A STUDY OF THE PREVALENCE OF METABOLIC SYNDROME AND ITS ASSOCIATION WITH STRESS AMONG PERIODONTAL DISEASE PATIENTS VISITING KOMFO ANOKYE TEACHING HOSPITAL

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by

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DECLARATION

The experimental work described in this thesis was carried out at the Department of Molecular Medicine, KNUST. This research is entirely my work and has not been submitted or published for any other degree elsewhere.

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ABSTRACT

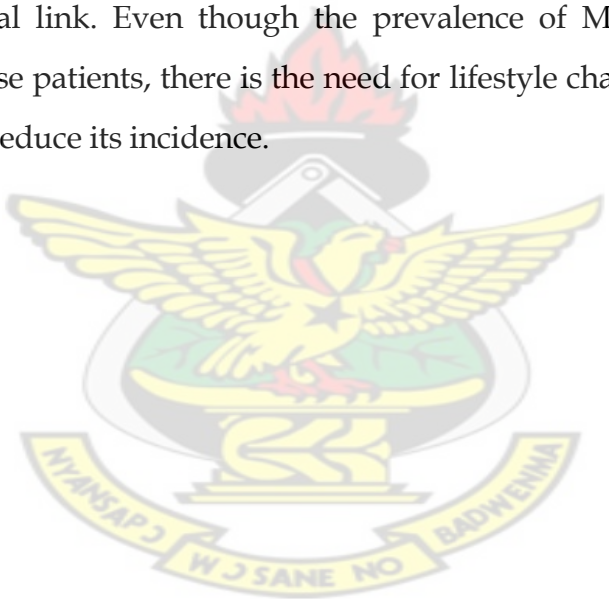
Background: Metabolic Syndrome (Met S) is a cluster of synergistically interacting cardiovascular risk factors consisting of insulin resistance, dyslipidaemia, obesity and hypertension. Studies indicate that Periodontal Disease (PD) shares similar risk factors and root cause with Met S. There are various links between periodontal disease and Met S which presupposes that patients with both clinical conditions may have a higher risk of the development of cardiovascular disease. Globally PD has been associated with Met S however, there is paucity of information on its prevalence among periodontal disease patients in Ghana. This research sought to find the prevalence of Met S in patients with PD and evaluate the relationship between the extent of PD and the various components of Met S.

Materials and Method: This study was conducted at the Restorative Unit of the Department of Oral Health, Komfo Anokye Teaching Hospital (KATH) from March 2011 to February 2012. A total of 302 participants were recruited into the study consisting of 206 patients diagnosed with PD and 96 participants without PD as controls. The participants were selected after having undergone thorough Oral examination and met the criteria for inclusion. The participants then completed a questionnaire that captured their Socio-demographic, Oral Hygiene and PD modifying factors. This study assessed the Lipid Profile, Cardiac Enzymes, cortisol levels and Oxidative Stress among periodontal disease patients using Malondialdehyde, vitamin C and Uric Acid as oxidative stress markers. The haematological profile of each study participant was also evaluated.

Results: Using the World Health Organization (WHO), National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATP III) and International Diabetes Federation (IDF) criteria for defining Met S, the prevalence of Met S among periodontal disease patients were 0.97%, 10.19% and 7.28% respectively. Prevalence of Met S increased with progression of the Periodontal disease with the highest in the advanced periodontitis study population. Hypertension emerged as

the most predictive metabolic risk factor for periodontitis compared to controls from this study using the NCEP ATPIII (50% vs. 37.5%, $p<0.05$) while the WHO (42.72% vs.30.21%) and IDF (45.63% vs. 34.37%) criteria revealed abdominal obesity as the most predictive metabolic risk factor. Neutrophil and Monocyte count among persons with periodontal disease were significantly high in the study population. Cortisol levels were significantly high and increased with disease progression indicating some level of stress. Oxidative stress was associated with periodontal disease as confirmed by the significantly high levels of Malondialdehyde, Uric Acid and low levels of Vit C among the PD patients. Dyslipidaemia was also found to be associated with PD

Conclusion: PD is associated with Met S and cardiovascular disease with Oxidative stress as a mutual link. Even though the prevalence of Met S is small among periodontal disease patients, there is the need for lifestyle changes and a good oral health to further reduce its incidence.



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Chapter 1
INTRODUCTION

1.1 BACKGROUND

Metabolic Syndrome (Met S) is a multisystem disorder characterized by a cluster of metabolic abnormalities that increases the risk of developing Cardiovascular Disease. These abnormalities include Diabetes, Hypertension, Obesity and Dyslipidaemia (Holt *et al.*, 2004; Toalson *et al.*, 2004; Meyer *et al.*, 2005; Blaha and Elasy, 2006; Lieberman *et al.*, 2006). It has been estimated that a quarter of the world's population is affected by Met S (Cameron *et al.*, 2004). The predisposing factors of Met S include genetics, stress, age, lower socioeconomic status, sedentary lifestyle and smoking (Tsigos and Chrousos, 2002). Cardiovascular disease is a major cause of death in the western countries (WHO, 2005).

