SUBGINGIVAL FUSOBACTERIUM NUCLEATUM IN MOTHERS WITH PRETERM LOW BIRTH WEIGHT INFANTS AT KIAMBU LEVEL 5 HOSPITAL AND ITS RELATIONSHIP TO PERIODONTAL DISEASE

DR. KYALE DAVID SUMBI - BDS (Nbi)

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DECLARATION OF ORIGINALITY

I **David Sumbi Kyale,** of the University of Nairobi, School of Dental Sciences, declare that this report titled, "**Subgingival Fusobacterium Nucleatum and Preterm Low Birth Weight among Postpartum Women at Kiambu Level 5 Hospital**" is my original work.

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SUPERVISORS APPROVAL

I, Dr. Kyale Sumbi David, hereby submit this research report with the approval of my supervisors:

Dr. Veronica W. Wangari. BDS (Nbi), MDS Periodontology (Nbi)

Department of Periodontology/ Community and Preventive Dentistry,

School of Dental Sciences.

University of Nairobi.

Signature: _____ Date: _____

Dr. Hudson Alumera. BDS (Nbi), MDS periodontology (Nbi)

Department of Periodontology/ Community and Preventive Dentistry,

School of Dental Sciences.

University of Nairobi.

Signature: _____ Date: _____

Prof. Loice W. Gathece. BDS., MPH., PhD. (Nbi)

Department of Periodontology/ Community and Preventive Dentistry,

School of Dental Sciences.

University of Nairobi.

Signature: _____ Date: _____

DEDICATION

I dedicate this thesis to my wife Mercy Sumbi, my son Mulumu Sumbi and to my parents, Mr Charles Kyale Kisumbi and Dr. Bernina Kyale Kisumbi.

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LIST OF ACRONYMS

CAL	Clinical attachment loss
CDC/AAP	Centre's for Disease Control and Prevention/ American Academy of Periodontology
DNA	Deoxyribonucleic acid
GI	Gingival index
KNH	Kenyatta National Hospital
LBW	Low birth weight
MDS	Masters of Dental Surgery
MPH	Masters of Public Health
NBW	Normal birth weight
NBI	Nairobi
PB	Preterm birth
PCR	Polymerase chain reaction
qPCR	Quantitative real-time PCR
PDL	Periodontal ligament
PLBW	Preterm Low Birth Weight
PPROM	Preterm premature rupture of membranes
PS	Plaque score
PT	Preterm birth
ROM	Rupture of membranes
SPSS	Statistical Package for Social Sciences
UoN	University of Nairobi
WHO	World Health Organization

DEFINITION OF TERMS

Clinical attachment loss: The distance between the cemento-enamel junction to the bottom of the periodontal pocket[1]

Dental plaque: Soft deposits that form the biofilm adhering to the tooth surface or other hard tissues in the oral cavity (including removable and fixed prostheses)[1].

Fusobacterium Nucleatum A gram negative anaerobic oral commensal and periodontal pathogen associated with a wide spectrum of human diseases

Gingivitis: Refers to gingival inflammation without loss of connective tissue attachment[1]**.**

Gingival recession: The distance between the cemento-enamel junction (CEJ) and the gingival margin[1].

Low birth weight: This is an infant weighing less than 2500g at birth[2]

Periodontitis: An inflammatory disease of the supporting tissues of the teeth. It is caused by various microorganisms and results in progressive destruction of the periodontal ligament and alveolar bone with pocket formation recession or both. (CDC/AAP 1997)

Preterm birth: This is the delivery of an infant after 23 weeks gestation but less than 37 weeks of gestation[2].

Probing pocket depth: The distance between the gingival margin and the bottom of the pocket to the nearest whole millimetre[1].

ABSTRACT

Background

Preterm birth (PB) and low birth weight (LBW) are some of the main contributors to morbidity and mortality in neonates. Risk factors for preterm birth and low birth weight (PLBW) are tobacco, maternal body-mass index, socioeconomic disparities, older age of giving birth, multiple pregnancies and infections including periodontal disease. *Fusobacterium Nucleatum* is among the periodontal pathogens that have been isolated in amniotic fluid and associated with PLBW. It is proposed that it may spread to the placenta during a transient bacteraemia following periodontal disease.

Main objective

To investigate the relationship between occurrence of subgingival *Fusobacterium nucleatum* DNA and PLBW infants in postpartum mothers at Kiambu Level 5 Hospital.

Study design

This was an unmatched case control study.

Materials and Methods

A screening form was used to exclude mothers who did not fit the inclusion criteria. Sociodemographic data was collected and filled in a questionnaire. Weight at birth, gestation age at birth of the new-born was retrieved from the participant's medical records and recorded in the clinical examination forms. Cases were mothers who delivered a new-born of less than 37 weeks gestation age (preterm), while controls were mothers who delivered a new-born of 37 weeks gestation age and above (term). Plaque score (Silness and Loe 1964), gingival index (Loe and Silness 1963), periodontal probing depth, and the level of clinical attachment was used to determine the periodontal status of the participants. Plaque samples were collected from the participants using sterilised size 30 endodontic paper points. Plaque

was collected from the Ramfjord teeth then each of the six specimens was then pooled in a micro centrifuge and stored at -20^{0}C. The samples were then transferred to the laboratory and stored at -80^{0}C until processing. Occurrence of DNA of *F.* nucleatum in the plaque samples was then measured using quantitative real time PCR. Data was analysed using IBM SPSS 20 and Microsoft excel software. Chi-square, Fisher's tests, Independent t-tests and ANOVA tests were used for analysis. Data was presented in the form of tables and figures. Ethical approval was sought from the UoN-KNH ethics review committee and clearance to conduct the research from Kiambu County Health Research and Development Unit.

Results

A total of 108 mothers were included in the study, 54 cases and 54 controls. The mothers' age ranged 16-40 with a mean of 25.42 years ($\pm$ 5.67 years SD). The gestation age of the new-borns ranged between 23 – 43 weeks with a mean of 36.23 ($\pm$ 4.44 SD). The weight of the new-borns ranged between 800 – 4100 grams with a mean of 2739.89 ($\pm$ 700.05 grams). A statistically significant association was found between gestation age and term and preterm birth (p= <0.001), this could be due to the fact that term infants had a much higher mean gestational age than the preterm infants. Majority (64.8%) of the participants had never visited a dentist. The education level of the mothers when compared with plaque scores was found to be statistically significant (p= 0.010), in that the plaque scores got progressively lower as the education level increased. The prevalence of *F. nucleatum* among the participants was 85%. The prevalence was higher among the cases 89% than the controls 82%. Cases had more than double the concentration of Fusobacterium nucleatum (160.67 copies/ul) as compared to the controls (73.41 copies/ul), however this was not statistically significant (p= 0.063. Cases were more likely (odds ratio [OR] 1.81, 95% CI 0.61 to 5.42) of being exposed to *F. nucleatum* than controls, though this was not statistically significant. Pre-term delivery mothers (cases) had a higher mean gingival index of (1.29 $\pm$ 0.31),

compared with term delivery mothers (controls) (1.18 $\pm$ 0.24) and this was found to be statistically significant (p= 0.043). Using the CDC/AAP case definition 2012 for periodontitis 20 (18.5%) participants had severe periodontitis, 48 (44.4%) had moderate periodontitis, 16 (14.8%) had mild periodontitis while 24 (22.2%) did not have periodontitis. Cases were more likely (odds ratio [OR] 1.5, 95% CI 0.26 to 1.63) of being exposed to periodontal disease than those controls, this finding was however not statistically significant.

Conclusion

Cases had higher plaque scores, gingival index and *F. nucleatum* DNA concentration than controls. Twenty two percent of the participants had no periodontitis while 44% had moderate periodontitis using CDC/AAP 2012 criteria. Cases were more likely to be exposed to *F. nucleatum* and varying severities of periodontitis than controls.

Recommendations

During pregnancy, all women should have routine dental check-ups and periodontal care where necessary. Only focussing on those with disease yet *F. nucleatum* was found even in healthy individuals may not reap benefits all round.

CHAPTER ONE

1.0 INTRODUCTION

1.1 FUSOBACTERIUM NUCLEATUM

F. nucleatum is a periodontal pathogen, it is a gram-negative rod found in both periodontal health and disease. This periodontal pathogen has been isolated in chorioamniotic membranes, placentas and amniotic fluids in premature deliveries. Association between *F. nucleatum* and preterm birth was tested in an experiment with mice. *F. nucleatum* invaded the placenta and eventually the amniotic fluid, this led to premature delivery and still births. The model paralleled human infection and was the first evidence of the invasive nature of *F. nucleatum* and its ability to cause negative birth outcomes[3]. In humans *F. nucleatum* isolated in infected amniotic fluid was shown to have come from the oral cavity, this showed the ability of this periodontal pathogen to invade epithelial and endothelial tissues[4]. Another study found an association between detection of bacterial DNA including *F. nucleatum* and previous history of miscarriages, intrauterine death and preterm birth[5].

Periodontal disease is associated with preterm low birth weight (PLBW). *F. nucleatum* is a bacterium that is associated with periodontitis and it has also been associated with PLBW. Hence this study aimed to examine the existence and nature of the relationship between *F. nucleatum* and PLBW. Results of this study could be used to formulate prevention strategies so as to reduce the incidence of PLBW which is high locally compared with the rest of the world.

1.2 PRETERM BIRTH AND LOW BIRTH WEIGHT

Preterm birth is a delivery occurring at less than 37 weeks of gestation but after 23 weeks, a weight of less than 2500 grams is low birth weight. PLBW is one of the major factors associated with morbidity and mortality of neonates. It is estimated that 9.6% of all births are preterm

amounting to 12.9 million births globally. The rates are highest in Africa at 11.6%. Europe at 6.2% of births has the lowest rates[6]. In Eastern Africa the rate is estimated to be 14.3%, while in Kenya it has been reported to be 12%. Of the estimated 3.1 million neonatal deaths that occurred globally in 2010, 1 million (35%) were directly related to preterm births[6]. It is postulated that preterm birth has several causes including infections, uteroplacental ischemia and uterine over distention, however half the causes of preterm birth are unknown. Preterm birth risk factors include multiple gestation, maternal age and parity, previous preterm birth, inter-pregnancy interval, antenatal care attendance, maternal nutritional status, antepartum haemorrhage, pregnancy induced hypertension, maternal infections, foetal gender, and congenital anomalies among others. Many of the risk factors result in amplification of the inflammatory and infection pathway, which could explain why preterm birth is associated with multiple factors. Intrauterine infections account for 25-40% of all preterm births[7]. Periodontal disease was first proposed to a be risk factor for preterm birth in 1996[8]. Periodontal disease is a broad term used to describe a chronic inflammatory disease that destroys the tooth supporting structures. In Kenya gingivitis has a prevalence of up to 90% whereas it is 1-10% for severe chronic periodontitis [9]. A Kenyan survey reported that 98% of the Kenyan adult population had gingival inflammation, with the spread between men and women being equal[10]. Periodontal disease is associated with systemic diseases and conditions such as preterm birth, endocrine, respiratory and cardiac diseases. There is strong evidence that periodontal disease is associated with PLBW. The severity of periodontal disease is increased in pregnancy. Increased prevalence of severe periodontal disease is associated with PLBW while the opposite is true. Higher severity and prevalence of periodontal disease leads to an increased incidence of PLBW[11].

PLBW and periodontal disease have similar risk factors including smoking, ethnicity, alcohol use, level of education and socioeconomic status and are thus potential confounders[11]. It is then

best to account for these by considering the age, systemic and environmental factors of pregnant women[11]. With confounders such as smoking, alcohol and drug abuse being accounted for, a Sri Lanka study was able to demonstrate the association of periodontal disease with PLBW. Pregnant women with periodontal disease showed an increased risk of preterm births[12].

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 FUSOBACTERIUM NUCLEATUM AND ITS ROLE IN PRETERM AND LOW BIRTH WEIGHT

The oral cavity has over 500 species of bacteria that co-exist with each other. *F. nucleatum* is a gram negative anaerobic rod found in both health and disease[13]. *F. nucleatum* is both a commensal and pathogen, among the orange complex of periodontal pathogens. Periodontal disease is a broad term used to describe a chronic inflammatory disease that destroys the tooth supporting structures; the bone, periodontal ligament and gingiva. Infections confined to gingiva are termed gingivitis. Gingivitis has a prevalence of between 50-90%, it is however reversible by basic oral hygiene practice. When the infection spreads to the underlying bone and supporting connective tissue it is called periodontitis. Mild periodontitis has a prevalence of 22%, while moderate and severe has a prevalence of 13%[14].

The oral cavity is home to hundreds of bacterial commensals and pathogens of both aerobic and anaerobic types. When dental plaque matures gram negative and anaerobic forms increase relative to other forms. Certain bacteria are associated with periodontal disease, they include *Porphyromonas gingivalis, Tannerella forsythius, Treponema denticola, Fusobacterium nucleatum* among others[14]. Infection of periodontal tissue by these bacteria and others causes release of bacterial leucotoxins, collagenases and fibrinolysis. These agents have been shown to have systemic effects[14]. Biofilms that cause gingivitis and periodontitis are complex polymicrobial communities whose virulence makes them resistant to host defences and antimicrobial agents. Some individuals are more susceptible to periodontitis than others. Periodontitis progression is influenced not only by the immune system and inflammatory response of the host, but also by environmental and genetic factors[15].

Periodontal disease it is associated with other systemic diseases such as adverse pregnancy outcomes (preterm birth, still birth, pre-eclampsia, chorioamnionitis), Alzheimer's disease, infections of the respiratory tract, colorectal cancer and cardiovascular disease[16]. *F. nucleatum* is a very common oral bacteria in both health and disease with a prevalence of between 85-96% recorded using both PCR and culture techniques[17,18]. Distinct subgroups exist within the species with five so far being recognised*: polymorphum, nucleatum, animalis, fusiforme* and v*incentii*[19,20]. *Ss nucleatum* is associated with disease while *ss fusiforme* and *ss vicentii* are associated with health[16].

The prevalence of *F. nucleatum* is high in both health and disease. It is implicated in gingivitis, chronic periodontitis, localised and generalised aggressive periodontitis. It is also associated with periapical periodontitis and pulp necrosis[21]. *F. nucleatum* levels are increased in saliva and serum in patients with periodontitis[16].

As the levels of *F. nucleatum* increase so does the severity of periodontal disease, inflammation and pocket depth[22,23]. *F. nucleatum* levels are increased in smokers and in type 2 diabetic patients in both periodontal health and disease. Experimental studies in mice have proven that F. nucleatum causes periodontitis and induces bone loss and abscess formation[16]. *F. nucleatum* facilitates the aggregation and establishment of *Tanneralla forsythia* and *Porphyromonas gingivalis* among other oral species, this creates an infective synergy that enhances the virulence of these periodontal pathogens[24].

F. nucleatum is among the most highly implicated species in PLBW and adverse pregnancy outcomes overall and among the oral species[16]. *F. nucleatum* is an important neonatal pathogen, its prevalence of detection in cord blood from neonatal sepsis is equal or higher than two well-known neonatal pathogens *E. coli* and group B *streptococcus. F. nucleatum* has been detected

in cord blood, amniotic fluid, and foetal lung and stomach associated with adverse pregnancy outcomes such as preterm birth, still birth and pre-eclampsia[25].

F. nucleatum has been shown to induce preterm birth in pregnant mice [3]. A case report reported isolation of *F. nucleatum* bacteria species from a still birth infant which after PCR analysis localised the source to be the subgingival flora[5]. In another study involving a case report of a term stillbirth, *F. nucleatum* was identified as the causative agent and was shown to have trans-located from subgingival plaque to the placenta leading to inflammation which eventually led the death of the foetus. Genetic studies identified the subgingival clone to be the same one found in the placenta[25].

A recent study that retrospectively examined term and preterm placentas of mothers after delivery showed a higher rate of detection of *F. nucleatum* in term than preterm birth, concluding that *F. nucleatum* is not associated with preterm births. The study however did not have information on the periodontal status of the participants making it unclear if periodontitis was associated with preterm births[26].

