome by the direct and/or indirect effect of periodontal pathogens on the developing foetus.

Chi-squared test of association showed a highly statistically significant association between pregnancy outcomes (i.e. LBW vs NBW) for BANA-positive and BANA-negative tests (p-value <0.000001). BANA showed a sensitivity of 87% and a NPV of 91% in detecting LBW, while PD showed a sensitivity of 96% and NPV of 97%. Using LBW as the golden standard, a specificity of 91% was found for BANA.

6.4.3. Birth Weight for Gestational Age (BWGA)

No significant associations were observed between BWGA and age, location, education and pregnancy stage. However, BWGA was significantly associated with plaque index and gingival index ($p<0.001$).

Although the mean BWGA decreased with increased PPD in the present study, the association was not significant ($p= 0.2265$). CAL showed a similar but significant association with decreasing BWGA ($p = 0.002$).

Santos-Pereira et al., (2007) studied 124 women between the ages of 15-40 to determine if chronic periodontitis increased the risk of experiencing preterm labour (PTL) using the same parameters as the present study where chronic periodontitis was described as one site with clinical attachment loss (CAL) > 1 mm with gingival bleeding and the severity of periodontitis was classified as early (CAL <3mm), moderate (CAL > 3 mm and < 5 mm), and severe (CAL >5mm). The extent of periodontitis was either localized, CAL < 30%, or generalized CAL > 30%. They, too, concluded that chronic periodontitis increased the risk of having PTLBW infants as was demonstrated in the present study when a significant association ($p=0.002$) between PTLBW and periodontal disease was established. No significant risk of PTB or small-for-gestational-age infant was found when adjusting for smoking, race/ethnicity, socioeconomic status, BMI, history of preterm delivery, presence of genitourinary infection, weekly weight gain, and history of dental check-ups by Pitiphat et al., (2008), nor was an association found between poorer periodontal health and either PTB or LBW in a cohort study of 1793 women with no history of smoking (Farrell et al., 2006). However, the latter reported an association between higher mean probing depth and late miscarriage.

Mumghaba and Manji (2006) examined the relationship between carious pulpal exposure and preterm low-birth-weight (PTLBW) and found no evidence for carious pulpal exposure being

significant risk factors in PTLBW infant delivery. DMFT for PTLBW and FTLBW did not differ significantly in the present study either.

Plaque Index for the PTLBW and FTLBW differed significantly (p =0.0445) in the study by Mumghaba and Manji (2006), but this was not the case in our study.

Buduneli *et al.* (2005) evaluated the number of sites with bleeding on probing and found no significant differences between PTLBW and FTLBW. However, PTLBW was significantly associated with periodontal disease and BANA when compared with FTLBW (p<0.00001) in our study. In this study the distributions of PTLBW and BANA-negative were 9 (12.7%), and 62 (87.3%) for PTLBW and BANA-positive. Significant differences were also observed between PTLBW and FTNBW for PPD (p <0.0001) and CAL (p =0.0006) in our study, as has previously been reported in other studies (Williams et al. 2000; Offenbacher et al. 2001; Jarjoura et al. 2005).

6.5. Conclusions and recommendations

BANA is a rapid and effective diagnostic aid and has shown to correlate well with the clinical indices used to diagnose periodontal disease (Loeshe et al., 1997a, b; Grisi et al., 2001). There was no significant difference in the socio-demographic attributes of the participants observed in this research. Exclusion criteria for participants were relevant confounders, such as tobacco use, multiple pregnancies chronic diseases like hypertension, diabetes mellitus and previous periodontal treatment, thus avoided bias of confounding.The findings of this study indicate a significant correlation that a positive BANA test result and the presence of periodontal disease during pregnancy have a significant association with negative pregnancy outcomes such as preterm birth and low birth weight.

Calculation of sensitivity, specificity, PPV and NPV for PD and BANA in predicting PTD showed that a diagnosis of PD and BANA hydrolysis could both predict PTD and that PTD was not likely to occur in mothers without PD or a negative BANA test. It can be concluded that the present study showed a significant association between PD and PTD and that PD is independently associated with PTD and LBW. Limitations of the study include the difficulty in making comparisons with other studies due to differences in (amongst others) sampling techniques, diagnostic criteria, pregnancy stage at recruitment, ethnicity and geographical location.

Within the limitations of the study the findings of this study proves the null hypothesis wrong and confirms that periodontal disease and a BANA positive test was associated with low birth weight and preterm birth. The findings support the hypothesis that a positive BANA test result and the presence of periodontal disease during pregnancy have a significant association with negative pregnancy outcomes such as miscarriage, preterm birth (gestation that lasts less than 37 weeks) and low birth weight (LBW) - a weight less than2.5 kilograms. The findings of this study suggests that the risk for LBW or PTB infants may be potentially reduced by microbial monitoring of potential colonization of periodontal pathogens and establishing oral hygiene and dental preventive measures in pregnant women. It can be suggested that the role of the microbial load and maternal immune response as related to pregnancy outcomes needs to be explored in other studies. This study also demonstrated the usefulness of the BANA test as a screening test for PTD. Its implementation as a chairside screening test for preterm delivery during antenatal visits should be considered.

REFERENCES

Abati S, Villa A, Cetin I, Dessole S, Lugliè PF, Strohmenger L, Ottolenghi L, Campus GG. (2013). "Lack of association between maternal periodontal status and adverse pregnancy outcomes: a multicentric epidemiologic study". *J Matern Fetal Neonatal Med.*Mar; 26(4):369-72.

ACOG Practice Bulletin. (2001). Assessment of risk factors for preterm birth. Clinical management guidelines for obstetrician-gynaecologists. Number 31, Obstet Gynecol. Oct; 98(4):709-16.

ACOG practice bulletin (2003). Management of preterm labour. Number 43, Int J Gynaecol Obstet.Jul; 82(1):127.

Africa CW (2011).Oral colonization of Gram-negative anaerobes as a risk factor for preterm delivery.Virulence. Nov-Dec; 2(6):498-508.

Africa CWJ (2012). Chapter 1: The microbial aetiology of periodontal diseases.

In: Periodontal Diseases: A Clinician's Guide; ISBN 978-953-307-818-2, Publishers INTECH, Rijeka, Croatia (Commissioned).

Africa CW, Bayingana C, Yasin-Harnekar S (2009). The use of Perioscan as a potential screening test for mothers at risk for delivery of pre-term and low birth-weight infants.J Int Acad Periodontol. Apr; 11(2):193-9.

Agueda A, Ramon JM, Manau C, Guerrero A, Echeverria JJ (2008).Periodontal disease as a risk factor for adverse pregnancy outcomes: a prospective cohort study. J Clin Periodontol.; 35(1):16-22.

Ainamo J, Barmes D, Beagrie G, Cutress T, Martin J, Sardo-Infirri J (1982). Development of the World Health Organization (WHO) community periodontal index of treatment needs (CPITN). Int Dent J.; 32: 281–91.

Albandar JM, Brunelle JA, Kingman A (1999). Destructive Periodontal Disease in Adults 30 Years of Age and Older in the United States, 1988-1994. J Periodontal; 70(1):13-9.

Albandar J and Rams T (2002) .Global epidemiology of periodontal diseases: an overview. Periodontol 2000; 29: 7–10.

Al-Shammari KF, Al-Ansari JM, Moussa NM, Ben-Nakhi A, Al-Arouj M, Wang HL (2006). Association of periodontal disease Severity with diabetes duration and diabetic complications in patients with type 1 diabetes mellitus. J Int Acad Periodontol.; 8(4):109-14.

Al-Zahrani MS, Bissada NF, Borawskit EA (2003). Obesity and periodontal disease in young, middle-aged, and older adults. J Periodontol; 74:610-615.

American Academy of Periodontology (2004). Statement regarding periodontal management of the pregnant patient. J Periodontol.75 (3):495.

Andrade JA, Feres M, Figueiredo LC, Salvador SL, Cortelli SC (2010) . The ability of the BANA Test to detect different levels of P. gingivalis, T. denticola and T. forsythia.Braz Oral Res.; Apr-Jun; 24(2):224-30.

Andrews WW,Goldenberg RL,Mercer B , Iams J, Meis P, Moawad A, et al (2000). The Preterm Prediction Study: Association of second-trimester genitourinary chlamydia infection with subsequent spontaneous preterm birth Am J Obstet Gynecol.; 183:662-8.

Annells MF, Hart PH, Mullighan CG, Heatley SL, Robinson JS, Bardy P, McDonald HM (2004). Interleukins-1, -4, -6, -10, tumor necrosis factor, transforming growth factor-beta, FAS, and mannose binding protein C gene polymorphisms in Australian women: risk of preterm birth. American Journal of Obstetrics and Gynaecology; 191, 2056–2067.

Armitage GC (2004). Periodontal diagnoses and classification of periodontal diseases. Periodontol 2000; 34:9-21.

Avula H, Mishra A, Arora N, Avula J (2013). KAP assessment of oral health and adverse pregnancy outcomes among pregnant women in Hyderabad, India.OralHealth Prev Dent.; 11(3):261-70.

Azarpazhooh A and Leake JL (2006). Systematic review of the association between respiratory diseases and oral health. J Periodontol; 77(9):1465-82.

Baelum V and Lopez R (2003). Defining and classifying periodontitis: Need for a paradigm shift? Eur J Oral Sci.; 111:2-6.