Prolonged stress has been implicated as an underlying cause of Met S characterized by a disordered hormonal balance of the hypothalamic pituitary-adrenal axis which triggers excess production of cortisol. Cortisol increases blood sugar through gluconeogenesis and also increases blood pressure by increasing sensitivity of the vasculature to epinephrine and nor epinephrine. It causes insulin mediated effects on adipose tissue, ultimately promoting visceral adiposity (Rosmond and Björntorp, 2000).

Periodontal Disease (PD) is a chronic inflammatory disease that affects the Periodontium (tooth supporting tissues) which is associated with apical migration of the junctional epithelium leading to destruction of the connective tissue attachment and alveolar bone loss (Flemming, 1999). Periodontal infection has been implicated as a possible risk factor for serious systemic vascular disease (Kuo *et al.*, 2008). There is evidence linking periodontitis to diabetes (Ford *et al.*, 2007) and CVD (Paquette *et al.*, 2007).

Patients with PD have shown increased levels of inflammatory cytokines such as Tumor Necrosis Factor alpha (TNF-α) and Interleukin 6 (IL-6) which leads to

insulin resistance (Tilg and Moschen, 2008), hypertension, dyslipidaemia and cardiovascular disease (Sesso *et al.*, 2003).

It is important to emphasize the influence of periodontitis on serum or plasma oxidative stress markers in humans. Several studies have demonstrated an increase in the product of oxidative damage in peripheral blood from patients with periodontitis compared with control individuals (Montebugnoli *et al.*, 2004; Baltacioglu *et al.*, 2008). Moreover, there has been evidence of a decreased anti-oxidant capacity in persons with periodontitis evaluated by different assays (Akalin *et al.*, 2007; Chapple *et al.*, 2007). This is in agreement with the report which demonstrates that an increase in reactive oxygen species (ROS) production precedes insulin resistance (Bullon *et al.*, 2009).

Obesity has emerged as a risk indicator of PD (Saito *et al.*, 2004) and individuals with PD reported higher blood pressure than individuals without it (Reeves *et al.*, 2006). Further studies have reported PD to be more prevalent in persons with diabetes and abnormal lipid metabolism (Losche *et al.*, 2000; Noack *et al.*, 2000; Katz *et al.*, 2002b).

The prevalence of metabolic syndrome has been estimated among specific Ghanaian populations; in diabetics, Met S was 55.9 % (Titty *et al.*, 2008), Pregnancy Induced Hypertension 62.0% (Turpin *et al.*, 2008), healthy active sports men 3.9% and in healthy sedentary workers 14.0 % (Owiredu *et al.*, 2011) using the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) criteria. No prevalence study on Met S in periodontal disease patients has been documented in Ghana. This study thus aims to find the prevalence of Met S among Periodontal disease patients and also to evaluate the association between the diagnosis and extent of PD and the various components of Met S.

1.2 JUSTIFICATION

There is evidence linking CVD with periodontal disease as documented by a number of authors. Several studies on the relationships and similar risk factors between Met S and PD have been conducted in other countries. However there is

an acute lack of recent, valid, reliable and comparable data on PD in Ghana where there are differences in cultures, geographical location, genetics and poor dental hygiene. The dentist population is generally low and also access to oral health services that are affordable and of appropriate quality is limited (Charlotte, 2005). This research seeks to find the relationships between Met S, Oxidative stress which predisposes an individual to develop CVD in periodontal disease patients. Knowledge of the prevalence and pathological mechanisms will help influence a healthy way of life through diet, exercise, good sleep and good oral hygiene ultimately ensuring a reduction in its incidence in Ghana. Data derived from this research will also explore the interrelationship of PD, Met S and Oxidative stress among Ghanaians. It will as well strengthen oral health units with the sole aim of improving the diagnosis, treatment and prevention of periodontal disease.

1.3 AIM OF THE STUDY

The primary aim of this study is to assess the prevalence and associations of Metabolic Syndrome, its individual components and oxidative stress among periodontal disease patients using the three major criteria for classifying Metabolic Syndrome namely: World Health Organization (WHO), International Diabetes Federation (IDF) and the National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATP III).

1.4 OBJECTIVES

- To examine the relationship between Met S and its individual components with Periodontal Disease.
- To evaluate the relationship between oxidative stress and Periodontal Disease.
- Find the risk factors associated with periodontal disease and its relation with cardiovascular disease.
- To evaluate the association between the diagnosis and extent of PD and the various components of Met S.

Chapter 2

LITERATURE REVIEW

2.1 PERIODONTITIS

Periodontitis is an inflammatory disease associated with infection from bacteria (Pihlstrom *et al.*, 2005). It is characterized by inflamed gingival, bleeding on probing, resorption of alveolar bone and attachment loss between the tooth and its surrounding alveolar bone. In periodontitis there is an increased secretion of inflammatory mediators leading to disruption of anabolic and catabolic processes starting with modifications in the morphology of periodontal tissue. When this imbalance occurs there is ulceration of the pocket epithelium and also inflammation coupled with healing process which results in local tissue damage of the gingival and periodontium with possible dissemination of microbial products and host inflammatory mediators (Page, 1998).