2.2 PRETERM, LOW BIRTH WEIGHT AND ITS CONSEQUENCES ON NEW-BORNS

Preterm birth is a delivery that occurs after 23 gestational week but before 37 gestational weeks. Low birth weight is a weight at birth below 2500 grams. PLBW is associated with increased neonatal morbidity and death. In a study in England and Wales it was found that preterm birth had mortality rates of 42 per 1000 live births, while babies born between 37-41 weeks had a morality rate of 1.8 per 1000 live births[27]. For babies born at 37 weeks and above, the mortality rate of those weighing above 2500 grams was 0.8 per 1000, while for those born between 1500- 2499 grams was 5.3 per 1000[27].

Preterm birth has consequences long after birth, these include behaviour disorders, neurological conditions and chronic health disorders. Morbidity is inversely proportional to gestational age. Some consequences of preterm birth are hidden and they include difficulties in learning and behaviour problems. These can extend into adolescence but regress into adulthood. Due to the high risk of neurological impairment, children born preterm need monitoring[28].

In 2005 the incidence of preterm birth was 9.6% globally with regional disparities: 10.6% in North America, 6.2% in Europe, 9.1% in Asia and 11.9% in Africa. Within Africa the highest rates were found in Eastern and Southern Africa 14.3% and 17.5% respectively. These were the highest rates globally[6].

Risk factors for PLBW include age of less than 17 years or above 35 years[29], multiple pregnancies, tobacco use, social and economic status, ethnicity and body mass index. Offenbacher et al 1996 proposed that periodontal disease is also a risk factor for PLBW[11].

2.3 PERIODONTITIS AND PRETERM BIRTH AND LOW BIRTH WEIGHT

Periodontal disease is associated with endocrine, respiratory, cardiac as well as other systemic diseases. Women with periodontitis before pregnancy suffer from a more severe form of periodontitis during pregnancy. Several authors have suggested that there is evidence of association of maternal periodontitis and PLBW. Increased severity and prevalence of periodontitis in pregnancy is associated with PLBW, while the opposite is true. Women in populations with high prevalence of periodontal disease are hence at risk of delivering preterm babies. Risk factors for preterm birth are similar to risk factors for periodontal disease, for example smoking, alcohol use, African descent, socioeconomic status and educational levels and are thus potential confounders[11].

Some authors have however found no association between preterm birth and periodontal disease[30,31]. A study conducted in Kenya found no association between periodontitis and PLBW, the study was conducted in mothers aged 26-30 years and the prevalence of severe periodontitis was 3.7%[32].

2.4 INFLUENCE OF PREGNANCY HORMONES ON PERIODONTAL DISEASE

Pregnancy, puberty, menstrual cycle and oral contraceptives are all associated with periods of self-limiting gingivitis due to increased plasma concentrations of ovarian hormones, oestrogens and progesterone[33]. In the second trimester of pregnancy progesterone causes some individuals to experience increased gingival inflammation but without attachment loss[34]. The clinical features seen in gingivitis are amplified by corticosteroids, androgen, oestrogens and progesterone. This is thought to occur due to an increase in the activity of fibroblasts, bone resorption, immune suppression and fluid exudation[34]. In pregnancy a shift occurs in the predominant types of bacteria with an increase of anaerobic types such as *Prevotella intermedia.* The amount of plaque does not increase in pregnant women, what changes is an increase in the proportions of anaerobic types of bacteria. The levels of these bacteria increase as pregnancy progresses and reduce towards the later stages of pregnancy. After parturition gingival inflammation and the proportions of these bacteria return to pre-pregnancy levels as the levels of sex hormones decline[33,35].

Progesterone increases the permeability of the gingival microvasculature resulting in oedema and increased accumulation of inflammatory cells. The production of prostaglandins which are inflammatory mediators increases in the presence of oestrogen and progesterone[33].

2.5 BIOLOGICAL THEORIES FOR PRETERM BIRTH AND LOW BIRTH WEIGHT

There are various theories that explain the link between periodontal disease and PLBW. Bacterial spreading theory postulates that periodontal pathogens travel systematically and affect the fetoplacental unit via translocation of periodontal pathogens. This theory is given strength by the isolation of some oral pathogenic species such as *P. Gingivalis, Eikenella, Fusobacterium nucleatum* and *Bergeyella* in amniotic fluid and placenta[36]. In the inflammatory dissemination theory, it is postulated that inflammatory cytokines such as PGE-2 and IL-1 beta produced in the periodontal tissues during inflammation are trans located to the placenta and cause an inflammatory response. This theory is the most plausible as it is the way in which periodontal disease is thought to influence other systemic diseases and conditions[37].

The third theory postulates that the feto-maternal unit mounts an immune response against oral pathogens. The presence of a certain Ig-M phenotype is associated with amplified inflammatory reaction by the placenta and hence increased risk of PLBW. A study examining this found that 35.2% of samples tested positive for Ig-M for one oral pathogen[37]. Detection of *F. nucleatum* required a method that was both highly sensitive and specific. Several methods exist in detecting oral bacteria from samples such as dental plaque, tissue biopsy, gingival crevicular fluid, saliva and blood. These methods include bacterial culture-based cultivation, microscopy, DNA probe hybridisation, specific antibody immunofluorescence, fluorescent oligonucleotide probe hybridisation and gene amplification through PCR (polymerase chain reaction)[38].

Conventional methods like culture and sensitivity and microscopy are good but have some short comings for example only 50-60% of subgingival bacteria can be cultured[39]. They also have

low sensitivity and require complex interpretations. Molecular methods such as PCR bridge some of these short comings.

PCR is a DNA amplification method. Other methods include NASBA (nucleic acid sequence-based amplification and LAMP (loop mediated isothermal amplification)[40].

Polymerase chain reaction involves denaturisation of DNA strands using heat, which promotes DNA synthesis. DNA is amplified and these amplification products are visualised by a camera or via fluorescence[41].

This study employed real-time PCR (qPCR). The advantages of qPCR over conventional PCR is that an agarose gel is not needed to view the results after the experiment as the reaction is monitored in real time via the melt curve analysis. Real time PCR can perform truly quantitative analysis whereby it can estimate the DNA concentration of a specific bacteria, unlike conventional PCR that gives the DNA concentration of the entire sample but not of individual bacteria and is hence semi-qauntitative[42]. Boutaga et al 2005 compared the selectivity, specificity and quantification ability of qPCR and culture. They found that qPCR was fast, selective and specific. It was deemed to have a role in overcoming some of the shortfalls of culture such as inability to culture some microorganisms. Out of the 5 periodontal pathogens studied among them *F. nucleatum,* it was found that real time PCR detected a higher quantity than culture for each pathogen[43]. Decat et al in 2012 after analysing 8 periodontal pathogens using real time PCR concluded that this method was able to detect bacterial loads of bacteria among them *Fusobacterium* genus and was a suitable method for assessing periodontal risk[41].

2.6 PROBLEM STATEMENT

Eighteen percent of all births in Kenya are preterm[44]. In Africa, the rate is 14.3% while globally it is 9.6%. Of the 3.1 million neonatal deaths globally in 2010, 1 million were directly related to PLBW[6]. PLBW is strongly associated with morbidity and death in the neonatal period.

Despite the heavy disease burden and health implications 25% of the causes of PLBW are not accounted for[8]. Preterm birth is caused by infections, uteroplacental ischemia, and uterine over distention among other factors. The known risk factors for preterm birth include chronic diseases such as diabetes mellitus, cardiovascular disorders, renal disease, asthma, HIV/AIDS and periodontitis[7]. Periodontitis has been associated with systemic illnesses such a diabetes mellitus, PLBW, respiratory and cardiac diseases. Offenbacher et al 1996 concluded that periodontitis during pregnancy leads to seven times increased risk for PLBW[8].

F. nucleatum is implicated in gingivitis, chronic periodontitis, localised and generalised aggressive periodontitis[21]. *F. Nucleatum* is an oral commensal found in 86% of individuals[18], it however has a higher rate of detection in destructive periodontitis, wounds and periapical infections[45]. It is part the orange complex of periodontal organisms and is strongly associated with causation of periodontitis. An infective synergy exists whereby *F. nucleatum* creates a favourable environment for the establishment of the most virulent periodontal bacteria *Tannerella forsythia* and *Porphyromonas gingivalis*[24]. *F. nucleatum* is not only associated with oral but with adverse pregnancy outcomes (preterm birth, still birth, pre-eclampsia, chorioamnionitis), respiratory diseases, cardiac diseases and gastrointestinal conditions such as cancer of the colon and rectum[16].

In pregnancy the rate of maternal periodontitis is estimated to be 73% showing a high prevalence among pregnant women[46]. Authors have also demonstrated that periodontitis during pregnancy increases the risk of PLBW and that a high prevalence of periodontitis is associated with increased risk, while low prevalence is not[12]. More than half of the causes of PLBW are not known. Periodontitis and *F. nucleatum* have both been associated with PLBW, it is therefore necessary to investigate their role[46].

2.7 STUDY JUSTIFICATION

Preterm birth is caused by different factors such as infections, ischemia of the placenta and over distention of the uterus. Most of these factors ultimately involve amplification of the inflammatory and infection pathway, which could explain why preterm birth is associated with multiple factors. Intrauterine infections account for 25-40% of all preterm births[7]. Periodontitis is a risk factor for preterm birth and low birth weight and has been estimated to increase the risk seven fold[8]. A higher prevalence of periodontitis increases the risk while a low prevalence does not[12].

F. nucleatum is one of the causative organisms of periodontal disease and furthermore has been implicated in PLBW. Among the recognized periodontal pathogens it is the one most commonly associated in other clinical infections such as infections of the blood, ovary, lungs and kidney[21]. It is associated with the destructive forms of periodontal diseases, it assists other oral pathogens in establishing oral infections such as periapical infections, pulp necrosis and periodontitis[45]. Some studies have isolated *F. nucleatum* in the placentas of women who had preterm birth[5,25]. The same *F. nucleatum* clone in the sub gingival tissues was found to be the same as the one in the placenta of babies born preterm[4]. A direct causal relationship was established in a mouse model where inoculation with *F. nucleatum* led to PLBW in pregnant mice[3]. Despite all these studies, the data on the relationship between periodontitis and PLBW is inconclusive. The relation between *F. nucleatum* and PLBW has not been adequately studied and there is scanty data on the topic worldwide and locally.

2.8 STUDY OBJECTIVES

2.8.1 Main Objective

To investigate the relationship between occurrence of subgingival *Fusobacterium nucleatum* DNA and preterm low birth weight infants among postpartum women at Kiambu Level 5 Hospital.

2.8.2 Specific Objectives

1. To determine the gestational age of new-borns.
2. To determine the weight at birth of new-borns.
3. To assess the periodontal disease status of the postpartum mothers.
4. To determine the occurrence of subgingival *F. nucleatum* DNA of the postpartum mothers.

2.9 HYPOTHESIS

2.9.1 Null hypothesis

There is no association between occurrence of subgingival *F. nucleatum* in postpartum women and delivery of PLBW new-borns.

2.9.2 Alternate hypothesis

There is an association between occurrence of subgingival *F. nucleatum* in postpartum women and delivery of PLBW new-borns.

2.10 STUDY VARIABLES

2.10.1 Independent variables

VARIABLE	MEASUREMENT
Mother's Age	Number of years
Mother's Education level	Highest Level attained
Fusobacterium Nucleatum	Absent or present
Fusobacterium nucleatum DNA concentration	Copies per microliter

2.10.2 Intervening variables

Periodontal status	Periodontitis – CDC/AAP 2012 case definition
	Gingival index – Loe and Silness 1963
Oral hygiene status	Plaque score- Silness and Loe 1964
Oral health seeking behaviour	Frequency of dental visits
Oral hygiene practice	Frequency of brushing

2.10.3 Dependent variables

Infants born less than 37weeks old gestation	Gestational age in weeks
Infant weighing less than 2500g	Weight in grams

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY DESIGN

This was an unmatched case control hospital-based study. Cases were mothers who gave birth to preterm babies of less than 37 weeks gestational age while controls were mothers who gave birth to babies of normal gestational age of 37 weeks and above.

3.2 STUDY AREA

The study was conducted in Kiambu County which has a population of 1,623,282 and recorded 62,476 births in 2016. The county is served by 108 public health facilities, fourteen of which are Tier 4 and above, of those 11 are tier 4 hospitals and 3 are Tier 5. This study was done at Kiambu Level 5 Hospital which is one of the 3 level five hospitals in Kiambu County.

3.3 STUDY POPULATION

The study population consisted of women who delivered at Kiambu Level 5 Hospital between January 2019 and March 2019 who fit the inclusion criteria.

3.4 INCLUSION CRITERIA

- All the postpartum women who consented to be enrolled into the study
- Women who gave birth to new-borns of less than 37 weeks gestational age
- Women who gave birth to new-borns above 37 weeks gestational age
- Postpartum women who had a singleton pregnancy
- Women who were less than 72 hours postpartum[11]

3.5 EXCLUSION CRITERIA

Criteria for excluding an individual from the study involved the following:

- Multiple gestation in current pregnancy
- Still born infants
- Participants who required antibiotic cover for invasive dental procedures
- Periodontal treatment in the last 3 months
- Antibiotics use in the last 4 weeks

3.6 CASE DEFINITION

Preterm birth implies delivery of a baby that occurs before 37 weeks gestation period while low birth weight implies a baby born weighing less than 2500 grams[2]. Mothers were classified as cases if their new-born baby was delivered before the 37^{th} gestational week. Conversely, mothers whose delivery occurred after the 37^{th} gestational week were classified as controls.

3.7 SELECTION OF STUDY PARTICIPANTS

The study population were women who delivered at Kiambu Level 5 Hospital which is one of the three level 5 hospitals in Kiambu County. The hospital attracts a diverse population of middle to low income status population, which also includes mothers from Nairobi County due to its proximity and relatively less congestion compared to Kenyatta National Hospital. Participants were admitted into the study within 72 hours after child birth after giving written informed consent. Two research assistants were trained on how to identify cases and controls from the hospitals daily birth register. The method of assessing the gestation age was based on the last menstrual period. The research assistants' minimum qualification was a certificate of record keeping or equivalent with training as a record clerk. Every case that was found was selected. Controls were randomly selected (using the table of random numbers) every time a

case was found. The principal investigator was blinded as to the case-control status by not participating in the selection to avoid bias.

3.8 SAMPLE SIZE DETERMINATION

The sample size was calculated using Kelsey's formula[47]. The Prevalence of *F. nucleatum* is 92% in periodontal health and 96% in periodontitis[18].

$$n = \frac{\left(Z_{\propto/2} + Z_{1-\beta}\right)^2 \bar{p}\bar{q}(r+1)}{r(p_1 - p_2)^2}$$

And:

$$n_2 = rn_1$$

Whereas

$n_1 =$ number of cases

$n_2 =$ number of controls

$Z_{\propto/2} =$ standard normal deviate for two $-$ tailed test based on alpha level (confidence level)

$Z_{1-\beta} =$ standard normal deviate for one $-$ tailed test based on beta level (power level)

$r =$ ratio of unexposed to exposed

$p_1 =$ proportion (percentage)of cases with exposure

$p_2 =$ proportion (percentage)of controls with exposure

$\bar{p} = \frac{p_1 + rp_2}{r+1}$ And $\bar{q} = 1 - \bar{p}$

Therefore:

$$n = \frac{(1.96 + 0.80)^2(0.94)(0.06)(1+1)}{1(0.96 - 0.92)^2} = 537$$

The estimated population size of the cases per month was 20, bringing the total in 3 months to N=60.

$$nf = n/(1 + \frac{n}{N})$$

nf= desired sample size (for population < 10,000),

n= desired sample size (for population >10,000),

N= estimate of population size.

$$nf = 537/(1 + \frac{537}{60})$$

$$nf = 54$$

The study investigator enrolled 54 cases and 54 controls.