Baelum V and Papapanou PN (1996). CPITN and the epidemiology of periodontal disease. Community Dent Oral Epidemiol; 24: 367–8.

Baelum V, Manji F,Wanzala P, Federskof O, (1995). Relationship between CPITN and periodontal attachment loss in an adult population. J Clinical Periodontol. Feb 22 (2): 146 - 52.

Bassani DG, Olinto MTA, Kreiger N (2007). Periodontal disease and perinatal outcomes: a case-control study.J Clin Periodontol, 34:31–39.

Barak S, Oettinger-Barak O, Oettinger M, Machtei EE, Peled M, Ohel G. (2003). Common oral manifestations during pregnancy: a review. Obstet Gynecol Surv.; 58(9):624-8.

Barak S, Oettinger-Barak O Machtei E. E, Sprecher H, Ohel G (2007). Evidence of Periopathogenic Microorganisms in Placentas of Women with Preeclampsia. Journal of Periodontology; April, Vol. 78, No. 4, Pages 670-676.

Barros F C, Victoria C G, Vaughan J P, Tomasi E, Horta B L, Cesar JA, Menezes MB, Halpern R, Post C L, del Mar Garcia M (2001).The epidemiological transition in maternal and child health in a Brazilian city, 1982–93: a comparison of two population-based cohorts. Paediatr.Perinat. Epidemiol.; 15: 4–11.

Bayingana C (2005). The prevalence of members of the "red complex" in pregnant women as revealed by PCR and BANA hydrolysis. Thesis.

Bearfield C, Davenport ES, Sivapathasundaram V, Allaker R P (2002). Possible association between amniotic fluid microorganism infection and microflora in the mouth. British Journal of Obstetrics and Gynaecology;109:527–33.

Beck JD, Eke PI, Heiss G, et al (2005). Periodontal disease and coronary heart disease: a reappraisal of the exposure. Circulation. (1); 112:19-24.

Behrman R, Stith-Butler A (2006). Preterm Birth: Causes, Consequences, and Prevention. Washington, D.C., The National Academies Press; 329.

Billings F (1912). Chronic focal infections and their etiologic relations to arthritis and nephritis. Arch Intern Med.;9:484-498.

Blondel B, Macfarlane A, Gissler M, Breart G, Zeitlin J (2006). Preterm birth and multiple pregnancy in European countries participating in the PERISTAT project. British Journal of Obstetrics and Gynaecology;113, 528–535.

Bobetsis A, Barros S, Offenbacher S (2006). Exploring the relationship between periodontal disease and pregnancy complications J Am Dent Assoc.; Vol. 137, No suppl_2, 7S-13S.

Boggess KA, Beck JD, Murtha AP, Moss K, Offenbacher S (2006). Maternal periodontal disease in early pregnancy and risk for a small-for-gestational-age infant. Am J Obstet Gynecol.; 194:1316–22.

Boggess KA, Madianos PN, Preisser JS, Moise KJ Jr, Offenbacher S (2005). Chronic maternal and fetal Porphyromonas gingivalis exposure during pregnancy in rabbits. Am J Obstet Gynecol; 192(2):554-7.

Boggess KA, Lieff S, Murtha AP, Moss K, Beck J, Offenbacher S (2003).Maternal periodontal disease is associated with an increased risk for preeclampsia. Obstet Gynecol.; 101(2):227-31.

Boggess KA, Moss K, Madianos P, Murtha AP, Beck J, Offenbacher S (2005). Fetal immune response to oral pathogens and risk of preterm birth. Am J Obstet Gynecol.; 193(3 part 2):1121-6.

Bogies KA, Beck JD, Murtha AP, et al (2006). Maternal periodontal age infant. Am J Onsted Gynecol.; 194(5): 1316–22.

Borrell L and Papapanou PN (2005). Analytical epidemiology of periodontitis. J Clinic Periodontol: 32(6 suppl) 132-158.

Bright Futures in Practice (2004). Oral Health Pocket Guide: National Maternal and Child Oral Health Resource Center; Georgetown University.

Buduneli N, Baylas H, Buduneli E, Turkoglu O, Kose T, DahlenG (2005). Periodontal infections and pre-term low birth weight: a case-control study. J Clin Periodontol; 32 (2):174-81.

Carey J C, Klebanoff MA, Hauth JC, Hillier S L, Thom EA, Ernest JM,Heine R P, Nugent R P, Fischer ML,Leveno KJ, Wapner R, Varner M (2000). Metronidazole to prevent preterm delivery in pregnant women with asymptomatic bacterial vaginosis. National institute of child health and human development network of maternal-fetal medicine units. New England Journal of Medicine; 342, 534–540.

Carlos JP, Wolfe MD, Kingman A (1986). The extent and severity index: a simple method for use in epidemiologic studies of periodontal disease. J Clin Periodontol.; 13: 500–5.

Carta G, Persia G, Falciglia K, Iovenitti P (2004).Periodontal disease and poor obstetrical outcome. Clinical and Experimental Obstetrics and Gynecology; 31, 47–49.

Champagne CM, Madianos PN, Lieff S, Murtha AP, Beck JD, Offenbacher S (2000). Periodontal medicine: emerging concepts in pregnancy outcomes. J Int Acad Periodontol.; 2: 9–13.

Chan H,Wu C, Welch KB, Loesche WJ (2010). Periodontal Disease Activity Measured by the Benzoyl-DL-Arginine-Naphthylamide Test Is Associated With Preterm Births . Journal of Periodontology.; July, Vol. 81, No. 7, 982-991.

Chi DL, Milgrom P, Carle AC, Huebner CE. Mancl LA (2014). Multilevel factors associated with dentists' counseling of pregnant women about periodontal health. Special Care in Dentistry, 34: 2–6.

Collins JG, Windley HW 3rd, Arnold RR, Offenbacher S (1994). Effects of a Porphyromonas gingivalis infection on inflammatory mediator response and pregnancy outcome in hamsters. Infect Immun.; 62(10):4356-61.

Collins JG, Smith MA, Arnold RR, Offenbacher S (1994). Effects of Escherichia coli and Porphyromonas gingivalis lipopolysaccharide on pregnancy outcome in the golden hamster. Infect Immun.; 62(10):4652-5.

Costerton W, Veeh R, Shirtliff M, Pasmore M, Post C, Ehrlich G (2003). The application of biofilm science to the study and control of chronic bacterial infections. Journal of Clinical Investigation. ; 112, 1466–1477.

Cowling P, McCoy DR, Marshall RJ, Padfield CJ and Reeves DS (1992). Bacterial colonization of the non-pregnant uterus: a study of pre-menopausal abdominal hysterectomy specimens. European Journal of Clinical Microbiology and Infectious Diseases; 11, 204–205.

Cronin CM, Shapiro CR, Casiro OG and Cheang MS (1995). The impact of very low-birth-weight infants on the family is long lasting. A matched control study. Archives of Pediatrics and Adolescent Medicine. ; 149,151–158.

Cuttress TW (1986). Periodontal health and periodontal disease in young people: Global epidemiology. Int Dent J.; 36:146–152.

D'Aiuto F, Parkar M, Nibali L, Suvan J, Lessem J, Tonetti MS (2006) . Periodontal infections cause changes in traditional and novel cardiovascular risk factors: results from a randomized controlled clinical trial. Am Heart J.; 15 :(5):977-84.

Dasanayake AP (1998). Poor periodontal health of the pregnant woman as a risk factor for low birth weight. Ann Periodontol.; 3(1):206-12.

Dasanayake AP (2010). Periodontal disease is related to local and systemic mediators of inflammation. J Evid Based Dent Pract; 10:246-7.

Dasanayake AP, Boyd D, Madianos PN, Offenbacher S, Hills E (2001).The association between Porphyromonas gingivalis-specific maternal serum IgG and low birth weight. J Periodontol; 72:1491–7.

Dasanayake AP, Russell S, Boyd D, et al (2003). Preterm low birth weight and periodontal disease among African Americans.Dent Clin North Am.; 47(1):115-25.

Davenport ES, Williams CE, Sterne JA, Murad S, Sivapathasundram V, Curtis MA (2002) .Maternal periodontal disease and preterm low birth weight: case-control study. J Dent Res.; 81: 313–18.

DeStefano F, Anda RF, Kahn HS, et al (1993). Dental disease and risk of coronary heart disease and mortality. BMJ; Mar 13; 306 (6879): 688-91.

Dorrington R, Bradshaw D, Johnson L, Budlender D (2004). The demographic impact of HIV/AIDS in South Africa. National indicators for 2004. Cape Town: Centre for Actuarial Research, South African Medical Research Council and Actuarial Society of South Africa.

Dortbudak O, Eberhardt R, Ulm M, Persson GR (2005). Periodontitis: a marker of risk in pregnancy for preterm birth. J Clin Periodontol.; 32(1):45-52.

Duff P, Gibbs RS, Blanco JD, St Clair PJ (1983). Endometrial culture techniques in puerperal patients. Obstetrics and Gynecology; 61, 217–222.

Ellenberg JH (1996). Intent to treat versus as treated analysis. Drug Information Journal; 30, 535–544.

Eskenazi B, Marks AR, Catalano R, Bruckner T, Toniolo PG (2007). Low birth weight in New York City and upstate New York following the events of September 11th. Human Reproduction; 22, 3013–3020.