Sample size = **108** participants.

3.9 SAMPLE DESIGN AND PROCEDURE

Data collection commenced in January 2019 and was completed after 3 months in March 2019. Participants were drawn from Kiambu Level 5 Hospital and were selected according to the selection criteria and designated as either cases or controls by the research assistants. Mothers who consented and fit the inclusion criteria in the maternity ward in Kiambu Level 5 Hospital were included in the study. The principal investigator was blinded as to the case control status. He administered the questionnaire to collect sociodemographic data, then performed a clinical examination to assess periodontal status and collect sub gingival plaque samples from the participants. The two research assistants recorded the data as the principal investigator performed the clinical examination. The plaque samples collected were stored in a cooler box at -4^{0}C, then in a portable freezer at -20^{0}C on the same day and delivered to BIO-ZEQ LTD

weekly where they were stored at -80^{0}C until processing. Quantitative real time PCR analysis was done at BIO-ZEQ Kenya LTD laboratories at Kenya Aids Vaccine Initiative (KAVI) in Kenyatta National Hospital.

3.10 DATA COLLECTION AND MANAGEMENT.

3.10.1 Sociodemographic data collection

A screening form (APPENDIX II) was used whereby all the participants suffering from the conditions in the exclusion criteria were eliminated, those who fulfilled the inclusion criteria were selected according to the case-control definition until the desired sample size was reached. Sociodemographic data was collected from participants and filled in a questionnaire. Weight at birth and gestation age at birth of the new-born and the participants weight and height were retrieved from the patient‘s medical records and recorded in the clinical examination forms.

3.10.2 Periodontal status measurements

The principal investigator was blinded as to case-control status by not participating in the recruitment and being blind as to whether a participant was a case or control during examination. Clinical examination was done in the post labour wards with the participants sitting upright on an office chair in the examination room. Illumination was from a head torch. Disposable gloves, masks, gauze, oral dental mirrors and sterile Michigan ‘O’ periodontal probes were used for examination. Supragingival plaque scores were taken using Silness and Loe index (1964) and gingival index using the Loe and Silness index (1963) on Ramfjord’s index teeth (Appendix I). If a Ramfjord tooth was missing the neighbouring molar, premolar or incisor was examined[48].

A full mouth periodontal examination was done using sterile mouth mirrors and Michigan ‘O’ periodontal probes. Periodontal probing depths in millimetres were measured at 6 different sites

for every tooth: mesiobuccal and mesiolingual, mid-buccal and mid-lingual and distobuccal and distolingual, on all erupted teeth expect for the 3rd molars. Probing depths measurements were done using a sterile Michigan 'O' periodontal probe and recorded in the periodontal probing depth chart (Appendix IV) to the nearest millimetre. Gingival recession was measured in millimetres from the cemento-enamel junction to the top of the marginal gingiva. Clinical attachment loss was calculated from the probing depths and recession measurements. The CDC/AAP 2012 case definition criteria was used to make a periodontal diagnosis as illustrated below in table 3.1.

Table 1: Periodontal Diseases Case Definition (CDC/AAP case definition 2012[49])

Disease category	Clinical attachment loss	Periodontal probing depth
Severe periodontitis	≥2 interproximal sites with CAL of ≥6mm (not on the same tooth)	**AND** ≥1 interproximal site with PPD of ≥5mm
Moderate periodontitis	≥2 interproximal sites with CAL of ≥4mm (not on the same tooth)	OR ≥2 interproximal sites with PPD of ≥5mm (not on the same tooth)
Mild periodontitis	≥2 interproximal sites with CAL ≥ 3mm (Not on the same tooth)	**AND** ≥ 2 interproximal sites with PPD ≥ 4mm (Not on the same tooth) Or one site with PPD ≥ 5mm
No or mild periodontitis	No evidence of mild, moderate or severe periodontitis	

Third molars excluded. *PPD*> periodontal probing depth, *CAL> clinical attachment loss

3.10.3 Subgingival plaque sample collection and storage.

Supragingival plaque was first removed using sterile cotton pellets and isolation achieved using cotton rolls. Subgingival plaque samples were then collected using sterile size 30 endodontic paper points(GAPADENT® Co. Ltd, XiQing district, P.R China) which were inserted for 20 seconds[50] in the gingival sulcus of the Ramfjord teeth. All the six paper points from each participant were pooled in a micro centrifuge tube labelled with the participant's serial number and stored in cooler box at -4^{0}C and then into a portable freezer at -20^{0}C and transferred to the laboratory weekly. The specimens were then stored in the laboratory at minus 80^{0}C as shown in Figure 1.

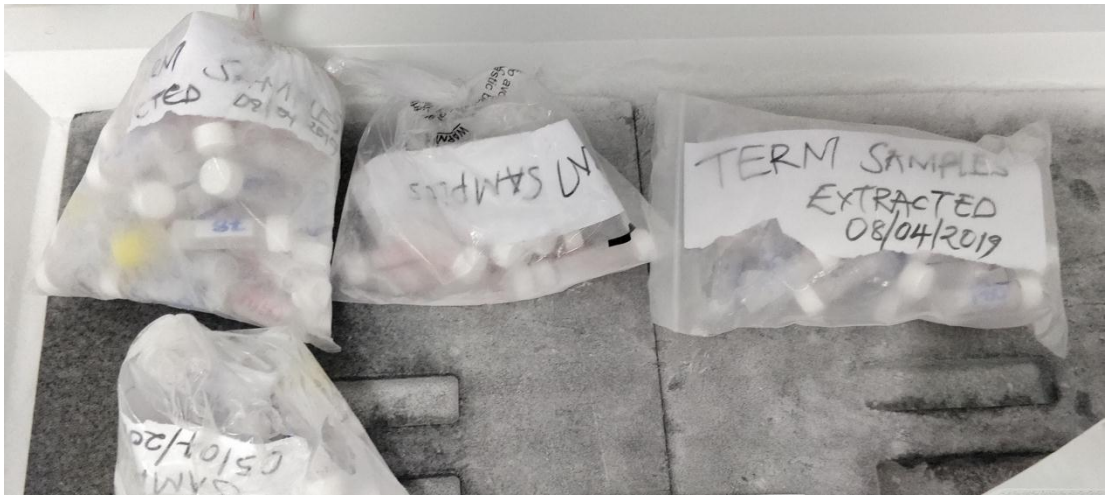

Figure 1: Plaque samples in micro centrifuge tubes stored at -80^{0}C.

3.10.4 Laboratory procedure

To copy DNA PCR requires DNA (template) forward and reverse primers which are sequences at the borders of the DNA molecule to be copied and deoxynucleotides (dNTPs). The primer sequences must be known and can be manufactured in the lab or bought commercially from suppliers. The PCR test involved three main steps, denaturation or unwinding of DNA by heating to 90- 96^{0}C, hybridization where the primers were bound to their complementary bases on the unwound single-stranded DNA and finally extension or DNA synthesis by a polymerase. A DNA probe was then used to determine the quantity of DNA template.

3.10.5 DNA extraction procedure

Plaque samples were let to thaw for 30 minutes before processing. DNA extraction was then done using QIAamp ® DNA mini kit (Qiagen GmbH, Hilden, Germany) (figure 2). One hundred and eighty µL of fast lysis buffer and 29 µL proteinase K was incorporated into the microcentrifuge containing the paper points from each participant. Vortexing and incubation at 56°C was done for 1-3 hours until complete lysis occurred. The microcentrifuge tubes were incubated at a temperature of 70 degrees Celsius for ten minutes, thereafter addition of 200µl ethanol (96–100%). This mixture was then centrifuged at 6000 x *g* (8000 rpm) for 1 min twice followed by centrifuging at full speed (20,000 x *g*; 14,000 rpm) for 3 min and finally placed in 1.5ml microcentrifuge then Centrifuged at 6000 x *g* (8000 rpm) for 1 min to elute the DNA. The collected supernatant was then used for quantitative PCR (qPCR) reaction. Some of the supernatant was stored at -80^{0}C as a backup.

Figure 2: QIAcube HT DNA extraction machine and QIAamp DNA extraction kit

3.10.6 Real time PCR and DNA quantification

The DNA template, genesig Real-time PCR detection kit for *F. nucleatum* and Oasig lyophilised 2 x qPCR Master Mix (Primer design LTD, York House, School lane, Chandler's Ford, UK) were thawed. After thawing they were mixed briefly by Vortexing. A reaction mix containing extracted DNA samples, *oasig* 2x qPCR Master Mix, F. nucleatum primer/probe mix, negative control (nuclease free water), positive control (containing *F. nucleatum* DNA) and standards 1,2,3 was prepared. Figure 3 shows the PCR detection kit and Master Mix.

Figure 3: Genesig Real-time PCR detection kit for F. nucleatum and *oasig* lyophilised 2x qPCR MasterMix

This mixture was then dispensed into PCR tubes before being placed into the qPCR cycler (Figure 4)

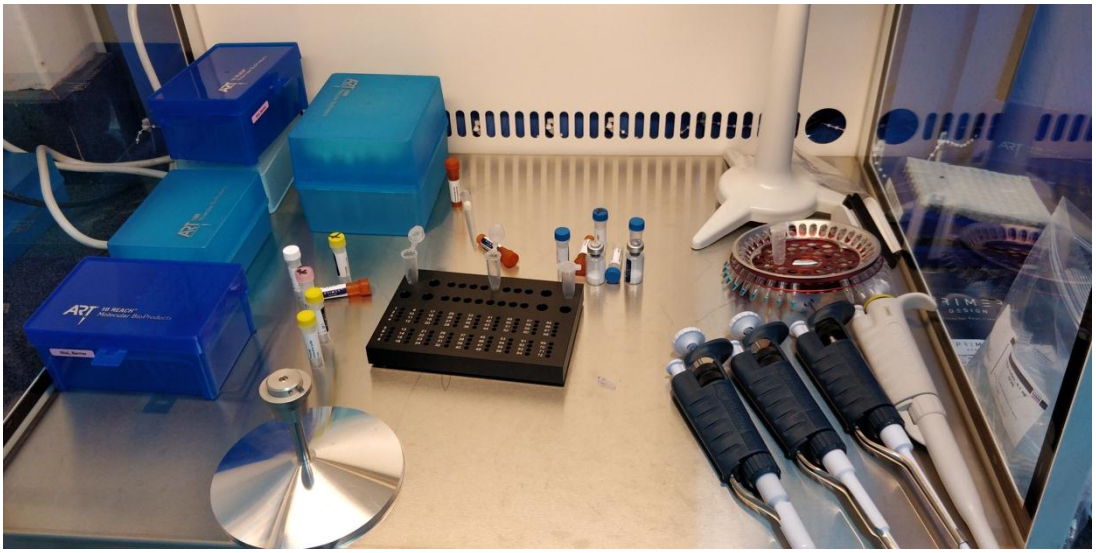

Figure 4: PCR Vacuum mixer workstation. PCR primers/probes, Master Mix, PCR tubes, centrifuge and pipettes holders.

The real-time PCR reaction was then carried out by QIAGEN's qPCR cycler, the Rotor-Gene Q (Qiagen GmbH, Hilden, Germany). (Figure 5 and 6)

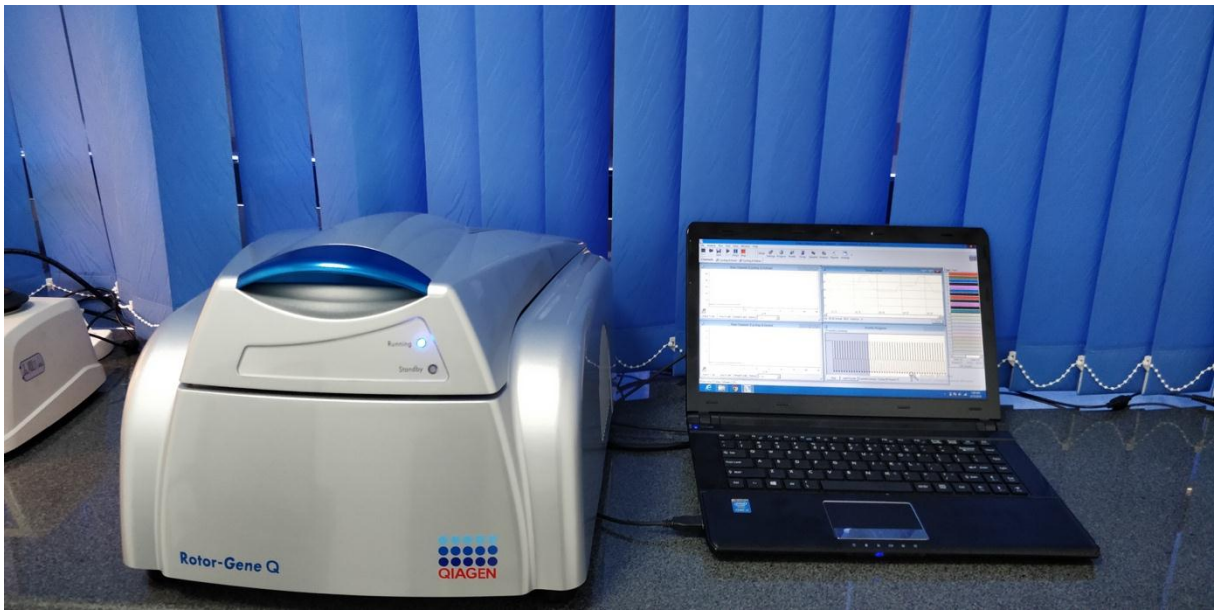

Figure 5: Rotor-Gene Q, QIAGEN Real-time PCR cycler

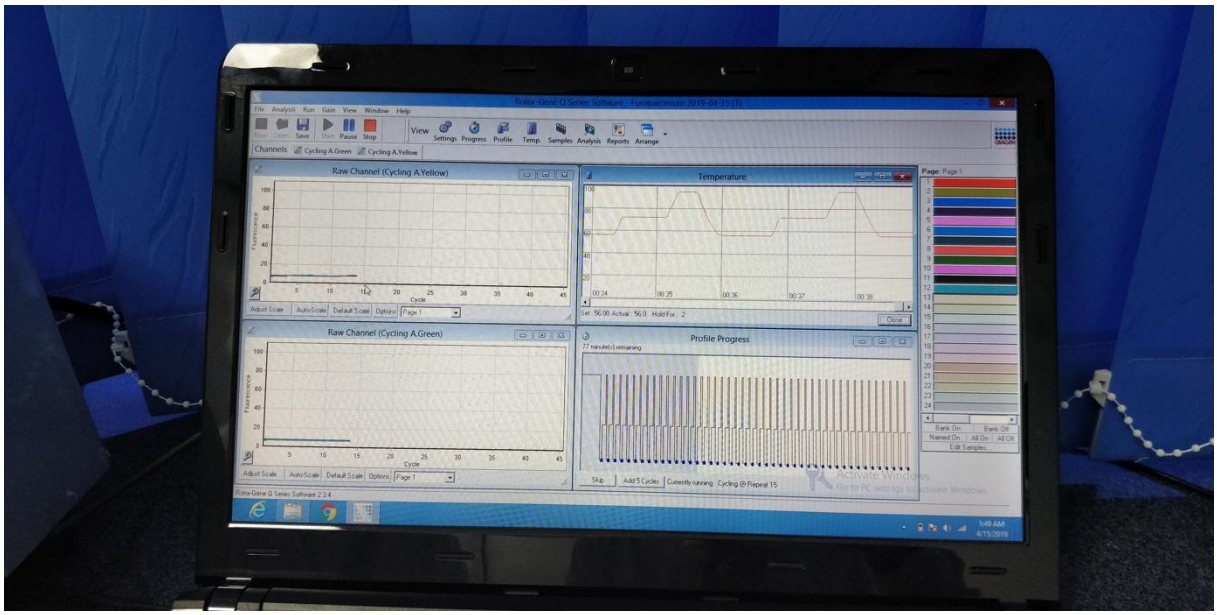

Figure 6: Rotor-Gene Q computer output display of high-resolution melting curve

The cycling program was as follows according to the manufacture's instruction:

Table 2: Optimized cycling conditions

Step	**Time**	**Temperature**
Initial denaturation	3 min	94°C
3 step cycling: Denaturation	0.5 to 1 minute	94°C
Annealing	0.5 to 1 minute	50-68°C
Extension	1 minute	72°C
Number of cycles	25-35	
Final extension	10 minutes	72°C

The Rotor-Gene Q software package was used to analyse the PCR reaction and produce output of DNA identification and quantification. Results were in the form of high-resolution melting curves showing number of copies produced by the reaction. The melting curves were output on a computer linked to the Rotor-Gene Q real time PCR cycler. After DNA amplification samples were stored at -80°C. PCR primers. Oasig lyophilised 2x qPCR Master Mix, DNA extraction kits and genesig Real-time PCR detection kit for *F. nucleatum* were sourced from (BIO-ZEQ Kenya LTD). Quantitative PCR analysis was done by BIO-ZEQ Kenya LTD.

3.11 CROSS INFECTION CONTROL

All the instruments and gauze used in the examination and plaque sample collection were sterilised in an autoclave. The examiner wore disposable face masks and used a different pair of clean gloves for examination of each participant. The examiner disinfected his hands with antiseptic solution in between every examination.

3.12 RELIABILITY AND VALIDITY

A pilot phase was carried out to ascertain the validity and reliability of questionnaires, clinical examination forms and instruments. All the clinical measurements were carried out by the principal investigator. Intra examiner reliability was determined through double

evaluation of every 10th patient by the principal investigator. For inter- examiner reliability, the principal investigator was calibrated by one of the supervisors who was a periodontologist. Inter and intra-examiner reliability was calculated using the Cohen's kappa score, whereby a score above 80% was acceptable.

3.13 DATA ENTRY, ANALYSIS AND PRESENTATION

Data was entered then cleaned and finally validated. Analysis was performed using SPSS (statistical packages for social sciences) version 20.0 (SPSS Inc, Chicago Illinois, USA) and Microsoft excel. Categorical data was measured using Chi-square and Fishers tests. Comparison of mean values was done using t-test and ANOVA. Logistic regression was used to test the multivariate dependence of preterm birth on demographic factors, socio-economic factors, smoking and periodontal status. Comparisons between periodontitis and occurrence of *F. nucleatum* in the case and control groups was analysed using Chi-square and odds ratios at 95% confidence limits. A significance level of $p < 0.05$ was used. Data was presented in the form of frequency diagrams, tables and pie charts.

3.14 ETHICAL CONSIDERATION

Ethical approval was sought from the Kenyatta National Hospital-University of Nairobi Ethics Research Committee reference number: P650/09/2018. Permission to conduct the study was sought from Kiambu Level 5 Hospital. Written informed consent was obtained from the participants after all risks and benefits had been explained (APPENDIX VI). All the participants who met the inclusion criteria had an equal chance of been enrolled into the study. There was no coercion or victimisation to the participants who choose not to participate in the study. Participants were at liberty to terminate their participation at any point during the study without victimisation. Data collected in the study was treated with utmost confidentiality and stored in a secure place where only authorised persons were able

to access it. There was no financial benefit to the participants, participation was on a voluntary basis. Participants who needed periodontal treatment were treated at no cost. All the instruments used in examining the participants were autoclaved and aseptic technique was practised while examining the participants. There was no risk of harm to the patients during examination and plaque collection as these were non-invasive procedures. The samples collected were cleared by the ethics research committee to be stored safely and used for future studies.

CHAPTER FOUR

4.0 RESULTS

4.1 SOCIODEMOGRAPHIC CHARACTERISTICS

In this study cases were mothers who delivered an infant of less than 37 gestational weeks (pre-term) while controls were mothers who delivered an infant of 37 gestational weeks and above (term). A total of one hundred and twenty participants were examined, however data was incomplete for 12 which were subsequently expunged leaving a study sample n=108 (90%), with 54 being cases and 54 being controls. There were a total of 108 new-borns of whom 60 (55.6%) were females while 48 (44.4%) were males. The mothers' age ranged between 16 – 40 years with a mean of 25.42 ($\pm$ 5.67 SD years). Forty-one (38%) mothers had achieved primary education, 53 (49%) secondary education and 14 (13%) tertiary education. The mothers who gave birth to term babies had a mean monthly income of 4,435 shillings, compared to 2,685 shillings for the mothers who gave birth to preterm babies, this was statistically significant ($t(106) = 2.47$, $p=0.015$). This could be due to the fact that socioeconomic status is a risk factor for preterm birth. Mothers in the age bracket (25-40) earned more (4,702 shillings) compared to those in the 16-25 age bracket (2,680 shillings), this difference was found to be statistically significant ($t(106)=2.86$, $p=0.0005$) table 3.

Table 3: Monthly income in shillings compared to characteristics of participants (n = 108)

Characteristics		n (%)	Monthly income in Shillings						
			95% Confidence Interval of Mean						
			Mean	SD	Lower	Upper	Df	Test	p
Birth type	Pre-Term	54 (50.0)	2,685	2,266	346	3,152	106	t = 2.47	0.015 *
	Term	54 (50.0)	4,435	4,678					
Gender	Female	60 (55.6)	4,025	3,956	-392	2,483	106	t = 1.44	0.152
	Male	48 (44.4)	2,979	3,459					
Weight (Grams)	800 – 2800	61 (56.5)	3,254	3,023	-745	2,152	106	t = 0.96	0.338
	2801 – 4100	47 (43.5)	3,957	4,553					
Mother's age (Years)	16 – 24	61 (56.5)	2,680	3,074	620	3,423	106	t = 2.86	0.005 *
	25 – 40	47 (43.5)	4,702	4,272					
Education	Primary	41 (38.0)	3,329	3,438	2,244	4,414	107	F = 1.71	0.185
	Secondary	53 (49.0)	3,283	3,866	2,217	4,349			
	Tertiary	14 (13.0)	5,286	4,084	2,928	7,644			
Brushing frequency	Once Daily	49 (45.4)	3,612	4,352	-1,353	1,544	106	t = 0.13	0.896
	> Once Daily	59 (54.6)	3,517	3,231					
Last dental visit (Years)	<= 5	18 (16.7)	3,944	3,669	2,119	5,769	107	F = 2.89	0.060
	> 5	20 (18.5)	5,200	4,047	3,305	7,094			
	Never	70 (64.8)	2,993	3,603	2,133	3,852			

Independent-Samples t test was used for birth type, gender, gestation age, weight of new born, mother's age, brushing frequency.

Analysis of Variance (ANOVA) was used for education and last dental visit. * $p < 0.05$.

4.2 GESTATION AGE OF NEW-BORNS

The gestation age of the new-borns ranged between 23-43 weeks with a mean of 36.23 (+- 4.44 SD). Half of the new-borns were term and had an average gestational age of 39.44 weeks (+- 2.13 SD). The remaining half of the new-borns were preterm and had an average gestational age of 33.02 weeks (+- 3.78 SD). Females had a higher gestation age 36.43 (+- 4.22 SD weeks) compared to males 35.98 (+- 4.72 SD weeks), this was not statistically significant (t= 0.527, p= 0.599) table 4.

Table 4: Pre-term and term status compared to characteristics of participants (n = 108)

Characteristics		n (%)	Pre-Term	Term	Test	P
Gender	Female	60 (55.6)	27 (50.0)	33 (61.1)	X^2 = 1.350	0.245
	Male	48 (44.4)	27 (50.0)	21 (38.9)		
Weight of new-borns (Grams)	800 – 2800	61 (56.5)	41 (75.9)	20 (37.0)	X^2 = 16.612	<0.001***
	2801 – 4100	47 (43.5)	13 (24.1)	34 (63.0)		
Mother's age (Years)	16 – 24	61 (56.5)	31 (57.4)	30 (55.6)	X^2 = 0.038	0.846
	25 – 40	47 (43.5)	23 (42.6)	24 (44.4)		
Education	Primary	41 (38.0)	22 (40.7)	19 (35.2)	X^2 = 1.381	0.501
	Secondary	53 (49.0)	27 (50.0)	26 (48.1)		
	Tertiary	14 (13.0)	5 (9.3)	9 (16.7)		

Pearson Chi-Square test of independence was used for all characteristics.

*** p< 0.001.

4.3 BIRTH WEIGHT OF NEW-BORNS

The birth weight of the new-borns ranged between 800-4100 grams with a mean of 2,739.89 grams (+- 700 SD). The term new-borns had an average weight of 3077.93 grams (+-564.13 SD), while the preterm new-borns had an average weight of 2401.85 grams (+-662.04 SD). A significant association was found between Pre-term and term status and weight of new-borns (X^2 = 16.612, p< 0.001).

4.4 ORAL HYGIENE PRACTICES AND ORAL HEALTH SEEKING BEHAVIOUR OF THE MOTHERS

Forty-nine (45%) of the participants brushed their teeth once daily, while the remaining 59 (55%) brushed twice daily. As regards the last dental visit majority 70 (64.8%) had never visited the dentist, 20 (18.5%) had visited a dentist more than 5 years ago, 14 (13%) had visited more than 1 year ago, while only 4 (3.7%) had visited 6 months- 1 year ago. Majority of the participants, 56(52%) reported not experiencing bleeding on brushing while the rest 52(48%) reported bleeding on brushing.

4.5 ORAL HYGIENE STATUS

All the participants had plaque. Majority of the participants, 61 (56.5%) had mild plaque accumulation while 44 (40.7%) had moderate plaque and 3 (2.8%) had severe plaque accumulation. The mean plaque score was (1.43 $\pm$ 0.49 SD). Cases had higher mean plaque scores (1.47 $\pm$ 0.55 SD) compared to controls (1.37 $\pm$ 0.41 SD), though this was not statistically significant, (t (106) = 1.088, p= 0.279). There was a statistically significant association between the education level of the mothers when compared with plaque scores (F=4.843, p 0.010). The plaque scores were progressively lower as the level of education increased. The other characteristics compared to the plaque score showed no significant findings and are summarised below (Table 5).

Table 5: Plaque scores compared to characteristics of participants (n = 108)

			Plaque score						
			95% Confidence Interval of Mean						
Characteristics		n (%)	Mean	SD	Lower	Upper	Df	Test	p
Birth type	Pre-Term	54 (50.0)	1.47	0.55	0.08	0.29	106	t = 1.088	0.279
	Term	54 (50.0)	1.37	0.41					
Gender	Female	60 (55.6)	1.41	0.49	0.22	0.15	106	t = 0.378	0.706
	Male	48 (44.4)	1.44	0.49					
Gestation age	23 – 37	58 (53.7)	1.48	0.53	0.07	0.31	106	t = 1.282	0.203
(Weeks)	38 – 43	50 (46.3)	1.36	0.43					
Weight	800 – 2800	61 (56.5)	1.41	0.45	0.21	0.16	106	t = 0.282	0.779
(Grams)	2801 – 4100	47 (43.5)	1.44	0.53					
Mother's age	16 – 24	61 (56.5)	1.45	0.49	0.12	0.25	106	t = 0.679	0.499
(Years)	25 – 40	47 (43.5)	1.39	0.49					
Education	Primary	41 (38.0)	1.58	0.46	1.43	1.72	2, 105	F = 4.843	0.010*
	Secondary	53 (49.0)	1.37	0.49	1.24	1.51			
	Tertiary	14 (13.0)	1.15	0.41	0.92	1.39			
Brushing	Once Daily	49 (45.4)	1.47	0.53	0.10	0.28	106	t = 0.971	0.334
frequency	> Once	59 (54.6)	1.38	0.45					
	Daily								
Last dental	<= 5	18 (16.7)	1.50	0.50	1.26	1.75	2, 105	F = 0.918	0.402
visit (Years)	> 5	20 (18.5)	1.30	0.37	1.13	1.47			
	Never	70 (64.8)	1.44	0.51	1.31	1.56			

Independent-Samples t test was used for birth type, gender, gestation age, weight of new born, mother's age, brushing frequency, bleeding gums.

Analysis of Variance (ANOVA) was used for education and last dental visit. * $p < 0.05$.

4.6 GINGIVAL INFLAMMATION

All the participants had gingivitis. The mean gingival index score for the participants was 1.23 ($\pm$0.29 SD). Despite the fact that all the participants had gingival inflammation, none had severe inflammation. Majority 88 (81.5%) had mild inflammation while 20 (18.5%) had moderate

inflammation. Pre-term delivery mothers had a higher mean gingival index of (1.29 ± 0.31 SD), compared with term delivery mothers (1.18 ± 0.24 SD) and this was found to be statistically significant ($t(106) = 2.048$, $p = 0.043$). The mean gingival index of mothers progressively reduced with increasing level of education as follows primary 1.30, secondary 1.23 and tertiary 1.04, this was found to be statistically significant (F= 4.666, p = 0.011). The association between bleeding gums and gingival index was found to be statistically significant (t = 2.322, 0.022). These and other comparisons of gingival index and characteristics of participants are captured in table 6 below.

Table 6: Gingival index compared to characteristics of participants (n = 108)

			Gingival index						
					95% Confidence Interval of Mean				
Characteristics		n (%)	Mean	SD	Lower	Upper	Df	Test	p
Birth type	Pre-Term	54 (50.0)	1.29	0.32	0.00	0.22	106	t = 2.048	0.043*
	Term	54 (50.0)	1.18	0.24					
Gender	Female	60 (55.6)	1.22	0.31	-0.14	0.08	106	t = 0.544	0.588
	Male	48 (44.4)	1.25	0.26					
Weight (Grams)	800 – 2800	61 (56.5)	1.22	0.28	-0.14	0.08	106	t = 0.532	0.596
	2801 – 4100	47 (43.5)	1.25	0.29					
Mother's age (Years)	16 – 24	61 (56.5)	1.22	0.29	-0.14	0.08	106	t = 0.532	0.596
	25 – 40	47 (43.5)	1.25	0.28					
Education	Primary	41 (38.0)	1.30	0.30	1.21	1.40	2, 105	F = 4.666	0.011*
	Secondary	53 (49.0)	1.23	0.29	1.15	1.31			
	Tertiary	14 (13.0)	1.04	0.15	0.96	1.13			
Brushing frequency	Once Daily	49 (45.4)	1.24	0.28	-0.09	0.13	106	t = 0.443	0.659
	> Once Daily	59 (54.6)	1.22	0.29					
Last dental visit (Years)	<= 5	18 (16.7)	1.26	0.33	1.10	1.42	2, 105	F = 2.500	0.087
	> 5	20 (18.5)	1.10	0.14	1.04	1.17			
	Never	70 (64.8)	1.26	0.30	1.19	1.33			

Independent-Samples t test was used for birth type, gender, gestation age, weight of new born, mother's age, brushing frequency, bleeding gums. Analysis of Variance (ANOVA) was used for education and last dental visit. * p< 0.05.

4.7 PERIODONTITIS AMONG PARTICIPANTS

Case definition for periodontitis was done according to CDC/AAP case definition 2012[49]. Using this case definition 20 (18.5%) had severe periodontitis, majority 48 (44.4%) had moderate periodontitis, 16 (14.8%) had mild periodontitis while 24 (22.2%) did not have periodontitis. Among the participants that had severe periodontitis 13/20 (65%) delivered pre-term babies, while 7/20(35%) delivered term babies, the association between periodontitis and preterm birth was not statistically significant (X^2 = 7.217, p 0.065). Only 4 (4.8%) of participants with periodontitis belonged to the tertiary level education group, while majority of those with no periodontitis 10 (41%) also belonged in this group. This was found to be statistically significant (X^2 = 22.840, p = <0.001). None of the participants who achieved tertiary education had severe periodontitis. This was statistically significant (FET = 28.915, p< 0.001). The participants that had never visited a dentist were found to be the majority 11 (46%) of those with no periodontitis and of those with 13 (65%) severe periodontitis (FET = 15.177, p< 0.013) (Table 7).