Espinoza J, Erez O, Romero R (2006).Preconceptional antibiotic treatment to prevent preterm birth in women with a previous preterm delivery. American Journal of Obstetrics and Gynecology; 194, 630–637.

Faria-Almeida R, Navarro A, Bascones A (2006). Clinical and metabolic changes after conventional treatment of type 2 diabetic patients with chronic periodontitis. J Periodontol; 77(4): 591-8.

Farrell S, Ide M, Wilson RF (2006).The relationship between maternal periodontitis, adverse pregnancy outcome and miscarriage in never smokers. J Clin Periodontol.; 33:115-120.

Feinberg AP (2007). Phenotypic plasticity and the epigenetics of human disease. Nature; 447:433-440.

Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S Woo P (1998). The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. Journal of Clinical Investigation. ; 102, 1369–1376.

Flynn CA, Helwig AL, Meurer LN (1999). Bacterial vaginosis in pregnancy and the risk of prematurity: a meta-analysis. Journal of Family Practice; 48, 885–892.

Foster JS and Kolenbrander PE (2004). Development of a multispecies oral bacterial community in a saliva-conditioned flow cell.Appl Environ Microbiol, Jul; 70(7):4340-8.

Galloway CE (1931). Focal Infection. Am J Surg.; 14(3):643-645.

Garcia RI, Henshaw MM, Krall EA (2001). Relationship between periodontal disease and systemic health. Periodontology 2000, 25: 21–36.

Garcia R, Henshaw M, Krall E (2001). Relationship between periodontal disease and systemic health. Periodontol 2000; 25: 21–36.

Gardosi JO (2005). Prematurity and fetal growth restriction. Early Human Development; 81, 43–49.

Gettinger G, Patters MR, Testa MA, Loe H, Anerud A, Boysen H, Robertson PB (1982). The use of six selected teeth in population measures of periodontal status. Journal of Periodontology. 54: 155-159.

Giannopoulou C, Cappuyns I, Mombelli A (2003). Effect of gingival crevicular fluid cytokine profile during experimental gingivitis. J Clin Periodontal; 30:996-1002.

Giannopoulou C, Kamma JJ, Mombelli A (2003). Effect of inflammation, smoking and stress on gingival crevicular fluid cytokine level. J Clin Periodontol; 30:145-153.

Gibbs RS, Romero R, Hillier SL, Eschenbach DA, Sweet RL (1992). A review of premature birth and subclinical infections. Am J Obstet Gynecol. 166(5): 1515-1528.

Genc MR, Onderdonk AB, Vardhana S,Delaney ML, Norwitz ER, Tuomala RE, Paraskevas LR,Witkin SS (2004). Polymorphism in 2 of the interleukin-1 receptor antagonist gene, local mid-trimester cytokine response to vaginal flora, and subsequent preterm birth. AmericanJournal of Obstetrics and Gynecology; 191, 1324–1330.

Genuit T, Bochicchio G, Napolitano LM, McCarter RJ, Roghman MC (2001). Prophylactic chlorhexidine oral rinse decreases Ventilator-associated pneumonia in surgical ICU patients. Surg Infect (Larchmt); 2(1):5-18.

Giannopoulou C, Kamma JJ, Mombelli A (2003). Effect of inflammation, smoking and stress on gingival crevicular fluid cytokine level. Journal of Clinical Periodontology; 30, 145–153.

Gibbs RS, Romero R, Hillier SL, Eschenbach DA, Sweet R L (1992). A review of premature birth and subclinical infection. American Journal of Obstetrics and Gynecology; 166, 1515–1528.

Gibbs R S (2001). The relationship between infections and adverse pregnancy outcomes:an overview. Annals of Periodontology; 6, 153–163.

Goepfert AR, Jeffcoat MK, Andrews WW, et al (2004). Periodontal disease and upper genital tract inflammation in early spontaneous preterm birth. Obstet Gynecol; 104(4):777-83.

Goldenberg RL, Culhane JF, Iams JD, Romero R (2008). Epidemiology and causes of preterm birth. Lancet; 371, 75–84.

Goldenberg RL, Hauth JC, Andrews WW (2000). Intrauterine infection and preterm delivery. N Engl J Med.; 342:1500-1507.

Grisi MFM, Correa Filho TA, Fanganiello CLS, Martins W, Silva-Neto CR, Salvador SL (2001). Relationship Between the Presence or Absence of Gingival Bleeding and the Enzymatic BANA Test. Braz Dent J.; 12(1): 23-26.

Hack M, Taylor HG, Klein N, Eiben R, Schatschneider C, Mercuri-Minich N (1994). School-age outcomes in children with birth weights under 750 g. New England Journal of Medicine; 331, 753–759.

Haerian-Ardakani A, Eslami Z, Rashidi-Meibodi F, Haerian A, Dallalnejad P, Shekari M, Moein Taghavi A, Akbari S (2013). Relationship between maternal periodontal disease and low birth weight babies.Iran J Reprod Med.; Aug; 11(8):625-30.

Han YW, Houcken W, Loos BG, Schenkein HA, Tezal M (2014). Periodontal Disease, Atherosclerosis, Adverse Pregnancy Outcomes, and Head-and-Neck Cancer.Adv Dent Res.; May; 26(1):47-55.

Han YW, Ikegami A, Bissada NF, Herbst M, Redline RW (2006).Transmission of an uncultivated Bergeyella strain from the oral cavity to amniotic fluid in a case of preterm birth. Journal of Clinical Microbiology; 44, 1475–1483.

Harmsen D and Ehmke B (2006). Current Microbiological Diagnostic Strategies in Periodontitis and Future Trends. Perio - Periodontal Practices Today; Volume 3, Issue 1.Pages: 63

Hasegawa K, Furuichi Y, Shimotsu A, Nakamura M, Yoshinaga M, Kamitomo M, et al (2003). Associations between systemic status, periodontal status, serum cytokine levels, and delivery outcomes in pregnant women with a diagnosis of threatened premature labor. Journal of Periodontology; 74:1764-1770.

Hauth JC, Goldenberg RL, Andrews WW, DuBard MB, Copper RL (1995). Reduced incidence of preterm delivery with metronidazole and erythromycin in women with bacterial vaginosis. New England Journal of Medicine; 333, 1732–1736.

Hillier SL, Nugent RP, Eschenbach DA, Krohn MA, Gibbs RS, Martin DH, Cotch MF, Edelman R, Pastorek JG. II, Rao AV, McNellis D, Regan J A, Carey JC, Klebanoff MA (1995). Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. The vaginal infections and prematurity stud group. New England Journal of Medicine; 333, 1737–1742.

Holbrook WP, Oskarsdottir A, Fridjonsson T, Einarsson H, Hauksson A, Geirsson RT (2004). No link between low-grade periodontal disease and preterm birth: a pilot study in a healthy Caucasian population. Acta Odontol Scand.; 62(3):177-9.

Holmlund A, Holm G, Lind L (2006). Severity of periodontal disease and numbers of remaining teeth are related to the prevalence of myocardial infarction and hypertension in a study based on 4,254 subjects. J Periodontol; 77(7):1173-8.

Horowitz S, Mazor M, Romero R, Horowitz J, Glezerman M (1995). Infection of the amniotic cavity with Ureaplasma urealyticum in the mid-trimester of pregnancy. Journal of Reproductive Medicine. ; 40, 375–379.

Hujoel PP, Drangsholt M, Spiekerman C, DeRouen TA (2002). Periodontitis-systemic disease associations in the presence of smoking—causal or coincidental? Periodontol 2000; 30: 51–60.

Hujoel PP, Lydon-Rochelle M, Robertson PB, del Aguila MA (2006). Cessation of periodontal care during pregnancy: effect on infant birthweight. Eur J Oral Sci, 114:2-7.

Hyman J (2006). The importance of assessing confounding and effect modification in research involving periodontal disease and systemic diseases. J Clin Periodontol.; 33(2):102–103.

Iftime C, Galuscan A, Jumanca D, Podariu A, Oancea R, Popovici R , Sava-Rosianu R (2008). Health education and prophylactic measures needed during pregnancy. Medicine in evolution; Nr. 4117.

Ishira K, Naitoy, Kato T, et al (1991). A sensitive enzymatic method (SK-013) for detection and quantification of specific periodontopathogens. J Periodont Res.; 27: 81-91.

Jared H and Boggess KA (2008). Periodontal Diseases and Adverse Pregnancy Outcomes: A Review of the Evidence and Implications for Clinical Practice. Journal of Dental Hygiene; Vol. 82, No. 3, July 2008.

Jarjoura K, Devine PC, Perez-Delboy A, Herrera-Abreu M, D'Alton M, Papapanou PN (2005). Markers of periodontal infection and preterm birth. Am J Obstet Gynecol; 192(2):513-9.

Jason H, Lind Holm E, Lindh C, Groop L, Bratthall G (2006). Type2 diabetes and risk for periodontal disease: a role for dental Health awareness. J Clin Periodontol.; 33(6):408-14.

Jeffcoat MK (1994). Prevention of periodontal diseases in adults: strategies for the future. Prev Med.; 23(5):704-8.

Jeffcoat MK, Hauth JC, Geurs NC, Reddy MS, Cliver SP, Hodgkins PM, Goldenberg RL (2003). Periodontal disease and preterm birth: results of a pilot intervention study. Journal of Periodontology; 74, 1214–1218.

Jeffcoat MK, Geurs NC, Reddy MS, Cliver SP, Goldenberg R, Hauth JC (2001). Periodontal infection and preterm birth: results of a prospective study. JADA; 132(7):875-80

Jensen J, Liljemark W, Bloomquist C (1981). The effect of female sex hormones on subgingival plaque. J Periodontol; 52(10):599-601.

Kanaparthy R, Kanaparthy A, Dev K (2012). BANA Hydrolysis as a Comparative Tool in the Evaluation of Amoxicillin and Azithromycin in the Treatment of Chronic Periodontitis, J Periodontol Implant Dent; 4(2):43-47.

Katz J, Chegini N, Shiverick K, Lamont RJ (2009). Localization of P. gingivalis in preterm delivery placenta. J Dent Res.; Jun; 88(6):575-8.

Katzenellenbogen J and Joubert G (1997). Data Collection and Measurement. In: Epidemiology A manual for South Africa. Oxford University Press Southern Africa. 82-92.

Kekki M, Kurki T, Pelkonen J, Kurkinen-Raty M, Cacciatore B, Paavonen J (2001). Vaginal clindamycin in preventing preterm birth and peripartal infections in asymptomatic women with bacterial vaginosis: a randomized, controlled trial. Obstetrics and Gynecology; 97, 643–648.

Kenagy G, Linsk N, Bruce D, Warnecke R, Gordon A, Wagaw F , Densham A (2003). Service Utilization, Service Barriers, and Gender among HIV-Positive Consumer in Primary Care. AIDS Patient Care and STD's.; 17 (5): 235 -244.

Kinane D and Bouchard P (2008). Group E of European Workshop on Periodontology. J Clin Periodontol.; Sep; 35(8 Suppl):333-7.

Kinane DF (2001). Causation and pathogenesis of periodontal disease Periodontol 2000, 25:8-20.

Kingman A, Albandar JM (2002). Methodological aspects of epidemiological studies of periodontal diseases. Periodontol 2000, 29**:**11-30.

Kolenbrander PE (2011). Multispecies communities: interspecies interactions influence growth on saliva as sole nutritional source.Int J Oral Sci.; 2011 Apr; 3(2):49-54.

Kolenbrander PE (2011). Multispecies communities: interspecies interactions influence growth on saliva as sole nutritional source.Int J Oral Sci.; Apr; 3(2):49-54.

Komiya Ito A, Ishihara K, Tomita S, Kato T, Yamada S (2010). Investigation of subgingival profile of periodontopathic bacteria using polymerase chain reaction.Bull Tokyo Dent Coll.; 51(3):139-44.

Konopka T, Rutkowska M, Hirnle L, Kopec W, Karolewska E (2003). The secretion of prostaglandin E2 and interleukin 1-beta in women with periodontal diseases and preterm low-birth-weight. Bulletin du Groupement International Pour la Recherche Scientifiqueen Stomatologie et al Odontologie; 45, 18–28.

Kornman KS and Loesche WJ (1980). The subgingival microbial flora during pregnancy. Journal of Periodontal Research; 15, 111– 122.

Kornman K, Page R, Tonetti M (1997).The host response to the microbial challenge in periodontitis: assembling the players. Periodontol 2000; 14: 33–53.

Kothiwale SV, Desai BR, Kothiwale VA, Gandhid M, Konin S (2014). Periodontal disease as a potential risk factor for low birth weight and reduced maternal haemoglobin levels.Oral Health Prev Dent.; 12(1):83-90.

Kramer MS (1987). Determinants of low birth weight, Methodological assessment and meta-analysis. Bulletin of the World Health Organization. ; 65(5):663-737.

Kramer MS, Séguin L, Lydon J, Goulet L (2000). Socio-economic disparities in pregnancy outcome: why do the poor fare so poorly? Paediatr Perinat Epidemiol.; 14:194–210.

Lamon DF (2003). Changes in vaginal flora after 2% clindamycin vaginal cream in women at high risk of preterm birth. British Journal of Obstetrics and Gynaecology; 110,788–789.

Lamont RF (1998). New approaches in the management of preterm labour of infective aetiology. Br J Obstet Gynaecol. Feb.; 105(2):134-7.

Langhoff-Roos J, Kesmodel U, Jacobsson B, Rasmussen S, Vogel I (2006). Spontaneous preterm delivery in primiparous women at low risk in Denmark: population based study. BMJ. ; 332, 937–939.

Lawn JE, Cousens S, Zupan J (2005). 4 Million neonatal deaths: when? Where? Why? Lancet;365, 891–900.

Leal MC, da Gama SG, Ratto KMN, Cunha CB (2004). Use of the modified Kotelchuck index in the evaluation of prenatal care and its relationship to maternal characteristics and birth weight in Rio de Janeiro, Brazil. Cad Saúde Pública.;20 (Suppl 1):63S-72S.

Lieff S, Boggess, AK, Murtha PA, Jared H, Madianos NP, Moss K, Beck J ,Offenbacher S (2004). The Oral Conditions and Pregnancy Study: Periodontal Status of a Cohort of Pregnant Women. J Periodontol;75(1): 116-126.

Leitich H, Bodner-Adler B, Brunbauer M, Kaider A, Egarter C, Husslein P (2003a). Bacterial vaginosis as a risk factor for preterm delivery: a meta-analysis. American Journal of Obstetrics and Gynecology.; 189, 139–147.

Leitich H, Brunbaue M, Bodner-Adler B, Kaider A, Egarter C, Husslein P (2003b). Antibiotic treatment of bacterial vaginosis in pregnancy: a meta-analysis. American Journal of Obstetrics and Gynecology.; 188, 752–758.

Leon R, Silva N, Ovalle A, Chaparro A, Ahumada A, Gajardo M, Martinez M, Gamonal J (2007). Detection of Porphyromonas gingivalis in the amniotic fluid in pregnant women with a diagnosis of threatened premature labor. Journal of Periodontology; 78, 1249–1255.

Lin D, Moss K, Beck J D, Hefti A, Offenbacher S (2007). Persistently high levels of periodontal pathogens associated with preterm pregnancy outcome. Journal of Periodontology; 78, 833–841.

Lin D, Smith MA, Champagne C, Elter J, Beck J, Offenbacher S (2003). Porphyromonas gingivalis infection during pregnancy increases maternal tumor necrosis factor alpha, suppresses maternal interleukin-10, and enhances fetal growth restriction and resorption in mice. InfectImmun; 71(9):5156-62.

Loe H and Silness J (1963). Periodontal Disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand; 21: 533–51.

Loe H and Silness J (1966). Periodontal disease in pregnancy. 2. Prevalence and Severity. Acta Odontol Scand; 21:533-549.

LoescheWJ (1997). Association of the oral flora with important medical diseases.Current Opinion in Periodontology; 4:21-8.

Loesche WJ (1993). Bacterial mediators in periodontal disease.Clin Infect Dis. Jun; 16 Suppl 4:S203-10.

Loesche WJ (1979). Clinical and microbiological aspects of chemotherapeutic agents used according to the specific plaque hypothesis. J Dent Res.; 58:2404-2412.

LoescheWJ (1999).The antimicrobial treatment of periodontal disease: changing the treatment paradigm.Critical reviews in oral biology and medicine: an official publication of the American Association of Oral Biologists.; 10(3):245-75.

Loesche WJ (1987). The bacterial etiology of periodontal disease: The specific plaque hypothesis. In: Clinical dentistry. Clark JW, editor. Ch. 12. Hagerstown, MD: Harper & Row Publishers, pp. 1-11.

Loesche WJ (1986). The identification of bacteria associated with periodontal disease and dental caries by enzymatic methods. Oral Microbiol Immunol; 1:65-72.

Loesche WJ, Bretz WA, Kerschensteiner D, Stoll J, Socransky SS, Hujoel PP, et al (1990a). Development of a diagnostic test for anaerobic periodontal infections based on plaque hydrolysis of Benzoyl-DL-Arginine-Naphthylamide. J Clin Microbiol; 28:1551-1559.

Loesche WJ, Bretz WA, Lopatin DE, Stoll J, Rau CF, Hillenburg KC, et al (1990b). Multi-center clinical evaluation of a chairside method for detecting certain periodontopathic bacteria in periodontal disease.J Periodontol; 61:189-196.

LoescheW J and GiordanoJ R (1997). Disease. Compendium of continuing education in dentistry (Jamesburg, N.J.: 1995); 18(3):221-6, 228-30.

Loesche WJ, Giordano J, Hujoel PP (1990c). The utility of the BANA test for monitoring anaerobic infections due to spirochetes (Treponema denticola) in periodontal disease. J Dent Res.; 69:1696-702.

Loesche WJ and Grossman NS (2001). Periodontal disease as a specific, albeit chronic infection: Diagnosis and Treatment. Clinical Microbiology Reviews; 14: 727-752.

Loesche W J, Kazor C E, Taylor G W (1997).The optimization of the BANA test as a screening instrument for gingivitis among subjects seeking dental treatment.Journal of clinical periodontology. ; 24(10):718-26.

Loesche WI and Lopatin DE (1998). Interactions between periodontal disease, medical diseases and immunity in the older individual. Periodontol 2000. ; 16:80-105.