Table 7: Comparison between periodontitis and characteristics of participants (n = 108)

Characteristics		Periodontitis				Test	P
		None	Mild	Moderate	Severe		
Birth type	Pre-Term	10 (41.7)	4 (25.0)	27 (56.2)	13 (65.0)	X^2 = 7.217	0.065
	Term	14 (58.3)	12 (75.0)	21 (43.8)	7 (35.0)		
Gender	Female	16 (66.7)	11 (68.8)	21 (43.8)	12 (60.0)	X^2 = 5.197	0.158
	Male	8 (33.3)	5 (31.2)	27 (56.2)	8 (40.0)		
Weight	800 – 2800	14 (58.3)	6 (37.5)	30 (62.5)	11 (55.0)	X^2 = 3.104	0.376
(Grams)	2801 – 4100	10 (41.7)	10 (62.5)	18 (37.5)	9 (45.0)		
Mother's age	16 – 24	13 (54.2)	11 (68.8)	30 (62.5)	7 (35.0)	X^2 = 5.494	0.139
(Years)	25 – 40	11 (45.8)	5 (31.2)	18 (37.5)	13 (65.0)		
Brushing	Once Daily	8 (33.3)	10 (62.5)	21 (43.8)	10 (50.0)	X^2 = 3.521	0.318
frequency	> Once Daily	16 (66.7)	6 (37.5)	27 (56.2)	10 (50.0)		
Last dental	<= 5	4 (16.7)	2 (12.5)	5 (10.4)	7 (35.0)	FET = 15.177	0.013*
visit (Years)	> 5	9 (37.5)	3 (18.8)	8 (16.7)	0		
	Never	11 (45.8)	11 (68.8)	35 (72.9)	13 (65.0)		
		No Periodontitis		Periodontitis			
Education	Primary	5 (20.8)		36 (42.9)		X^2 = 22.840	<0.001***
	Secondary	9 (37.5)		44 (52.4)			
	Tertiary	10 (41.0)		4 (4.8)			

Pearson Chi-Square test of independence was used for characteristics.

Fisher's Exact test (FET) was used for characteristics.

*** p< 0.001. * p< 0.05

There were more cases (44) who had periodontitis than controls (40), and less cases (10) who had no periodontitis than controls (14). The odds of exposure to periodontitis was 1.5 times higher among the cases than the controls (odds ratio [OR] 1.5, 95% CI 0.26 to 1.63), this finding was however not statistically significant (Table 8).

Table 8: Association between preterm birth and periodontitis (n = 108)

		Case (n = 54)	Controls (n = 54)	Odds Ratio (95% CI)	P value
Periodontitis	No Periodontitis	10 (18.5)	14 (25.9)	1.5 (0.26, 1.63)	0.355
	Periodontitis	44 (81.5)	40 (74.1)		

Odds Ratio was calculated for all characteristics.

P value for heterogeneity from X^2 test.

4.8 PREVALENCE OF FUSOBACTERIUM NUCLEATUM

Real time PCR was used to detect the presence and quantify the concentration of *F. nucleatum* DNA in the plaque samples of the participants. The graphs below (figures 7-9) show Rotor-Gene Q software output of melting curve for the samples. Y axis – normalised fluorescence, X axis- Cycle threshold. It shows that most of the samples were positive for F. nucleatum as the amplification occurred before cycle 35, which was the threshold of a result to be deemed positive. Estimation of F. nucleatum DNA concentration done by calculating area under the graph which was by the Rotor-gene Q software.

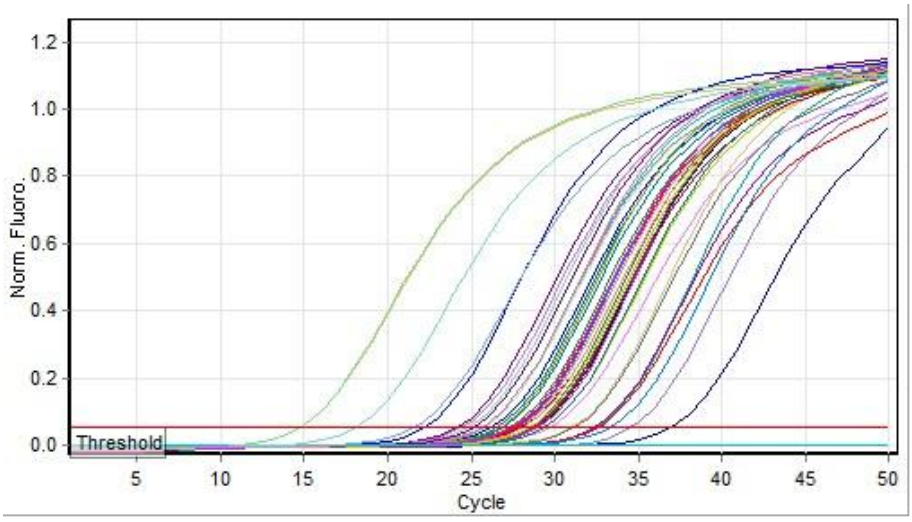

Figure 7: High resolution melting curve for *F. nucleatum (samples 2-39, 73-102)*

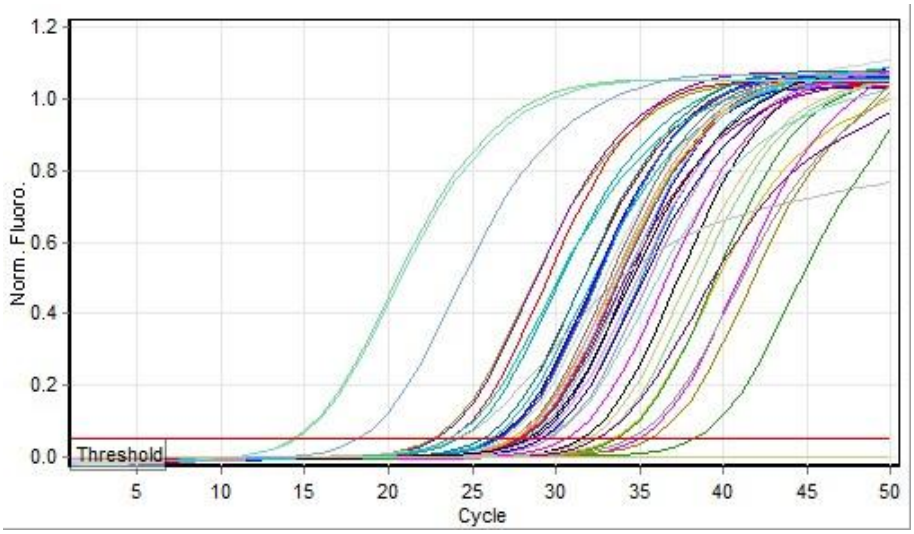

Figure 8: High resolution melting curve for *F. nucleatum (samples 40-71, 96-131)*

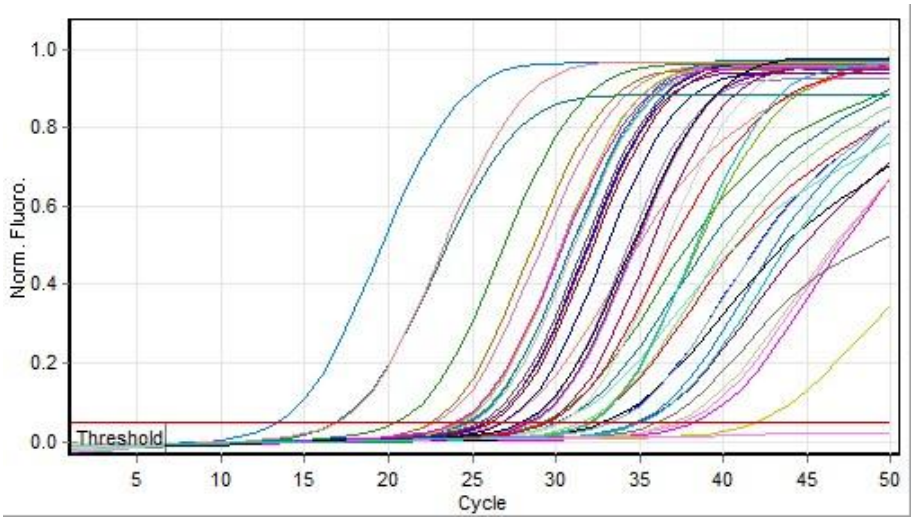

Figure 9: High resolution melting curve for *F. nucleatum (samples 1-69,72-83, 87-91)*

A standard curve estimated the reaction efficiency by comparing the standards (first 3 dots on the right) to the samples. The slopes of the standard curves ranged from -3.22 to -3.76 which translated to reaction efficiencies ranging from 84% to 100%. Y axis- number of cycle, X axis- concentration (figures 10-12).

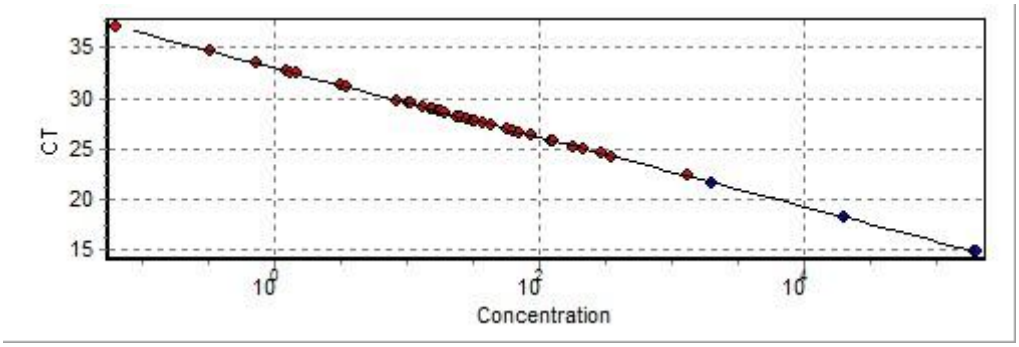

Figure 10: Standard curve- ***F. nucleatum (samples 2-39, 73-102)***.

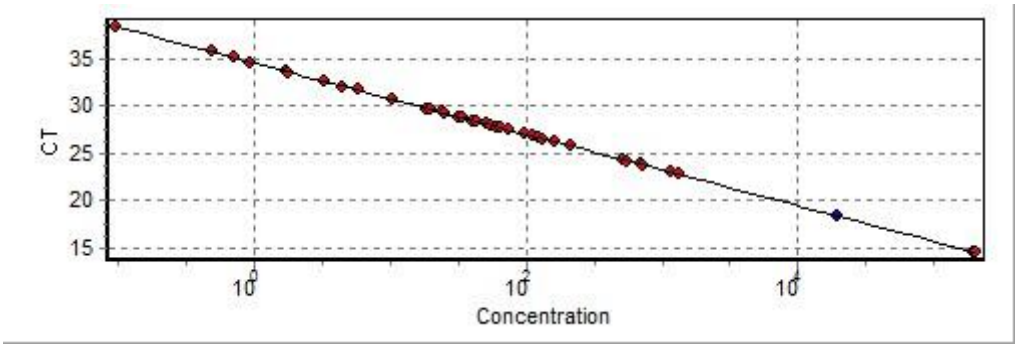

Figure 11: Standard curve- ***F. nucleatum (samples 40-71, 96-131)***.

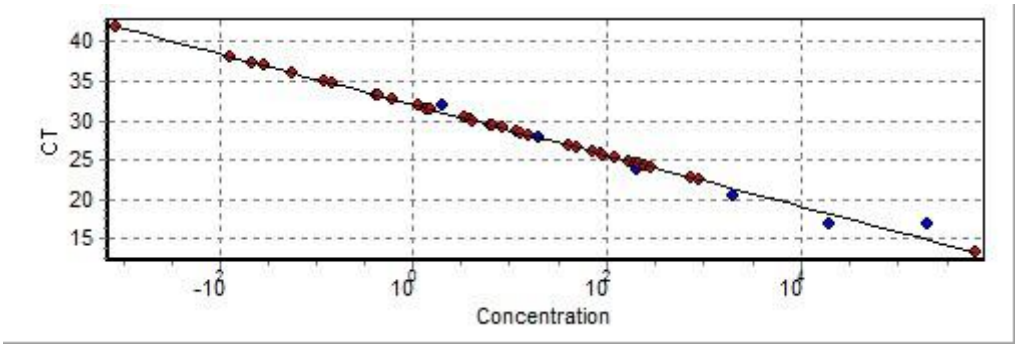

Figure 12: Standard curve- ***F. nucleatum (samples 1-69,72-83, 87-91)***.

The tables below (9-11) show the concentration of *F. nucleatum* DNA as calculated by Rotor-gene Q real time PCR machine. Controls and standards with known varying concentrations of F. nucleatum DNA were used in the run to validate the experiment and show efficiency of the run. Negative control of nuclease free water ruled out contamination of the runs.

Table 9: *F. nucleatum* DNA concentration (copies/ul) for samples (2-39, 73-102)

No.	Color	Name	Type	Ct	Ct Comment	Given Conc (copies/ul)	Calc Conc (copies/ul)
1		2	Unknown	27.76			33
2		3	Unknown	29.49			10
3		5	Unknown	26.31			88
4		6	Unknown	24.24			351
5		8	Unknown	29.79			8
6		9	Unknown	27.81			32
7		10	Unknown	26.62			71
8		12	Unknown	27.99			28
9		13	Unknown	28.56			19
10		14	Unknown	29.11			13
11		16	Unknown	28.91			15
12		17	Unknown	25.83			121
13		18	Unknown	31.13			3
14		19	Unknown		NEG (NTC)		
15		20	Unknown	24.96			217
16		23	Unknown		NEG (NTC)		

No.	Color	Name	Type	Ct	Ct Comment	Given Conc (copies/ul)	Calc Conc (copies/ul)
17		28	Unknown		NEG (NTC)		0
18		29	Unknown	24.51			293
19		31	Unknown	25.80			123
20		33	Unknown	28.14			26
21		34	Unknown		NEG (NTC)		
22		37	Unknown	26.81			63
23		38	Unknown	32.58			1
24		39	Unknown	26.97			56
25		102	Unknown		NEG (NTC)		0
26		99	Unknown	32.42			1
27		98	Unknown	28.72			17
28		97	Unknown	25.83			121
29		95	Unknown		NEG (NTC)		
30		94	Unknown	25.75			128
31		93	Unknown	31.25			3
32		92	Unknown	28.90			15
33		88	Unknown	32.65			1
34		85	Unknown	28.65			18
35		86	Unknown	22.29			1,305
36		84	Unknown	25.21			183
37		80	Unknown		NEG (NTC)		

No.	Color	Name	Type	Ct	Ct Comment	Given Conc (copies/ul)	Calc Conc (copies/ul)
38		79	Unknown	33.44			1
39		77	Unknown	29.47			10
40		76	Unknown	28.22			24
41		75	Unknown	27.37			43
42		74	Unknown	27.55			38
43		73	Unknown		NEG (NTC)		
44		Negative	Negative Control		NEG (NTC)		
45		Positive	Positive Control	14.84			193,358
46		Standard	Standard	14.82		200,000	196,984
47		Standard	Standard	18.18		20,000	20,617
48		Standard	Standard	21.67		2,000	1,970

Table 10: *F. nucleatum* DNA concentration (copies/ul) for samples *(40-71, 96-131)*

No.	Color	Name	Type	Ct	Ct Comment	Given Conc (copies/ul)	Calc Conc (copies/ul)
1		71	Unknown	27.95			56
2		70	Unknown	33.56			2
3		68	Unknown	28.74			34
4		67	Unknown	26.65			124
5		65	Unknown		NEG (NTC)		