Loesche WJ, Lopatin DE, Giordano J, Alcoforado G, Hujoel PP (1992). Comparison of the Benzoyl-DL-Arginine-Naphthylamide (BANA) test, DNA probes, and immunological reagents for ability to detect anaerobic periodontal infections due to Porphyromonas gingivalis, Treponema denticola, and Bacteroides forsythus.J Clin Microbiol. ; 30:427-433.

Loesche WJ, Syed SA, Stoll J (1987). Trypsin-like activity in subgingival plaque. A diagnostic marker for spirochetes and periodontal disease? J Periodontol; 58:266-273.

Loesche W J, Taylor G, Giordano J R, Hutchinson R, Rau C F, Chen YM, Schork M A (1997). A logistic regression model for decision to perform access surgery. Journal of clinical periodontology; 24(3):171-9.

Lopez, NJ, Da Silva I, Ipinza J, Gutierrez J (2005). Periodontal therapy reduces the rate of preterm low birth weight in women with pregnancy-associated gingivitis. Journal of Periodontology; 76, 2144–2153.

Lopez NJ, Smith PC, Gutierrez J (2002) .Higher risk of preterm birth and low birth weight in women with periodontal disease. J Dent Res.; 81(1):58-63.

Lopez N J, Smith P C, Gutierrez J (2002b). Periodontal therapy may reduce the risk of preterm low birth weight in women with periodontal disease: a randomized controlled trial. Journal of Periodontology.; 73,911–924.

Louro PM, Fiori HH, Filho PL, Steibel J, Fiori RM. (2001). Periodontal disease in pregnancy and low birth weight. J Pediatr (Rio J); 77(1):23-8.

Lovegrove J (2004). Dental plaque revisited: bacteria associated with periodontal disease. J N Z Soc Periodontol. ; (87):7-21.

MacDorman MF, Callaghan WM, Mathews T J, Hoyert D L, Kochanek KD (2007) .Trends in preterm-related infant mortality by race and ethnicity, United States,1999–2004. International Journal of Health Services; 37, 635–641.

Madianos PN, Lieff S, Murtha AP, et al. (2001). Maternal periodontitis and prematurity, II: maternal infection and fetal exposure. Ann Periodontol.; 6(1):175-82.

Mahulja-Stamenkovic V, Beleznay O, Stamenkovic M, Samardzija R, Makis J (1993). IgG, IgM and IgA umbilical blood concentrations in normal singleton vaginal deliveries with stinking amniotic fluid or intrapartal maternal febrility 4or 5 38 degrees C or duration of delivery 412 h. Zentralblatt fu¨r Gynakologie; 115, 33–35.

Manau C, Echeverria A, Agueda A, Guerrero A, Echeverria JJ (2008). Periodontal disease definition may determine the association between periodontitis and pregnancy outcomes. Journal of Clinical Periodontology; 35: 385–397.

Marsh P and Martin M. (1992). Oral Microbiology. London, New York, Tokyo, Melbourne, Madras: Chapman & Hall: pp167-196.

Marshall C and Rossman G (1995). Designing Qualitative Research. 2nd Edition. London: SAGE Publications.

Martin JA, Hamilton BE, Sutton PD, Ventura SJ, Menacker F,Kirmeyer S, et al (2007). Births: final data for 2005. Natl Vital Stat Rep.; 56:1–103.

Matteson SR, Joseph LP, Bottomley W, et al (1991).The report of the panel to develop radiographic selection criteria for dental patients. Gen Dent.; 39(4):264-70.

Mattila KJ, Valtonen WV, Nieminen MS, Huttunen IK (1995). Dental infection and the risk of new coronary events: prospective study of patients with documented coronary artery disease. Clin Infect Dis; 20:588-592.

Mattsby-Baltzer I, Platz-Christensen J J,Hosseini N, Rosen P (1998) . IL-1beta,IL-6, TNF alpha, fetal fibronectin, and endotoxin in the lower genital tract of pregnant women with bacterial vaginosis. Acta Obstetricia et Gynecologica Scandinavica; 77, 701–706.

McCormick M (2003). The contribution of low birth weight to infant mortality and childhood morbidity. N Engl J Med.; 1985; 312: 82–90.

McDonald H, Brocklehurst P, Parsons J (2005). Antibiotics for treating bacterial vaginosis in pregnancy. The Cochrane Database of Systemic Reviews; Issue 1. Art. No.: CD000262.

McDonald HM, O'Loughlin, JA, Vigneswaran R, Jolley PT, Harvey JA, Bof A, McDonald PJ (1997). Impact of metronidazole therapy on preterm birth in women with bacterial vaginosis flora (Gardnerella vaginalis): a randomised, placebo controlled trial. British Journal of Obsteterics and Gynaecology; 104, 1391–1397.

Menon R and Fortunato SJ (2007). Infection and the role of inflammation in preterm premature rupture of the membranes. Best Practice and Research Clinical Obsteterics and Gynaecology. ; 21, 467–478.

Michalowicz BS, Hodges JS, DiAngelis AJ, Lupo VR, Novak MJ, Ferguson JE, Buchanan W, Bofill J, Papapanou PN, Mitchell DA, Matseoane S, Tschida PA (2006) . OPT Study: Treatment of periodontal disease and the risk of preterm birth.N Engl J Med, 355:1885-1894.

Miller WD (1891). The human mouth as a focus of infection. Dent Cosmos; 33: 689-713.

Minkoff H. (1983) Prematurity: infection as an etiologic factor. Obstet Gynecol.; 62: 137–44.

Mitchell-Lewis D, Engebretson SP, Chen J, et al (2001). Periodontal infections and pre-term birth: early findings from a cohort of young minority women in New York. Eur J Oral Sci.; Feb; 109(1):34-9.

Miyazaki H, Pilot T, Leclercq MH, Barmes DE (1991). Profiles of periodontal conditions in adults measured by CPITN.Int Dent J, 41:74–80.

Miyazaki H, Yamashita Y, Goto-Kimura K, et al (1991). Periodontal condition of pregnant women assessed by CPITN. J Clin Periodontol.; Nov; 18(10):751-4

Mokeem SA, Molla GN, Al-Jewair TS (2004) .The prevalence and relationship between periodontal disease and pre-term low birth weight infants at King Khalid University Hospital in Riyadh, Saudi Arabia. J Contemp Dent Pract; 5(2):40-56.

Moller BR, Kristiansen FV, Thorsen P, Frost L, Mogensen SC (1995). Sterility of the uterine cavity. Acta Obstetricia et Gynecologica Scandinavica.; 74, 216–219.

Moore S, Ide M, Coward PY, et al (2004). A prospective study to investigate the relationship between periodontal disease and adverse pregnancy outcome. Br Dent J.; 197:251-8; discussion 247.

Moore S, Randhawa M, Ide M (2005) .A case-control study to investigate an association between adverse pregnancy outcome and periodontal disease. J Clin Periodontol.; 32(1):1-5.

Morales WJ, Schorr S, Albritton J (1994). Effect of metronidazole in patients with preterm birth in preceding pregnancy and bacterial vaginosis: a placebo-controlled, double-blind study. American Journal of Obstetrics and Gynecology; 171, 345-349.

Morgan M, Crall J, Goldenberg R, and Schulkin J (2009). Oral health during pregnancy. J Matern Fetal Neonatal Med.; Sep; 22 (9):733-9.

Moutquin JM (2003). Classification and heterogeneity of preterm birth. British Journal of Obstetrics and Gynaecology.; 110 (Suppl. 20), 30–33.

Mukhopadhaya N and Arulkumaran S (2007). Reproductive outcomes after in-vitro fertilization. Current Opinion in Obstetrics and Gynecology.; 19, 113–119.

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

Nishihara T and Koneke T (2004). Microbial aetiology of periodontitis. Periodontology 2000; 36:14-26.

Noack B, Klingenberg J, Weigelt J, Hoffmann T (2005). Periodontal status and preterm low birth weight: a case control study. Journal of Periodontal Research; 40, 339–345.

Nordstrom ML, Cnattingius S (1996). Effects on birthweights of maternal education, socio-economic status, and work-related characteristics. Scand J Soc Med.; Mar; 24(1):55-61.

Offenbacher S, Barros S, Beck J (2008). Rethinking Periodontal Inflammation. Journal of Periodontology.; Vol. 79, No. 8s, Pages 1577-1584,

Offenbacher S and Beck J (2007). Has Periodontal Treatment Failed to Reduce Adverse Pregnancy Outcomes? The Answer May Be Premature. Journal of Periodontology; 78:2, 195-197.

Offenbacher S, Beck JD, Jared HL, Mauriello S M, Mendoza L C, Couper D J, Stewart DD, Murtha AP et al (2009). Effects of Periodontal Therapy on Rate of Preterm Delivery. Obstetrics & Gynecology.; 114:3, 551-559.

Offenbacher S, Boggess KA, Murtha AP, et al (2006).Progressive periodontal disease and risk of very preterm delivery.Obstet Gynecol.; 107(1): 29-36.

Offenbacher S, Jared HL, O'Reilly PG, Wells SR, Salvi GE, Lawrence HP et al (1998).Potential pathogenic mechanisms of periodontitis associated pregnancy complications. Annals of Periodontology; 3:233-250.

Offenbacher S, Katz V, Fertik G, et al (1996). Periodontal infection as a possible risk factor for preterm low birth weight. J Periodontol; 67(supplement 10):1103-13.

Offenbacher S, Lieff S, Boggess KA, Murtha AP, Madianos PN, Champagne CME, Mckaig RG, Jared HL, Mauriello SM, Auten RL Jr, Herbert WNP, Beck JD (2001). Maternal periodontitis and prematurity. Part I: Obstetric outcome of prematurity and growth restriction. Ann Periodontol, 6:164–174.

Offenbacher S, Lin D, Strauss R, McKaig R, Irving J, Barros SP, Moss K, Barrow DA, Hefti A, Beck JD (2006). Effects of periodontal therapy during pregnancy on periodontal status, biologic parameters, and pregnancy outcomes: a pilot study.J Periodontol; 77(12):2011-24.

Offenbacher S, Madianos PN, Suttle M (1999). Elevated human IgM suggests in utero exposure to periodontal pathogens. J Dent Res.; 78:2191.

Offenbacher S, Riche EL, Rabbors SP, Bobetsis YA, Lin D, BeckJD (2005). Effects of maternal Campylobacter rectus infection on murine placenta, fetal and neonatal survival, and brain development. J Periodontol; 76(supplement 11):2133-43.

Oittinen J, Kurki T, Kekki M, Kuusisto M, Pussinen P, Vilkuna-Rautiainen T, Nieminen A, Asikainen S, Paavonen J (2005). Periodontal disease and bacterial vaginosis increase the risk for adverse pregnancy outcome. Infect Dis Obstet Gynecol.; 13(4):213-216.

Organization for Economic Co-operation and Development (OECD) (2001). OECD Health Data 99: A Comparative Analysis of 29 Countries. OECD; Paris, France:

Paige DM, Augustyn M, Adih WK, et al (1998). Bacterial vaginosis and preterm birth: a comprehensive review of the literature. J Nurse Midwifery.; Mar-Apr; 43(2):83-9. Review.

Page RC and Kornman KS (1997). The pathogenesis of human periodontitis: An introduction. Periodontol 2000; 14:9-11.

Paquette DW (2002) .The periodontal infection-systemic disease link: a review of the truth or myth. J Int Acad Periodontol.; 4: 101–19.

Pararas M, Skevaki C, Kafetzis D (2006). Preterm birth due to maternal infection: Causative pathogens and modes of prevention. Eur J Clin Microbiol Infect Dis. Sep; 25 (9):562-9.

Perioscan, Oral-B Laboratories Inc., Redwood City, CA.

Persson R, Hitti J, Verhelst R, Vaneechoutte M, Persson R, Hirschi R, Weibel M, Rothen M, Temmerman M, Paul K, Eschenbach D (2009).The vaginal microflora in relation to gingivitis. BMC Infect Dis. Jan 22; 9:6.

Philstrom BL, Lowenguth RA, et al (1995). Evaluation of periodontal treatments using controlled-release tetracycline fibers: clinical response. Periodontol; 66:692-699.

Philstrom BL, Michalowixz BS, Johnson NW (2005) .Periodontal Diseases. Lancet; 366(9499):1809-20.

Pitiphat W, Joshipura K J, Gillman M W, Williams P L, Douglass C W, Rich-Edwards J W (2008). Maternal periodontitis and adverse pregnancy outcomes. Community Dentistry and Oral Epidemiology, 36: 3–11.

Platz-Christensen JJ, Mattsby-Baltzer I, Thomsen P, Wiqvist N (1993). Endotoxin and interleukin-1 alpha in the cervical mucus and vaginal fluid of pregnant women with bacterial vaginosis. ; 169, 1161–1166.

Precht DH, Andersen PK, Olsen J (2007). Severe life events and impaired fetal growth: a nation-wide study with complete follow-up. Acta Obstetricia et Gynecologica Scandinavica.; 86, 266–275.

Preshaw PM (2009). Definitions of periodontal disease in research.J Clin Periodontol.; Jan; 36 (1):1-9

Preshaw PM, Hefti AF, Jepsen S, Etienne D, Walker C, Bradshaw MH. (2004). Subantimicrobial dose doxycycline as adjunctive treatment for periodontitis: a review. J Clin Periodontol.; 31:697-707.

Radnai M, Gorzo I, Nagy E, Urban E, Novak T, Pal A (2004). A possible association between preterm birth and early periodontitis: a pilot study. J Clin Periodontol; 31(9):736-41.

Rajapakse PS, Nagarathne M, Chandrasekra KB, Dasanayake AP (2005). Periodontal disease and prematurity among non-smoking Sri Lankan women. J Dent Res.; 84(3):274-7.

Raju TN (2008). Late-preterm births: challenges and opportunities. Pediatrics; 121(2); 402-3.

Ramfjord SP (1959). Indices for prevalence and incidence of periodontal disease. J Periodontol; 30: 51–59.

Ramfjord SP, Emslie RD, Greene JC, Held AJ, Waerhaug J (1968). Epidemiological studies of periodontal diseases. Amer. J. Pub. Health.; 58:1713-22.

Reddy BV, Tanneeru S, Chava VK (2014). The effect of phase-I periodontal therapy on pregnancy outcome in chronic periodontitis patients.J Obstet Gynaecol.; Jan; 34(1):29-32.

Reddy UM, Wapner RJ, Rebar RW, Tasca RJ (2007). Infertility assisted reproductive technology, and adverse pregnancy outcomes: executive summary of a National Institute of Child Health and Human Development workshop. Obstetrics and Gynecology. ; 109, 967–977.

Redman CW, Sacks GP, Sargent IL (1999). Preeclampsia: an excessive maternal inflammatory response to pregnancy. Am J Obstet Gynecol.; 180: 499–506.

Romero BC, Chiquito CS, Elejalde LE, Bernardoni CB (2002). Relationship between periodontal disease in pregnant women and the nutritional condition of their newborns. J Periodontol; 73(10):1177-83.

Romero R, Chaiworapongsa T, Kuivaniemi H, Tromp G (2004). Bacterial vaginosis, the inflammatory response and the risk of preterm birth: a role for genetic epidemiology in the prevention of preterm birth. American Journal of Obstetrics and Gynecology; 190, 1509 1519.

Romero R, Espinoza J, Kusanovic JP, Gotsch F, Hassan S, Erez O, Chaiworapongsa T, Mazor M (2006). The preterm parturition syndrome. British Journal of Obstetrics and Gynaecology: An International Journal of Obstetrics and Gynaecology.; 113, 17–42.

Romero R, Kadar N, Hobbins JC, Duff GW (1987a). Infection and labor: the detection of endotoxin in amniotic fluid. American Journal of Obstetrics and Gynecology; 157,815–819.

Romero R, Lafreniere D, Duff GW, Kadar N, Durum S, Hobbins JC (1987b) . Failure of endotoxin to cross the chorioamniotic membranes in vitro. American Journal of Perinatology; 4, 360–362.

Rosebury T (1962). Microorganisms indigenous to man. New York: McGraw-Hill, pp. 1-380.

Rosenstein IJ, Morgan D J, Lamont RF,Sheehan M, Dore CJ, Hay PE, Taylor-Robinson D (2000). Effect of intravaginal clindamycin cream on pregnancy outcome and on abnormal vaginal microbial flora of pregnant women. Infectious Diseases in Obstetrics and Gynecology; 8, 158-165.

Russell A L (1966). World epidemiology and oral health. Environmental variables in oral disease. (Kreshover S J & McClure F J, eds.) Washington. DC: American Association for the Advancement of Science; 21-39.

Sant'Ana AC, Campos MR, Selma CR, Maria LR, Sebastião LA, Passanezi E (2011). Periodontal treatment during pregnancy decreases the rate of adverse pregnancy outcome: a controlled clinical trial. Journal of Applied Oral Science; 19(2), 130-136.

Sadatmansouri S, Sedighpoor N, Aghaloo M (2006). Effect of periodontal treatment phase I on birth term and birth weight. J Indian Soc Pedod Prev Dent.; 24:23–6.

Saigal S, Burrows E, Stoskopf BL, Rosenbaum PL, Streiner D (2000a). Impact of extreme prematurity on families of adolescent children. Journal of Pediatrics. ; 137,701–706.

Saigal S, Hoult LA, Streiner DL, Stoskopf BL, Rosenbaum PL (2000b). School difficulties at adolescence in a regional cohort of children who were extremely low birth weight. Pediatrics; 105, 325–331.

Santos-Pereira SA, Giraldo PC, Saba-Chujfi E et al. (2007) Chronic periodontitis and pre-term labour in Brazilian pregnant women: an association to be analysed. J Clin Periodontol.; 34(3):208-13.

Saunders, Mark NK, Lewis P, Thornhill A (2000). Research Methods for Business Students. 2nd Edition:Financial Times/Prentice Hall, Harlow, England.

Scannapieco FA (2005). Systemic effects of periodontal diseases. Dent ClinNorth Am.; 49:533-550.

Scannapieco FA, Bush RB, Paju S (2003). Periodontal disease as a risk factor for adverse pregnancy outcomes. A systematic review. Ann Periodontol.; 8:70–8.

Schmidt EF, Bretz WA, Hutchinson RA, Loesche WJ (1988). Correlation of the Hydrolysis of Benzoyl-Arginine Naphthylamide (BANA) by Plaque with Clinical Parameters and Subgingival Levels of Spirochetes in Periodontal Patients. J Dent Res; 67:1505-09.