No.	Color	Name	Type	Ct	Ct Comment	Given Conc (copies/ul)	Calc Conc (copies/ul)
9		63	Unknown	33.67			2
10		58	Unknown		NEG (NTC)		
11		57	Unknown	31.67			6
12		56	Unknown	26.18			166
13		55	Unknown	32.07			4
14		54	Unknown	32.61			3
15		52	Unknown	29.73			19
16		50	Unknown	28.35			44
17		48	Unknown		NEG (NTC)		
18		43	Unknown	29.65			20
19		42	Unknown	27.79			62
20		41	Unknown	25.76			213
21		40	Unknown		NEG (NTC)		0
22		131	Unknown	22.74			1,357
23		129	Unknown	24.33			512
24		128	Unknown		NEG (NTC)		

No.	Color	Name	Type	Ct	Ct Comment	Given Conc (copies/ul)	Calc Conc (copies/ul)
25		127	Unknown	29.25			25
26		126	Unknown	22.97			1,176
27		124	Unknown	28.06			52
28		123	Unknown	27.01			100
29		122	Unknown	24.20			555
30		121	Unknown	34.62			1
31		120	Unknown	27.46			75
32		119	Unknown	28.10			51
33		116	Unknown	23.76			729
34		115	Unknown	27.50			74
35		114	Unknown	26.53			133
36		113	Unknown	32.59			3
37		111	Unknown	28.87			32
38		110	Unknown	26.81			112
39		108	Unknown	25.77			213
40		105	Unknown	27.70			65
41		103	Unknown		NEG (NTC)		0
42		101	Unknown	30.70			10

No.	Color	Name	Type	Ct	Ct Comment	Given Conc (copies/ul)	Calc Conc (copies/ul)
43		100	Unknown	28.45			41
44		96	Unknown	23.81			703
45		Negative	Negative Control		NEG (NTC)		
46		Positive	Positive Control	14.52			208,032
47		standard	Standard	14.59		200,000	200,000
48		standard	Standard	18.35		20,000	20,000

Table 11: *F. nucleatum* DNA concentration (copies/ul) for samples (*1-69,72-83, 87-91)*

No.	Color	Name	Type	Ct	Ct Comment	Given Conc (copies/ul)	Calc Conc (copies/ul)
1		83	Unknown	28.48			40
2		82	Unknown		NEG (NTC)		
3		81	Unknown	30.25			8
4		78	Unknown	31.98			3
5		72	Unknown		NEG (NTC)		
6		69	Unknown	30.74			7
7		66	Unknown	27.03			64

No.	Color	Name	Type	Ct	Ct Comment	Given Conc (copies/ul)	Calc Conc (copies/ul)
8		64	Unknown	23.61			792
9		62	Unknown	26.08			198
10		61	Unknown		NEG (NTC)		
11		60	Unknown	29.74			9
12		59	Unknown	32.01			1
13		53	Unknown		NEG (NTC)		
14		51	Unknown	28.57			21
15		49	Unknown	27.09			96
16		47	Unknown	30.19			8
17		46	Unknown	28.20			48
18		45	Unknown	28.56			39
19		44	Unknown	22.77			1250
20		36	Unknown	26.05			72
21		35	Unknown	22.54			1283
22		32	Unknown	31.87			2
23		30	Unknown	24.54			212
24		27	Unknown	24.68			190
25		26	Unknown	26.83			61

No.	Color	Name	Type	Ct	Ct Comment	Given Conc (copies/ul)	Calc Conc (copies/ul)
26		25	Unknown	28.46			40
27		24	Unknown	29.39			17
28		22	Unknown	30.31			8
29		21	Unknown	24.88			249
30		11	Unknown	24.15			578
31		7	Unknown		NEG (NTC)		
32		1	Unknown	25.32			176
33		91	Unknown	29.44			14
34		90	Unknown	24.33			297
35		89	Unknown	25.82			128
36		87	Unknown	25.69			145
37		Negative	Negative Control		NEG (NTC)		
38		Positive	Positive Control	14.34			208,124
39		Standard	Standard	14.81		200,000	202,032
40		Standard	Standard	18.85		20,000	19,958
41		Standard	Standard	20.49		2,000	2,067
42		Standard	Standard	24.89		200	210

No.	Color	Name	Type	Ct	Ct Comment	Given Conc (copies/ul)	Calc Conc (copies/ul)
43		Standard	Standard	29.62		20	19
44		Standard	Standard	32.38		2	2

Legend:

NEG (NTC)- Negative sample (amplified past 35 cycles or did not amplify at all).

COLOUR- Colour code of sample or standard

CYCLE THRESHOLD- The number of PCR cycles the sample or standard run before amplifying

CALCULATED CONCENTRATION- copies/ul of *F. nucleatum* calculated from cycle threshold

This report was generated by Rotor-Gene Q Series Software 2.3.4 (Build 3)

The prevalence of *F. nucleatum* among the participants was 85%. The prevalence was higher among the cases 89% than the controls 82%. Among the mothers aged 16-24 years the prevalence was 82% compared to 89% in the 25-40 age bracket though this was not statistically significant (X^2 = 1.150, p= 0.284). When compared to the level of education of participants the prevalence was 88% among primary level, 81% among secondary level and 93% among tertiary level, this was also not statistically significant, (FET =1.251, p 0.560). The comparison of *F. nucleatum* with the various participant characteristics yielded no statistically significant results (Table 12).

Table 12: Comparison of *F. nucleatum* occurrence with the characteristics of participants

Characteristics		Fusobacterium		Test	P
		Presence	Absence		
Birth type	Pre-Term	48(52.2)	6 (37.5)	X^2 = 1.174	0.279
	Term	44 (47.8)	10 (62.5)		
Gender	Female	50 (54.3)	10 (62.5)	X^2 = 0.367	0.545
	Male	42 (45.7)	6 (37.5)		
Weight	800 – 2800	53 (57.6)	8 (50.0)	X^2 = 0.321	0.571
(Grams)	2801 – 4100	39 (42.4)	8 (50.0)		
Mother's age	16 – 24	50 (54.3)	11 (68.8)	X^2 = 1.150	0.284
(Years)	25 – 40	42 (45.7)	5 (31.2)		
Education	Primary	36 (39.1)	5 (31.2)	FET = 1.251	0.560
	Secondary	43 (46.7)	10 (62.5)		
	Tertiary	13 (14.1)	1 (6.2)		
Brushing	Once Daily	42 (45.7)	7 (43.8)	X^2 = 0.020	0.888
frequency	> Once Daily	50 (54.3)	9 (56.2)		
Last dental	<= 5	15 (16.3)	3 (18.8)	FET = 0.260	0.926
visit (Years)	> 5	17 (18.5)	3 (18.8)		
	Never	60 (65.2)	10 (62.5)		

Pearson Chi-Square test of independence was used for characteristics.

Fisher's Exact test (FET) was used for characteristics.

4.9 FUSOBACTERIUM NUCLEATUM DNA QUANTIFICATION

The Calculated concentration of Fusobacterium nucleatum DNA (copies/ul) ranged between 0.00 – 1305.00 copies/ul with a mean of 117.04 (± 244.21 SD). Mothers delivering pre-term babies had more than double the concentration of Fusobacterium nucleatum (160.67 copies/ul) as compared to mothers delivering term babies (73.41 copies/ul), however this was not statistically significant. Among the mothers delivering low birth weight babies the concentration was marginally higher at 118.05 (copies/ul) as compared with 116.42 (Copies/ul) in those who delivered normal birth weight babies, this was not statistically significant. When Fusobacterium nucleatum DNA concentration was compared with periodontitis it was found that among the participants with no periodontitis the mean concentration in copies/ul was 148.38 (281.22 SD), mild periodontitis 54.50 (81.27 SD) copies/ul, moderate periodontitis 114.54 (241.05 SD) copies/ul and severe periodontitis highest at 151.90 (309.20 SD) copies/ul. The association was however not statistically significant (r_s = -0.038, p = 0.697). *F. nucleatum* compared to demographic characteristics of participants showed no statistically significant results as shown in Table 13 below.

Table 13:*F. nucleatum* DNA (copies/ul) compared to characteristics of participants n=108

			Calculated Concentration (copies/ul)				
Characteristics		n (%)	Mean	SD	Df	Test	p
Birth type	Pre-Term	54 (50.0)	160.67	285.37	91.520	t = 1.878	0.063
	Term	54 (50.0)	73.41	187.31			
Gender	Female	60 (55.6)	134.78	301.84	88.098	t = 0.905	0.368
	Male	48 (44.4)	94.85	143.15			
Weight	<2500 grams	41 (38.0)	118.05	192.13	106	t = 0.034	0.973
(Grams)	>2500grams	67 (62.0)	116.42	272.60			
Mother's age	16 – 24	61 (56.5)	99.67	248.44	106	t = 0.841	0.402
(Years)	25 – 40	47 (43.5)	139.57	239.36			
Education	Primary	41 (38.0)	151.80	307.03	2, 105	F = 1.050	0.354
	Secondary	53 (49.0)	82.42	189.48			
	Tertiary	14 (13.0)	146.29	218.10			
Brushing	Once Daily	49 (45.4)	116.31	242.19	106	t = 0.028	0.978
frequency	> Once Daily	59 (54.6)	117.64	247.94			
Last dental	<= 5	18 (16.7)	122.72	295.94	2, 105	F = 0.015	0.985
visit (Years)	> 5	20 (18.5)	109.35	187.47			
	Never	70 (64.8)	117.77	247.38			

Independent-Samples t test was used for birth type, gender, gestation age, weight of new born, mother's age, brushing frequency, bleeding gums. Analysis of Variance (ANOVA) was used for education and last dental visit.

The concentration of *F. nucleatum* DNA was marginally higher in mothers with moderate gingival inflammation at 122.15copies/ul than in mothers with mild gingival inflammation 115.89 copies/ul, however this was not statistically significant. t = 0.103, p 0.918. These and other findings are summarised in Table 14 below.

Table 14: Concentration of *F. nucleatum* DNA (copies/ul) compared to clinical characteristics of participants (n = 108)

			F. nucleatum DNA Concentration (copies/ul)				
Characteristics		n (%)	Mean	SD	Df	Test	p
Plaque severity	Mild	61 (56.5)	122.44	258.05	2, 105	F = 0.196	0.822
	Moderate	44 (40.7)	104.68	224.13			
	Severe	3 (2.8)	188.33	317.57			
Gingival inflammation	Mild	88 (81.5)	115.89	255.37	106	t = 0.103	0.918
	Moderate	20 (18.5)	122.15	192.89			

Independent-Samples t test was used for gingival inflammation. *p<0.05

More cases (48) than controls (44) had exposure to *F. nucleatum*, while less cases (6) were not exposed as compared to controls (10). The odds of being exposed to *F. nucleatum* was 1.8 times higher among the cases than the controls (odds ratio [OR] 1.81, 95% CI 0.61 to 5.42), however this was not statistically significant (Table 15).

Table 15: Association between preterm birth and *F. nucleatum* (n = 108)

		Case (n = 54)	Controls (n = 54)	Odds Ratio (95% CI)	P value
F. Nucleatum	Absence	6 (11.1)	10 (18.5)	1.81 (0.61, 5.42)	0.245
	Presence	48 (88.9)	44 (81.5)		

Odds Ratio was calculated for all characteristics.

P value for heterogeneity from X^2 test.

Among the participants exposed to *F. nucleatum* most (68) had periodontitis while (24) did not have periodontitis. Among those who were not exposed to *F. nucleatum* none had periodontitis while (16) had periodontitis (Table 16).

Table 16: Association between *F. Nucleatum* and periodontitis Risk (n = 108)

		No Periodontitis (n = 24)	Periodontitis (n = 84)
F. Nucleatum	Absence	0 (0)	16 (19.0)
	Presence	24 (100)	68 (81.0)

The association between preterm birth, *F. nucleatum* and periodontitis was examined using a logistic regression analyses. There was no evidence for an association between increasing levels of *F. nucleatum*, periodontitis and preterm birth. Controlling for maternal age there was no significant association between increasing level of *F. nucleatum* and preterm birth (OR 2.045, 95% CI 0.641 to 6.523, p=0.227) and periodontitis (OR 1.184, 95% CI 0.416 to 3.36, p= 0.752) and preterm birth. These and other characteristics are shown in table 17 below.

Table 17: Association between preterm birth, F. nucleatum and measures of periodontal status

	Binary logistic regression				
	95% Confidence Interval for Odds ratio				
Characteristics	Odds Ratio	Lower	Upper	Wald test	p
F. nucleatum	2.045	0.641	6.523	1.461	0.227
Periodontitis	1.184	0.416	3.366	0.100	0.752
Mean gingival index	0.254	0.044	1.466	2.348	0.125
Mean plaque score	0.976	0.393	2.421	0.003	0.957
Mother‘s age	0.995	0.928	1.066	0.022	0.881

Adjusted for maternal age.

Wald test used to calculate significance for all characteristics.

CHAPTER FIVE

5.0 DISCUSSION

5.1 SOCIODEMOGRAPHIC VARIABLES

The study realised 100% of the intended sample n=108, further the participants were enrolled in a span of two and a half months. This may be explained by the fact that firstly the location of Kiambu level 5 makes it accessible to many patients residing in Kiambu and Nairobi counties and secondly perhaps it is the preferred maternity facility by the mothers in the communities. Thirty eight percent (38%) of the participants had achieved primary education level education, 49% secondary education while only 13% had received tertiary education. This low level of tertiary education is comparable to a similar study done in rural Tanzania among women aged 14-44 years visiting a maternity ward, where 85.7% had informal to primary with 14.3% having secondary to tertiary education[51]. This low level of education could be attributable to the fact that the literacy levels of women in Kenya are low at 58.9% as compared to men 64.1%[52]. As compared with primary and secondary levels of education, the mothers with tertiary education were the only group to deliver more term babies as compared to preterm babies. In the other two aforementioned groups primary and secondary the preterm babies were more than term babies. The term babies seen in those with tertiary education could be explained by their higher socio-economic status. Psychological factors such as stress related to pregnancy, depression and stressful life events have been shown to be a risk factor for pre-term delivery, levels of stress are likely to be higher in low socio-economic settings[36].

5.2 BIRTH WEIGHT AND GESTATIONAL AGE OF NEW-BORNS

The average birth weight among the case and controls was 2401 grams and 3077 grams respectively. This is highly comparable with a study done in Iran whereby the birth weight of cases and controls were 2815 grams and 3017 grams respectively[53]. The similarity could be due to the fact that in both studies it was singleton deliveries that were being compared and the

demarcation for cases (preterm babies) starts at (23-36) weeks gestation whereby infants are smaller and have a low birth weight pushing the average down, whereas the controls (term babies) range from (37-42 weeks gestation) which is associated with larger babies that weigh more.

5.3 ORAL HEALTH PRACTICES

As regards oral health practices the majority of the women in this study (55%) reported to brushing twice a day or more. This is higher than the Kenya National Oral Health Survey where (39%) of women brushed at least twice a day[10]. In a study done in India in a heterogeneous population of urban and rural pregnant women (36%) brushed twice daily[54]. The current study shows a higher percentage of brushing which may be attributed to the study being conducted in a semi-urban region while both the Kenya National Oral Health Survey and the study from India covered a mixed population of rural and urban women. Majority (68%) of the participants in this study had never visited a dentist, while only (3.7%) had visited a dentist in the last 6 months to one year. This is lower than in a study in Brazil where 20% of the pregnant women visited a dentist for treatment during pregnancy[54,55]. The difference may be attributable to the differing geographical, socioeconomic and other characteristics between the two populations. These percentages are low, women should be encouraged to seek elective dental treatment during the 2nd trimester and pain management whenever it is necessary. This differs with the Kenya National Oral Health Survey that reported that 27% of women had never visited a dentist, while 62% had visited a dentist in the last 5 years[10]. The difference could be due the fact that the aforementioned study had a much larger sample size and covered both urban and rural populations while this study was restricted to a semi-urban population. Overall these studies show a pattern of poor oral health seeking behaviour.

5.4 ORAL HYGIENE STATUS

In the present study the mean plaque scores for the cases was 1.47 while for controls it was 1.37. This is comparable to an Indian study whereby women at term (34-38 weeks) had a plaque score of 1.49 compared to 0.7 at 4-6 weeks postpartum[56]. The plaque levels were high at term and then reduced at 4-6 weeks post-partum when pregnancy hormones are known to return to pre-pregnancy levels. Other factors that could explain the generally poor hygiene is lack of access to tooth brushing during labour and that women often report nausea during pregnancy which may interfere with their ability to brush their teeth particularly the posterior teeth. This could account for the increased plaque scores that were recorded immediately after birth in this study.

5.5 GINGIVITIS

The mean gingival score was 1.29 among the mothers who delivered preterm babies and 1.18 among the mothers who delivered term babies. An Indian study on post-partum women reported a gingival index score of 1.00, this is lower than the score found in this study[57,58]. Some authors found no significant increase in the level of inflammation during pregnancy, they hypothesised that the increased levels of the hormones would not have an effect on periodontal tissues[59,60], but despite this what is not in contention is the presence of gingival inflammation during pregnancy. Pregnancy hormones increase the vascularisation of gingival tissue and generally there is increased blood in circulation to cater for the growing foetus, this causes the gingival tissues to be engorged with blood leading to easy bruising during mastication and oral hygiene procedures. This could explain the high gingival index during and immediately after pregnancy (72 hours postpartum) that promptly returns back to pre-pregnancy levels at 4-6 weeks postpartum.

5.6 PERIODONTAL STATUS

Generally periodontitis prevalence ranges from 10% to 60% depending on the diagnostic criteria used[61]. In this study the prevalence of periodontitis was (78%). This is higher than in other studies that had prevalence ranging from (44%) to (73%) [31,62,4]. These studies used much larger sample sizes than the current study which can explain the difference. Another study stated a higher prevalence of (94%)[46]. The percentage of participants with severe periodontitis (18.5%) is markedly higher than in a Kenyan study among post-partum women (3.7%). Sixty nine percent had mild or no periodontitis while in the current study it was (37%) of participants, (27%) had moderate periodontitis compared to (44%) in this study[32]. The difference may be attributed to the study location which was in an urban setting as compared to the study which was semi-urban. Mother's with severe disease were more likely to be less educated, mothers with the highest-level education had the lowest prevalence of severe periodontitis. A statistically significant association was not found between preterm birth and periodontitis, this differs with several authors who found an association[11,29,46]. The lack of association between periodontitis and preterm birth determined in this study is however in agreement with studies done in Britain and India[31,62]. Among other factors the lack of an association may be due to the difference in population characteristics, resource settings and healthcare systems as it known there are many risk factors for preterm birth with over half the causes unaccounted for.

5.7 PERIODONTITIS AND FUSOBACTERIUM NUCLEATUM

F. nucleatum was higher in severe periodontitis than in moderate or mild periodontitis. The group with no periodontitis recorded higher levels of *F. nucleatum* than in mild and moderate periodontitis, but lower than in severe periodontitis. This study found no statistically significant association between *F. nucleatum* and periodontitis. The high concentration of *F. nucleatum* among the healthy individuals is in line with a study that demonstrated that, putative periodontal pathogens are often found in healthy subjects in the absence of periodontal

disease[39]. This differs from a study that found *F. nucleatum* to be significantly higher in the periodontitis group than the healthy group[22]. A study detecting periodontal pathogens using PCR found that *F. nucleatum* was significantly more prevalent in chronic periodontitis than in those without periodontitis, the results may differ due to the difference in definition of periodontitis whereby this study based its comparison on the probing depths while current study categorised periodontitis according to the CDC/AAP 2012[23].

5.8 FUSOBACTERIUM NUCLEATUM AND PRETERM BIRTH

The current study found the prevalence of *F. nucleatum* among participants to be 85% using real time PCR method. Using conventional PCR a study reported an overall percentage of 77% for *F. nucleatum*, 90% among patients with generalised aggressive periodontitis, 83% among participants with chronic periodontitis and 58% among patients with no periodontitis[23]. A study using real time PCR, the same method used in the current study, reported a similar prevalence 86%[22], among the group with periodontitis the prevalence was 100%, healthy 81.3% and gingivitis 76.1%. A study among Rwandan women using PCR stated a prevalence of 86.2% of *F. nucleatum* which was also highly comparable with the prevalence determined in this study[17]. A study employing culture techniques reported a prevalence of 60%, this marked difference could be the use of a different detection technique which has shown low agreement with PCR[5]. In the current study the prevalence of *F. nucleatum* was slightly more in the cases at 89% compared to 82% in the controls, but this difference was not statistically significant. A similar pattern was seen quantitatively whereby the cases had more than double the mean concentration of *F. nucleatum* DNA in copies/ul as compared to the controls, this was also not statistically significant. The presence of periodontitis and a high bacterial load does also not always lead to negative pregnancy outcomes like preterm birth, hence the presence of *F. nucleatum* in the oral cavity does not necessarily indicate a risk for preterm birth[4].

5.9 CONCLUSION

This study concluded as follows, first and foremost that majority of the participants in this study (44%) had moderate periodontitis using the CDC/AAP 2012 criteria. Concentration and prevalence of *F. nucleatum* DNA was higher among the cases than the controls, though no significant association was found. The cases had a higher gingival index than the controls and this was statistically significant. Cases were more likely to be exposed to *F. nucleatum* (1.8 odds ratio) and varying severities of periodontitis (1.5 odds ratio) than controls, though this was not statistically significant.

5.10 RECOMMENDATIONS

Within the limits of this study the following are the recommendations:

- During pregnancy all women should have routine dental check-ups and periodontal care where necessary. Only focusing on those with disease yet *F. nucleatum* was found in even healthy individuals may not reap benefits all round.
- Pregnant women should maintain very good oral hygiene to prevent gingivitis, which was shown to be higher among the cases.

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APPENDICES

APPENDIX I: INDICES

Plaque score: Silness and Loe Plaque index 1964

Scores	Criteria
0	No plaque
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen in situ only after application of disclosing solution or by using the probe on the tooth surface.
2	Moderate accumulation of soft deposit s within the gingival pocket, or the tooth and gingival margin which can be seen with the naked eye.
3	Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin

Gingival index: Loe and Silness Index 1963

0- Normal, absence of oedema and no bleeding on probing

1- Presence of oedema with absence of bleeding

2- Oedema present, glazing and bleeding on probing

3- Oedema, ulcerations with spontaneous bleeding

APPENDIX II: SCREENING FORM

Screening Instructions:

1. Only proceed with screening after consent
2. Use a language the mother can understand use translator if necessary
3. Only record "yes" if there is medical evidence of disease, not subjective opinion from mother

Serial No._____ Age (Years): _____ Date:_____

Consent to screen: Yes _____ No_____

Condition	Yes	No
Active Infections e.g. Urinary tract infections, malaria, syphilis		
Chronic illnesses e.g. Diabetes, hypertension, Asthma, Heart disease, renal disease		
Multiple gestation in current pregnancy		
Delivery of live birth		
Conditions that would require prophylaxis before dental treatment		
Periodontal treatment in the last 3 months		
Use of antibiotics in the last 4 weeks		
Placental abnormalities, Pre-eclampsia, Cervical incompetence		

APPENDIX III: QUESTIONNAIRE

QUESTIONNAIRE

TITLE: SUBGINGIVAL FUSOBACTERIUM NUCLEATUM AND PRETERM LOW BIRTH WEIGHT AMONG POSTPARTUM WOMEN AT KIAMBU LEVEL 5 HOSPITAL

INSTRUCTIONS TO INTERVIEWERS:

Ensure the participants are the biological mothers of the child who was delivered in Kiambu Level 5 Hospital

1. Do not ask leading questions or suggest responses to the participants
2. For responses not among the choices, fill in the answer next to the question

Date: _____ Serial No._____

Age (Years): _____

1. Highest level of education of the mother: Primary ☐ Secondary ☐ Tertiary ☐

2. Highest level of education of the father: Primary ☐ Secondary ☐ Tertiary ☐

3. Household income per month in Kshs: _____

4. What is your ethnicity (race/ tribe)? _____

5. Tooth brushing habits: Once daily ☐ Twice daily ☐ Thrice daily ☐ Never ☐

6. What do you use to brush your teeth? Commercial toothbrush ☐ Chewing stick ☐

Fingers ☐ Others (specify) ☐

7. Last dental visit: 3-6 months ago ☐ 6 months -1 year ago ☐ >1 year ago ☐

> 5 years ago ☐ >10 years ago ☐ Never ☐

8. Smoking Habit: Smoker ☐ Non-smoker ☐ Previous smoker ☐

9. Did you use alcohol during the pregnancy? Yes ☐ No ☐

10. Do your gums bleed on brushing? Yes ☐ No ☐

11. Did you have a gingival growth during this pregnancy? Yes ☐ No ☐

12. When was your last pregnancy (in months)? _____

APPENDIX IV: CLINICAL EXAMINATION FORMS: MOTHER'S ORAL HEALTH ASSESSMENT

Date: _____ Serial No._____

Mother's weight (kgs):_____Mother's height: _____ Mother's age (yrs.):_____

Gestation age of new-born (wks.): _____ Gender of new-born: _____

Weight of new-born at birth (grams): _____

Plaque score: Silness and Loe 1964

Tooth	16		11		24		36		31		44	
Surface	F	P	F	P	F	P	F	L	F	L	F	L
Score												

Total…… Mean……..

Gingival index: Loe and Silness 1963

Tooth	16		11		24		36		31		44	
Surface	F	P	F	P	F	P	F	L	F	L	F	L
Score												

Total……. Mean……..

Periodontal probing depth chart

Maxillary arch:

Tooth	17			16			15			14			13			12			11			21			22			23			24			25			26			27		
Palatal																																									
Recession (mm)																																									
CAL (mm)																																									
Facial																																									
Recession (mm)																																									
CAL (mm)																																									
Mobility																																									

Average CAL……

Mandibular arch:

Tooth	47			46			45			44			43			42			41			31			32			33			34			35			36			37		
Palatal																																									
Recession (mm)																																									
CAL (mm)																																									
Facial																																									
Recession(mm)																																									
CAL (mm)																																									
Mobility																																									

Average CAL……

APPENDIX V: LABORATORY FORM

Date of sample collection: _____ Participants Serial No: _____

Date and time sample received, centrifuged and supernatant stored

_____

Fusobacterium nucleatum: Real-time PCR results

Occurrence of *Fusobacterium nucleatum* (tick one)

Absent _____ Present _____

Levels of *Fusobacterium nucleatum* _____

Laboratory technologist: Signed _____

APPENDIX VI: CONSENT FORM ENGLISH

UNIVERSITY OF NAIROBI (UoN) COLLEGE OF HEALTH SCIENCES P O BOX 19676 Code 00202 Telegrams: varsity **(254-020) 2726300 Ext 44355**	**KNH-UoN ERC** **Email:** **uonknh_erc@uonbi.ac.ke** **Website:** **http://www.erc.uonbi.ac.ke** **Facebook:** **ttps://www.facebook.com/uonknh.erc** **Twitter: @UONKNH_ERC** **ttps://twitter.com/UONKNH_ERC**	**KENYATTA NATIONAL HOSPITAL (KNH)** **P O BOX 20723 Code 00202** **Tel: 726300-9** **Fax: 725272** **Telegrams: MEDSUP, Nairobi**

PARTICIPANT INFORMATION AND CONSENT FORM

ADULT CONSENT

FOR ENROLLMENT IN THE STUDY

Title of Study: SUBGINGIVAL FUSOBACTERIUM NUCLEATUM AND PRETERM LOW BIRTH WEIGHT AMONG POSTPARTUM WOMEN AT KIAMBU LEVEL 5 HOSPITAL

Principal Investigator\and institutional affiliation: Dr. David Sumbi Kyale, University of Nairobi.

Co-Investigators and institutional affiliation: Not Applicable

Introduction:

I would like to tell you about a study being conducted by the above listed researchers. The purpose of this consent form is to give you the information you will need to help you decide whether or not to be a participant in the study. Feel free to ask any questions about the purpose of the research, what happens if you participate in the study, the possible risks and benefits, your rights as a volunteer, and anything else about the research or this form that is not clear. When we have answered all your questions to your satisfaction, you may decide to be in the study or not. This process is called 'informed consent'. Once you understand and agree to be in the study, I will request you to sign your name on this form. You should understand the general principles which apply to all participants in a medical research:

i) Your decision to participate is entirely voluntary ii) You may withdraw from the study at any time without necessarily giving a reason for your withdrawal iii) Refusal to participate in the research will not affect the services you are entitled to in this health facility or other facilities. We will give you a copy of this form for your records.

May I continue? YES / NO

This study has approval by The Kenyatta National Hospital-University of Nairobi Ethics and Research Committee protocol no. _____

WHAT IS THIS STUDY ABOUT?

The researchers listed above are interviewing Mothers who have just delivered their babies. The purpose of the interview is to find out if Fusobacterium nucleatum, a bacteria found in the mouth, is associated with deliver of preterm babies. The study is for part of the requirements for the award of Masters of Dental Surgery in Periodontology degree. Participants in this research study will be asked questions about their oral hygiene practices, presence of gum bleeding and gum swelling during pregnancy and number of dental visits. Participants will also have the choice to undergo an oral examination to find out the health of their gums.

There will be approximately 108 participants in this study randomly chosen. We are asking for your consent to consider participating in this study.

WHAT WILL HAPPEN IF YOU DECIDE TO BE IN THIS RESEARCH STUDY?

If you agree to participate in this study, the following things will happen:

You will be interviewed by a trained interviewer in a private area where you feel comfortable answering questions. The interview will last approximately five minutes. The interview will cover topics such as your name, age, oral hygiene practices, gum health status, education level and number of dental visits.

After the interview has finished, an oral examination will be done where we will assess your gum oral health status by using dental instrument, dental plaque samples will then be collected from your mouth and later measured in a laboratory.

We will ask for a telephone number where we can contact you if necessary. If you agree to provide your contact information, it will be used only by people working for this study and will never be shared with others. The reasons why we may need to contact you is if we need to clarify any details or to refer you for dental treatment.

ARE THERE ANY RISKS, HARMS DISCOMFORTS ASSOCIATED WITH THIS STUDY?

Medical research has the potential to introduce psychological, social, emotional and physical risks. Effort should always be put in place to minimize the risks. One potential risk of being in the study is loss of privacy. We will keep everything you tell us as confidential as possible. We will use a code number to identify you in a password-protected computer database and will keep all of our paper records in a locked file cabinet. However, no system of protecting your confidentiality can be absolutely secure, so it is still possible that someone could find out you were in this study and could find out information about you.

Also, answering questions in the interview may be uncomfortable for you. If there are any questions you do not want to answer, you can skip them. You have the right to refuse the interview or any questions asked during the interview.

It may be embarrassing for you to have your mouth examined. We will do everything we can to ensure that this is done in private. Furthermore, all study staff and interviewers are professionals with special training in these examinations/interviews.

You may feel some discomfort during oral examination. In case of an injury, illness or complications related to this study, contact the study staff right away at the number provided at the end of this document. Sterile instruments will be used to examine you to ensure you are safe. The study staff will treat you for minor conditions or refer you when necessary.

ARE THERE ANY BENEFITS BEING IN THIS STUDY?

You may benefit by receiving free oral health check-up and information and you may be advised on where to seek treatment. We will refer you to a hospital for care and support where necessary. Also, the information you provide will help us better understand the reasons babies are born preterm. This information is a contribution to science and could help reduce this happening to other mothers in the future.

WILL BEING IN THIS STUDY COST YOU ANYTHING?

No. being in this study is free, it will not cost you any money.

WILL YOU GET REFUND FOR ANY MONEY SPENT AS PART OF THIS STUDY?

You will not spend any money as the interviewers will come to your hospital and won't require you to buy anything.

WHAT IF YOU HAVE QUESTIONS IN FUTURE?

If you have further questions or concerns about participating in this study, please call or send a text message to the study staff at the number provided at the bottom of this page.