Silva AAM, Barbieri MA, Gomes UA, Bettiol H (1998).Trends of low birth weight: a comparison of two birth cohorts separated by a 15 year interval in Ribeirão Preto, Brazil. Bull World Health Organ; 76:73-84.

Schenkein HA (2002). Finding genetic risk factors for periodontal diseases: Is the climb worth the view? Periodontol 2000; 30:79-90.

Schieve LA, Cohen B, Nannini A, Ferre C, Reynolds MA, Zhang Z, Jeng G,Macaluso M, Wright VC (2007). A population-based study of maternal and perinatal outcomes associated with assisted reproductive technology in Massachusetts. Maternal and Child Health Journal.; 11,517–525.

Schneider H, Husslein P, Schneider KTM (2006). Die Geburtshilfe. In: Obstetrics, 3rd edition, p. 1136. Springer Medizin Verlag, Heidelberg.

Sconyers JR, Crawford JJ, Moriarty JD (1973). Relationship of bacteraemia to toothbrushing in patients with periodontitis. J Am Dent Assoc.; 87: 616–22.

Sembene M, Moreau JC, Mbaye MM, et al (2000). Periodontal infection in pregnant women and low birth weight babies. Odontostomatol Trop.; 23(89):19-22.

Seymour RA and Steele JG (1998). Is there a link between periodontal disease and coronary heart disease? Dent J.; Jan 10; 184(1):33-8.

Shennan AH and Bewley S (2006). Why should preterm births be rising? BMJ.;

332, 924–925.

Silness P and Löe H (1964). Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand. 22: 121-124.

Simhan HN and Canavan TP (2005). Preterm premature rupture of membranes: diagnosis, evaluation and management strategies. British Journal of Obstetrics and Gynaecology.; 112 (Suppl. 1), 32–37.

Sjoberg I and Hakansson S (1991). Endotoxin in vaginal fluid of women with bacterial vaginosis. Obstetrics and Gynecology; 77, 265–266.

Slade GD, Ghezzi EM, Heiss G, Beck JD, Riche E, Offenbach (2003) . Relationship between periodontal disease and Creative protein among adults in the atherosclerosis risk in Communities study. Arch Intern Med.; 163(10):1172-9.

Smith LK, Draper ES, Manktelow BN, Field DJ (2009). Socioeconomic inequalities in survival and provision of neonatal care: population based study of very preterm infants. BMJ; 339:b4702.

Socransky SS and Haffajee AD (2005). Periodontal microbial ecology. Periodontology 2000; 38:135-187.

Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. (1998). Microbial complexes in subgingival plaque. J Clin Periodontol.; 25(2):134-44.

Sooriyamoorthy M and Gower DB (1989). Hormonal influences of gingival tissue relationship to periodontal disease. J Clin Periodontol.; 16:201-208.

Spahr A, Klein E, Khuseyinova N, et al (2006). Periodontal infections and coronary heart disease: role of periodontal bacteria and importance of total pathogen burden in the Coronary Event and Periodontal Disease (CORODONT) study. Arch Intern Med.; 166(5):554-9.

Srinivas S, Sammel M, Stamilio DM, Clothier B, Jeffcoat M, Parry S, Macones G, Elovitz M, Metlay J (2009) . Periodontal disease and adverse pregnancy outcomes: is there an association? Am J Obstet Gynecol.; May; 200(5):497.e1-8.

Stamilio DM, Chang JJ, Macones GA (2007). Periodontal disease and preterm birth: do the data have enough teeth to recommend screening and preventive treatment? Am J Obstet Gynecol.; 196:93–4.

Stavros P and Ziyah M et al (2003). The impact of preterm birth on hospital inpatient admissions and costs during the first 5 years of life. Pediatrics; 112:1290-1297.

Strebel R and Bucher HU (1994). Improved chance of survival for very small premature infants in Switzerland. Schweizerische Medizinische Wochenschrift.; 124, 1653–1659.

Sunita Bamanikar and Liew Kok Kee (2013).Knowledge, Attitude and Practice of Oral and Dental Healthcare in Pregnant Women. Oman Med J.; Jul; 28(4): 288–291.

Swartz L and Roux N (2004). A study of local government HIV/AIDS projects in South Africa. Journal of social aspects of HIV/AIDS.; 1(2):99-106.

Taani DQ, Habashneh R, Hammad MM, Batieha A (2003). The periodontal status of pregnant women and its relationship with socio-demographic and clinical variables. J Oral Rehabil;30(4): 440-445.

Tarannum F and Faizuddin M (2007). Effect of periodontal therapy on pregnancy outcome in women affected by periodontitis. J Periodontol; 78:2095–103.

Tatakis D and Kumar P (2005). Etiology and pathogenesis of periodontal diseases. Dent Clin North Am.;Jul; 49(3):491-516.

Taylor HG, Klein N, Minich NM, Hack M (2001). Long-term family outcomes for children with very low birth weights. Archives of Pediatrics and Adolescent Medicine.; 155, 155–161.

Terry SD, Jones JE, Brown HR (1994). Dental care experiences of people living with HIV/AIDS in Aotearoa New Zealand. New Zealand Dental Journal; 90:49 -55.

Tilakarantne A, Soory M, Ranasinghe AW, Crea SMX, Ekanayake SL, De Silva M (2000). Periodontal disease status during pregnancy and 3 months postpartum, in a rural population of Sri-Lankan women. J Clin Periodontol.; 27:787-792.

Thomas KM, Jared HL, Boggess K, Lee J, Moos M, Wilder RS (2008). Prenatal Care Providers' Oral Health and Pregnancy Knowledge Behaviors. J Dent Res.; 87(Spec Iss A).

Thomas JG and Nakaishi LA (2006) .Managing the complexity of a Dynamic biofilm. J Am Dent Assoc.; 137(supp):10S- 15S.

Tracy SK, Tracy MB, Dean J, Laws P, Sullivan E (2007). Spontaneous preterm birth of live born infants in women at low risk in Australia over 10 years: a population based study. British Journal of Obstetrics and Gynaecology.; 114, 731–735.

Tran SD. and Rudney JD (1999). Improved Multiplex PCR Using Conserved and Species-Specific 16S rRNA Gene Primers for Simultaneous detection of Actinobacillus actinomycetemcomitans, Bacteroides forsythus, and Porphyromonas gingivalis. Journal of Clinical Microbilogy; 37(11): 3504-3508.

Ugwumadu A, Manyonda I, Reid F, Hay P (2003). Effect of early oral clindamycin on late miscarriage and preterm delivery in asymptomatic women with abnormal vaginal flora and bacterial vaginosis: a randomised controlled trial. Lancet.; 361, 983–988.

Vergnes JN and Sixou M (2007). Preterm low birth weight and maternal periodontal status: a meta-analysis. Am J Obstet Gynecol.; 196:135–47.

Vettore MV, Lamarcagde A, Leao AT, Thomaz FB, Sheiham A, Leal MC (2006). Periodontal infection and adverse pregnancy outcomes: a systematic review of epidemiological studies. Cadernos de Saude Publica.; 22, 2041–2053.

von Dadelszen P and Magee LA (2002). Could an infectious trigger explain the differential maternal response to the shared placental pathology of preeclampsia and normotensive intrauterine growth restriction? Acta Obstet Gynecol Scand.; 81: 642–8.

Walker BR, McConnachie A, Noon JP, et al (1998). Contribution of parental blood pressures to association between low birth weight and adult high blood pressure: cross sectional study. BMJ; Mar 14; 316(7134):834-7.

Watson M, Bretz W, Loesche W (1994). Presence of Treponema denticola and Porphyromonas gingivalis in Children Correlated with Periodontal Disease of Their Parents. J Dent Res.; 73(10):1636-1640, October.

Wilder R, Robinson C, Jared HL, Lieff S, Boggess K (2007). Obstetrician's knowledge and practice behaviors concerning periodontal health and preterm delivery and low birth weight. J Dent Hyg; 81(4):81.

Williams CE, Davenport ES, Sterne JA, Sivapathasundaram V, Fearne JM, Curtis MA (2000).Mechanisms of risk in preterm low-birthweight infants. Periodontology 2000; 23:142-150.

Wimmer G and Pihlstrom B (2008). A critical assessment of adverse pregnancy outcome and periodontal disease. J Clin Periodontol.; 35(Suppl. 8): 380–397.

Witkin SS, Vardhana S, Yih M, Doh K, Bongiovanni AM, Gerber S (2003). Polymorphism in intron 2 of the fetal interleukin-1 receptor antagonist genotype influences mid-trimester amniotic fluid concentrations of interleukin-1beta and interleukin-1 receptor antagonist and pregnancy outcome. Am J Obstet Gynecol.; 2003 Nov; 189(5):1413-7.

WHO (2005). ICD-10 International Statistical Classification of Diseases and Related Health Problems, 2nd edition. WHO; Geneva.

WHO (2006). Neonatal and Perinatal Mortality: Country, Regional and Global Estimates. Geneva: World Health Organization; Geneva.

World Health Organization (1987). Oral health surveys: Basic methods. Geneva: World Health Organization.; Geneva.

WHO (2013). The 8th Global Conference on Health Promotion. WHO; Helsinki, Finland10–14 June.