For more information about your rights as a research participant you may contact the Secretary/Chairperson, Kenyatta National Hospital-University of Nairobi Ethics and Research Committee Telephone No. 2726300 Ext. 44102 email uonknh_erc@uonbi.ac.ke.

The study staff will pay you back for your charges to these numbers if the call is for study-related communication.

WHAT ARE YOUR OTHER CHOICES?

Your decision to participate in research is voluntary. You are free to decline participation in the study and you can withdraw from the study at any time without injustice or loss of any benefits.

CONSENT FORM (STATEMENT OF CONSENT)

Participant's statement

I have read this consent form or had the information read to me. I have had the chance to discuss this research study with a study counsellor. I have had my questions answered in a language that I understand. The risks and benefits have been explained to me. I understand that my participation in this study is voluntary and that I may choose to withdraw any time. I freely agree to participate in this research study.

I understand that all efforts will be made to keep information regarding my personal identity confidential. By signing this consent form, I have not given up any of the legal rights that I have as a participant in a research study.

I agree to participate in this research study: Yes /No

I agree to have oral plaque samples preserved for later study: Yes/ No

I agree to provide contact information for follow-up: Yes /No

Participant printed name: _____

Participant signature / Thumb stamp _____ Date _____

Researcher's statement

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has willingly and freely given his/her consent.

Researcher's Name: Dr. David Sumbi Kyale Date: _____

Signature_____

Role in the study: Principal investigator

For more information contact

Principal investigator

Dr. David Kyale Sumbi,

School of Dental Sciences, University of Nairobi,

Tel: 0733314150

Lead Supervisor

Dr. Veronica W. Wangari.

Department of Periodontology/Community and Preventive Dentistry, School of Dental Sciences. University of Nairobi.

Tel: 0721662199

Chairperson,

KNH- UoN ethics review committee

Telephone number: (254-020) 2726300-9

Email: uonknh_erc@uonbi.ac.ke

APPENDIX VI: CONSENT FORM KISWAHILI

UNIVERSITY OF NAIROBI (UoN) COLLEGE OF HEALTH SCIENCES P O BOX 19676 Code 00202 Telegrams: varsity **(254-020) 2726300 Ext 44355**	**KNH-UoN ERC** **Email: uonknh_erc@uonbi.ac.ke** **Website: http://www.erc.uonbi.ac.ke** **Facebook: ttps://www.facebook.com/uonknh.erc** **Twitter: @UONKNH_ERC** **ttps://twitter.com/UONKNH_ERC**	**KENYATTA NATIONAL HOSPITAL (KNH)** **P O BOX 20723 Code 00202** **Tel: 726300-9** **Fax: 725272** **Telegrams: MEDSUP, Nairobi**

FOMU YA RIDHAA

RIDHAA YA MTU MZIMA YA USAJILI WA UTAFITI

Mada ya utafiti: UWEPO NA IDADI YA BAKTERIA FUSOBACTERIUM NUCLEATUM UFIZINI NA UHUSIANO WAKE NA KUZALIWA KWA WATOTO WALIO NA UZANI WA CHINI KATIKA HOSPITALI YA KIAMBU LEVEL 5

Mkuu wa uchunguzi na uhusiano wa taasisi: Daktari David Sumbi Kyale,

Chuo kikuu cha Nairobi

Wachunguzi wenza na uhusiano wa taasisi: Haihusiki

Utangulizi:

Ningetaka kukueleza kuhusu utafiti unaofanywa na watafiti ambao wametajwa hapo juu. Lengo la fomu hii ya ridhaa ni kukuwezesha kufanya uamuzi wa iwapo utashiriki katika utafiti au la. Kuwa mwepesi wa kuuliza swali lolote kuhusiana na lengo la utafiti,nini hufanyika iwapo utashirikio kwenye utafiti, hatari na manufaa ya utafiti, haki yako kama mtu aliyejitolea kwa hiari na jambo jingine lolote kuhusiana na utafiti au fomu hii ambalo halijaeleweka. Baada ya kuyajibu maswali yako vilivyo, waweza kuamua kushiriki kwenye utafiti au kutoshiriki. Mchakato huu unafahamika kama ‘ridhaa inayofahamika’. Pindi tu utakapoelewa na kukubali kuwa kwenye utafiti, nitaomba ulinakili jina lako na kutia sahihi

kwenye fomu hii. Yafaa uelewe sharia za kawaida ambazo hutumiwa na washiriki wote katika utafiti wa kimatibabu: i) Uamuzi wako wa kushiriki ni wa hiari kabisa ii) Waweza kujiondoa kwenye utafiti wakati wowote bila kupatiana sababu ya kufanya hivyo. Iii) Kukataa kushirikio kwenye utafiti hakutaathiri wajibu uanaopaswa kutekeleza katika kituo hiki cha afya ama vituo vinginevyo. Tutakupa nakala ya fomu hii kwa ajili ya rekodi zako

Naweza kuendelea? NDIO / LA

Utafiti huu umeidhinishwa na hospitali ya Kitaifa ya Kenyatta-Kamati ya maadili na utafiti Chuo Kikuu Cha Nairobi, Nambari ya itifaki. _____

Utafiti huu unahusu nini?

Utafiti huu unanuwia kupata uwepo na idadi ya bakteria fusobacterium nucleatum ufizini na uhusiano wake na kuzaliwa kwa watoto walio na uzani wa chini katika hospitali ya Kiambu level 5 .Habari nitakazopata ni sehemu ya utafiti wangu wa tasnifu ambayo ni sehemu ya ukamilifu wa shahada ya uzamili katika upasuaji na afya ya ufizi.

Nitashiriki vipi?

Nitakuuliza maswali kuhusiana na unayofahamu kwenye afya ya kinywa.Nitakiangalia kinywa chako na niyanakili nitakayoyaona. Nitachukua sampuli ya uchafu ulio katika meno na ufizi wa wahusika na baadaye itachunguzwa.Uchunguzi utafanywa kwa kutumia vifaa safi na hakuna shurutisho litakalofanywa.

NI NINI KITAKACHOFANYIKA IWAPO UTAAMUA KUWEKO KWENYE UTAFITI?

Iwapo utakubali kushiriki kwenye utafiti, mambo yafuatayo yatafanyika:

Utahojiwa na mtu ambaye amepitia mafunzo katika mahali pa siri ambapo utaweza kuyajibu maswali. Mahojiano hayo yatachukuwa yapata muda wa dakika tano. Mahojiano hayo

yatahusisha mada kama vile usafi kinywani na ufahamu wa usafi kinywani na jinsi ya kufanya usafi huo. Mahojiano yalikamilika utachunguzwa mdomoni na daktari.

Tutakuuliza utupe nambari ya simu ambayo tutatumia kuwasiliana iwapo tutahitajika kufanya hivyo.Ukikubali kutupa nambari ya simu itatumiwa tu na watafiti katika utafiti huu na kamwe haitapewa mtu mwingine yeyote.Sababu yetu kuchukua nambari yako ya simu ni ili tuweze kuwasiliana nawe iwapo data itapotea.

JE, KUNA HATARI ZOZOTE AU MADHARA YANAYOHUSISHWA NA UTAFITI HUU?

Utafiti wa kimatibabu una uwezo wa kusababisha hatari za kisaikolojia, katika mahusiano, hisia na kimwili.Yafaa tujaribu tuwezavyo kupunguza hatari hizo.Hatari moja ambayo yaweza kutokea ni ukosefu wa siri.Yote utakayotuambia yatabaki kuwa siri.Tutatumia kodi fulani kukutambua katika tarakilishi iliyo na neno la siri. Data na nakala zetu zote tutazifungia kwa kabati. Hata hivyo,hakuna chombo cha kuhifadhi siri yako ambacho ni salama kabisa na huenda mtu akafumbua kwamba ulishiriki katika utafiti na apate habari kukuhusu.

Aidhaa kujibu maswali kwenye mahojiano huenda kukawa kugumu kwako.Iwapo kuna maswali hutaki kujibu waweza kuyaacha.Una haki ya kukataa mahojiano au swali lolote litakaloulizwa kwenye mahojiano.

Inawezekana liwe ni jambo la aibu kwako kufanyiwa uchunguzi.Tutahakikisha ya kwamba yote hayo yatafanyiwa mahali pa siri.Hali kadhalika watakaofanya mahojiano ni watu wenye weledi na ujuzi. Huenda usihisi vizuri wakati wa kukaguliwa kinywani. Pakitokea ya kwamba umejeruhiwa, umekuwa mgonjwa au shida nyingine inayohusiana na utafiti huu imetokea piga nambari utakayoona mwishoni mwa nakala hii haraka iwezekanavyo.Wahudumu watakutibu magonjwa madogo madogo au wakutume kwingineko iwapo itahitajika kufanya hivyo

KUNA MANUFAA YOYOTE KATIKA UTAFITI HUU?

Huenda utafaidika kwa kupata uchunguzi wa ufizi bila malipo.Tutakutuma hospitalini iwapo tutahitajika kufanya hivyo.Habari utakayotupa itasaidia kuelewa vyema uhusiano wa Fusbacterium nucleatum katika ufizi na uhusiano wake na kuzaliwa kwa watoto walio na uzani wa chini. Habari hiyo itachangia ufahamu katika sayansi na nia ya kupata na kudhibitisha ugonjwa kwa njia ya haraka. Ugonjwa ukishadhibitishwa papo hapo na daktari wataweza kuwachunguza zaidi na kuwatibu wagonjwa.

JE KUWEPO KATIKA UTAFITI HUU KUTAKUGHARIMU CHOCHOTE? : HAIHUSIKI

UTARUDISHWA PESA ZOZOTE UTAKAZOTUMIA KATIKA UTAFITI?

Hakuna jambo lolote litakalokupelekea wewe kutumia pesa, lakini iwapo pesa zako zitumike,utaregeshewa .

IWAPO UKUMBANE NA MASWALI SIKU ZA USONI

Iwapo utakuwa na maswali zaidi kuhusu utafiti huu tafadhali piga simu au utume arafa kwa nambari iliyoko mwishoni mwa nakala hii ili kuwasiliana na wahudumu wetu.

Kwa habari Zaidi kuhusu haki yako kama mshiriki wa utafiti waweza kuzungumza na katibu/Mwenye kiti, Hospitali ya Kitaifa ya Kenyatta-Kamati ya maadili na utafiti Chuo Kikuu cha Nairobi, Nambari ya simu 2726300 Ext. 44102 Barua pepe:uonknh_erc@uonbi.ac.ke.

Wahudumu watakulipa hela zako ukishatumia nambari hizi iwapo mawasiliano yatahusu utafiti

CHAGUO LAKO LINGINE NI LIPI?

Uamuzi wako wa kushiriki katika utafiti huu ni wa hiari.Una ruhusa ya kukataa kushiriki katika utafiti na waweza kujiondoa katika utafiti bila hasara yoyote na bila kukiukwa kwa haki yako.

FOMU YA RIDHAA

Kauli ya mshiriki

Nimeisoma fomu hii ya ridhaa ama nimesomewa ujumbe. Nilipata fursa ya kujadiliana kuhusu utafiti huu na mtafiti. Maswali yangu yamejibiwa kwa lugha ambayo naielewa. Nimeelezewa manufaa na hatari ziliwepo. Naelewa kuwa ushiriki wangu kwa utafiti huu ni wa hiari na naweza kujiondoawa wakati wowote.Nimekubali kwa hiari kushiriki katika utafiti huu.

Naelewa juhudi zitafanywa ili kuuhifadhi habari yangu wa kibinafsi.

Kwa kutia sahihi fomu hii ya ridhaa, sijaiacha haki zangu kisheria kama mshiriki katika utafiti.

Nimekubali kushiriki katika utafiti huu: Ndio La

Nimekubali mate yahifadhiwe yatumike baadaye: Ndio La

Nimekubali kupeana nambari za simu ili nifuatiliwe: Ndio La

Jina la mshiriki lililochapishwa: _____

Sahihi ya mshiriki / alama ya kidole _____Tarehe _____

Kauli ya mtafiti

Mimi, ambaye nimetia sahihi, nimetoa maelezo kamili kuhusiana na utafiti huu kwa mshiriki ambaye ametajwa hapo juuna naamini ya kwamba mshiriki ameelewa na akatoa ridhaa yake kwa hiari.

Jina la mtafiti: Dr. David Sumbi Kyale **Tarehe**: _____

Sahihi _____

Kazi yake katika utafiti: Mkuu wa uchunguzi

Kwa habari zaidi zungumza na:

Mkuu wa Uchunguzi

Dr. David Sumbi Kyale

Shule ya kisayansi ya meno, Chuo Kikuu Cha Nairobi,

Nambari ya simu: 0733314150.

Msimamizi mkuu

Daktari. Veronica W. Wangari.

Department of Periodontology/Community and Preventive Dentistry, School of Dental Sciences, University of Nairobi

Nambari ya simu: 0721662199

Katibu/ Mwenyekiti

Hospitali ya Kitaifa ya Kenyatta-Kamati ya maadili na utafiti Chuo Kikuu Cha Nairobi,

Nambari ya simu. (254-020) 2726300-9

Barua pepe: uonknh_erc@uonbi.ac.ke.

APPENDIX VII: ETHICAL APPROVAL

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

KENYATTA NATIONAL HOSPITAL APPROVED 25 JAN 2019 KNH/UoN-ERC P. O. BOX 20723 - 00202 NAIROBI

Ref: KNH-ERC/A/26

25th January, 2019

Dr. David Sumbi Kyale
Reg. No. V60/88693/16
Dept. of Periodontology/ Community and Preventive Density
School of Dental Sciences
College of Health Sciences
University of Nairobi

Dear Dr. Kyale,

RESEARCH PROPOSAL – SUBGINGIVAL FUSOBACTERIUM NUCLEATUM AND PRETERM LOW BIRTH WEIGHT AMONG POSTPARTUM WOMEN AT KIAMBU LEVEL 5 HOSPITAL (P650/09/2018)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above research proposal. The approval period is 25th January 2019 – 24th January 2020.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
f) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
g) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Yours sincerely,

PROF. M. L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Director, CS, KNH
The Chairperson, KNH- UoN ERC
The Assistant Director, Health Information, KNH
The Dean, School of Dental Sciences, UoN
The Chair, Dept. of Periodontology/ Community and Preventive Dentistry, UoN
Supervisors: Dr. Veronica W. Wangari, Dr. Hudson Alumera, Prof. Loice W. Gathece

COUNTY GOVERNMENT OF KIAMBU
DEPARTMENT OF HEALTH SERVICES

All correspondence should be addressed to
HEAD HRDU - HEALTH DEPARTMENT
Email address: mndiritu@gmail.com
mkwasa@live.com
Mobile: 0721641516
0721974633

HEALTH RESEARCH AND DEVELOPMENT UNIT
P. O. BOX 2344 - 00900
KIAMBU

Ref. No: KIAMBU/HRDU/AUTHO/2019/01/30/Kyale DS

Date: 30 Jan 2019

TO WHOM IT MAY CONCERN,

RE: CLEARANCE TO CONDUCT RESEARCH IN KIAMBU COUNTY

Kindly note that we have received a request by **Dr. David Sumbi Kyale** of **University Of Nairobi** to carry out research in Kiambu County, the research topic being on ***"Subgingival Fusobacterium Nucleatum And Preterm Low Birth Weight Among Postpartum Women At Kiambu Level 5 Hospital"***.

We have duly inspected his documents and found that he has been cleared by **Kenyatta National Hospital-University Of Nairobi** until **24 Jan 2020**. He thus does not need any further clearance with another regulatory body in order to conduct research within the county of Kiambu.

However, it is incumbent upon the facility in which the research is being carried out to ensure that they are conversant with the remit of the study and operate in line with their institutional norms on conducting research. This note also accords him the duty to provide feedback on his research to the county at the conclusion of his research.

DR. M. NDIRITU NDIRANGU
COUNTY HEALTH RESEARCH DEVELOPMENT UNIT
KIAMBU COUNTY