WHO (2005). ICD-10 International Statistical Classification of Diseases and Related Health Problems, 2nd edition.WHO; Geneva

Xiong X, Buekens P, Fraser W, Beck J, Offenbacher S (2006). Periodontal disease and adverse pregnancy outcomes: a systematic review BJOG: An International Journal of Obstetrics & Gynaecology. ; Volume 113, Issue 2: 135–143.

Zachariasen RD (1993). The affect of elevated ovarian hormones on periodontal health: oral contraceptives and pregnancy. Women health; 20(2):21-30.

Yalcin F, Eskinazi E, Soydine M, Basegmez C, Issever H, Isik G, Berber L, Has R, Sabuncu H, Onan U (2002). The effect of sociocultural status on

Periodontal conditions in pregnancy. J Periodontol; 73(2):178-182.

Yeo A, Smith MA, Lin D, et al (2005). Campylobacter rectus mediates growth restriction in pregnant mice. J Periodontol; 76(4):551-7.

Yokoyama M, Hinode D, Yoshioka M, Fukui M, Tanabe S, Grenier D, Ito HO (2008). Relationship between Campylobacter rectus and periodontal status during pregnancy. Oral Microbiol Immunol.; 23(1):55-9.

APPENDICES

Appendix 1: Consent Form

INFORMED CONSENT TO PARTICIPATE IN STUDY

Dear…….

I am a doctoral student in the department of medical biosciences from the Faculty of Sciences, University of the Western Cape. I would like to ask you a few questions about yourself and do a clinical examination which will take about 15 to 20 minutes. The purpose of the research project is to check for gum disease in the mouths of pregnant women in order to establish whether the presence of gum disease in the mouths of pregnant women can be used as an indicator for pre-term delivery or delivery of babies with low birth weights.

The clinical procedure will entail the measurement of oral clinical indices for use in making a clinical diagnosis of pregnancy gingivitis and the removal of plaque samples from selected teeth for laboratory investigation. The clinical measurements and sample collection are non-invasive and safe and will be carried out with the utmost care to ensure the comfort of the patient. You will be identified with a code number and your name will not appear on any form all the information you give us about yourself will be strictly confidential. You are completely free to take part or not to take part in the study. If you decide that you do not want to be part of the study, this will not be held against you. If you would like to take part in the study, please sign the form below to allow us to proceed with the interview. If you would like to withdraw from the study at any point for any reason, please feel free to do so and no questions will be asked.

If you have any questions or queries or would like more information about the study please contact Dr M Turton on telephone number (033) 3946 486; fax (033) 3946487; e-mail 8403619 @ uwc.ac.za or after hours on (084 7760436).

Thank you for your cooperation

Yours sincerely

Dr Mervyn Turton.

I understand what the study is about and agree to participate in the study.

My consent is subject to the following:

- The results will in no way disclose the identity of the participants.
- The results can be used or published for benefits in the oral health field.

Name:..... Signature:.....

(In block letters)

Date:.....

Witness:.....

Appendix 2A: Data Capture Sheet: Part 1

Part 1

Data Capture Sheet of Clinical Examination and BANA Analysis

Date of examination: _____ **Site**: _____

Demographic

<table>
<tr><td>File number</td><td></td><td>Number of previous pregnancies</td><td colspan="4"></td></tr>
<tr><td>Name</td><td></td><td>Pregnancy stage</td><td colspan="4"></td></tr>
<tr><td rowspan="2">Age</td><td rowspan="2"></td><td rowspan="2">Race group</td><td>BLK</td><td>CLRD</td><td>ASIAN</td><td>WHITE</td></tr>
<tr><td>1</td><td>2</td><td>3</td><td>4</td></tr>
</table>

Clinical assessment

Smoking	No 1	Yes 2	Sometimes 3
History of adverse pregnancy outcomes	No 1	Yes 2	
Weight	Under 1	Moderate 2	Over 3
Alcohol	None 1	Daily 2	Weekly 3
Level of Education	Primary 1	Secondary 2	Tertiary 3

DMFT

Decayed	Missing	Filled	Total

Plaque index (PI)

	M	D	Tot
16			
21			
24			
36			
41			
44			
	Score		

Gingival Index (GI)

	M	D	Tot
16			
21			
24			
36			
41			
44			
	Score		

Probing Depth (PD

None 1	Mild 2	Moderate 3	Severe 4

Clinical attachment Loss (CAL)

None 1	Mild 2	Moderate 3	Severe 4

Calculus

None 1	Mild 2	Moderate 3	Severe 4

Oral Lesions

No	1
Yes	2

Periodontal Disease

None	1
Mild	2
Moderate	3
Severe	4

BANA Analysis

No	1
Yes	2

Appendix 2B: Data Capture Sheet Part 2

Part 2

Data Capture Sheet of Pregnancy Outcomes

Date of examination:…… **Site:** ……

Demographic

File number	
Mothers Name	
Age	

Pregnancy Outcomes

Birth weight (kg)		
Gestational Age (weeks)		
Weight for Gestational Age		
Gender of Baby	Female	Male
	1	2
AGPAR score		

Appendix 3: Approval of Research Study by UWC Human Subjects Ethics Committee

OFFICE OF THE DEAN
DEPARTMENT OF RESEARCH
DEVELOPMENT

Private Bag X17
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Telegraph: UNIBOL
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Fax: +27 21 959 3170
Website: www.uwc.ac.za

To Whom it May Concern

I hereby certify that the Senate Research Committee of the University of the Western Cape has approved the methodology and the ethics of the following research project by Prof C Africa (Medical Biosciences)

Research Project: The Effect of Maternal Oral Health Care on Pregnancy Outcomes

Registration no: 11/11

Prof
Manager: Research Development Office
University of the Western Cape

Appendix 4: Adverse pregnancy outcomes that have been linked to periodontal disease

Adverse pregnancy outcomes that have been linked to periodontal disease include:

- Preterm low
- birthweight
- Preterm birth
- Low birthweight
- Pre-eclampsia
- Miscarriage
- Stillbirth

(McCormick 2003; Bobetsis, 2006).

Appendix 5: List of MOU's

List of MOU's where research was undertaken

- Edendale
- Hlengisizwa
- Cato Manor
- Ntseleni
- Empangeni
- Mbalenhle
- Caluza
- Embo
- Eastboom

Appendix 6: Definitions

This study used terminology from the World Health Organization International Statistical Classification of Diseases and Related Health Problems to ensure consistent with universally accepted definitions (WHO, 2005).

1. Birth: Complete expulsion or extraction of foetuses X500 g or X25 cm (if weight or length is unavailable, 22 weeks' gestation are considered equal to 500 g) (WHO, 2005).

2. Live birth: Complete expulsion or extraction from its mother of a product of conception, irrespective of the duration of the pregnancy, which, after such separation, breathes or shows any other evidence of life, such as beating of the heart, pulsation of the umbilical cord, or definite movement of voluntary muscles, whether or not the umbilical cord has been cut or the placenta is attached; each product of such a birth is considered live born (WHO, 2005).

3. Still birth: Complete expulsion or extraction from its mother of a product of conception, of at least 22 weeks gestation or 500 g, which after separation did not show any signs of life (WHO, 2005).

4. Foetal death: Death before the complete expulsion or extraction from its mother of a product of conception, irrespective of the duration of pregnancy; the death is indicated by the fact that after such separation the foetus does not breathe or show any other evidence of life, such as beating of the heart, pulsation of the umbilical cord or definite movement of voluntary muscles (WHO, 2005).

5. Birth weight: First weight of infant obtained afterbirth (WHO, 2005).

6. Duration of gestation: Time from the first day of the last normal menstrual period (LMP) (WHO, 2005).

7. Gestational age: Gestational age is expressed in completed days or completed weeks after first day of LMP (WHO, 2005).

8. Perinatal period: Time that commences at 22 completed weeks of gestation (the time when birth weight is normally 500 g), and ends at one completed week after birth (WHO, 2005).

9. Perinatal mortality rate: Number of deaths of foetuses weighing at least 500 g (or, when birth weight is unavailable, after 22 completed weeks of gestation or with a crown–heel length of 25 cm or more), plus the number of early neonatal deaths per 1000 total births (WHO, 2005).

10. Neonatal death: Death of a live-born infant during the first 28 completed days of life. May be subdivided into early neonatal death, occurring during the first 7 days of life, and late neonatal death, occurring after the seventh day but before 28 completed days of life (WHO, 2005).

11. Pre-term birth: Birth under 37 week's, gestational age (Martin et al., 2007).

12. Late pre-term birth: Birth at 34-36 week's, gestational ages (Martin et al., 2007).

13. Very pre-term birth: birth under 32 week's, gestational age (Martin et al., 2007).

14. Extremely pre-term birth: birth under 28 week's, gestational age (Martin et al., 2007).

15. Low birth weight (LBW) i.e. birth weight under 2500 g (up to and including 2499 g); very low birth weight (VLBW) i.e. under 1500 g (up to and including 1499 g); extremely low birth weight (ELBW) i.e. under 1000 g (up to and including 999 g) (WHO, 2005).

16. Pre-term labour: Regular uterine contractions accompanied by cervical change at under 37 weeks gestation (Goldenberg et al., 2008).

17. Pre-term premature rupture of membranes (PPROM): Spontaneous rupture of the membranes as under 37 weeks gestation at least 1 hour before the onset of contractions (Goldenberg et al., 2008).

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