The inflammatory response in the periodontal tissues in response to the challenge by dental biofilm is complex and involves a network of cytokines functioning in synergy. This could be influenced by either environmental factors or genetics (Heitz-Mayfield, 2005). The inflammatory response is characterized by localized production of various inflammatory markers and enzymes such as C-reactive proteins, Interleukin 1b, Interleukin 6, Tumor Necrosis Factor alpha (TNFα), Prostanoids and Metalloproteinase. These increased inflammatory cytokines are a contributing factor to bone loss in periodontitis (Flemming, 1999).

The balance between the protective host factors and microbial challenge is greatly influenced by environmental and genetic factors that have an impact on the immune-inflammatory response of the host (Kinane *et al.*, 2006).

The risk factors for periodontitis include age, smoking frequencies, diabetes mellitus and a poor oral environment, conditions associated with compromised immune responses (e.g. HIV), nutritional defects, osteoporosis, medications that

cause drug induced gingival overgrowth (e.g. calcium channel blockers, phenytoin, cyclosporin), genetic factors, and local factors (e.g. anatomical deficiencies in the alveolar bone) (Pihlstrom *et al.*, 2005) Stress has also been cited as a risk factor for Periodontitis recently (Khalaf and Shammani, 2005).

2.2 PATHOGENESIS OF PERIODONTAL DISEASE

Periodontal disease is caused by gram negative anaerobic (e.g. *Bacteriodes forsythus*, *Porphyromonas gingivalis*, *Provetella intermedia*) or micro aerophilic bacteria (e.g. *Campylobacter rectus*, *Actinobacillus actinomycetemcomitans*, *Eikenella corrodens*) in the periodontal pockets. However it is uncertain the extent of destruction of periodontal tissue caused by the direct effect of the bacteria and the extent of indirect effects mediated by the host's response to the bacteria. Several bacterial products are found in the periodontal pockets which have a great influence on the periodontium. The products include exotoxins, endotoxins, histiolytic enzymes and factors such as trauma, systemic conditions and diet interfere with cell function but are not necessarily toxic. Sulfides and ammonia released by most bacteria in the periodontal pockets may also be cytotoxic in the periodontium (Slots, 1979). Certain disorders and systemic conditions are now considered as initiators or progressors of periodontal disease rather than the primary causative factors. They make the disease worse by making bacteria activity increase and also impair healing and protection against periodontal infections. Systemic diseases and conditions have been shown to increase risk for periodontal disease. Examples include diabetes, environmental immunosuppresion, immunodeficiency diseases, renal dysfunction, pregnancy and osteoporosis (Garcia *et al.*, 2001). Studies have indicated that about 10% to 15% of the world's population is affected by periodontal disease which represents the greatest cause of tooth loss (Baelum and Lopez, 2004).

2.3 GENETIC AND ENVIRONMENTAL RISK FACTORS OF PERIODONTAL DISEASE

2.3.1 Genetic factors

Periodontal disease is a complex inflammatory disease with various aetiologies which include genetic polymorphism (GP), major histocompactibility complex (MHC) and familial aggregation (FA) (Stabholz *et al.*, 2010). Genes that are known to play a role in the pathogenesis of periodontitis enjoy an association with immune response which affects the expression of interleukin 1, interleukin-6, interleukin-10, FC- gamma receptor, CD14 and vitamin D receptor (loos et al., 2005). Meta-analysis revealed a weak link between interleukin-1 but indicated a strong relationship with polymorphisms associated with FC-gamma receptor genes in periodontal disease patients (Nikolopoulos *et al.*, 2008).

MHC is a group of genes that encodes the human leukocyte antigen responsible for cell recognition. They aid in the activation of T- cell response and antigen activation as well. The binding capacity of antigen peptides is affected when HLA polymorphism occurs thereby affecting antigen-specific T-cell response (Zinkernagel and Doherty, 1997). Patients having aggressive periodontitis enjoyed a positive association with HLA A9 but no association was found with HLA-A2 and HLA-B5 (Stein *et al.*, 2008). Biological mechanisms surrounding HLA association with periodontitis remains inconclusive which require monitoring in further studies.

Family studies conducted by (Marazita *et al.*, 1994) found that siblings of periodontal disease patients often suffered severe periodontitis and suggested autosomal dominant among African American and Caucasians as the mode of inheritance. Van der Velden *et al.* (1993) also conducted a study which suggested genetics as a basis for the development of a less aggressive periodontitis. (Michalowicz *et al.*, 2000) evaluated the contribution of genetic factors to the expression of periodontitis in adults with chronic periodontitis to be 50%.

2.3.2 Smoking

Numerous studies have reported a relationship between smoking and periodontitis (Rivera-Hidalgo, 2003; Laxman and Annaji, 2008).

Reports from other research have shown a higher prevalence of periodontitis and tooth loss among smokers. The effect of smoking has been shown to be dependent on its dosage (Castro *et al.*, 2006). Smoking causes recurrence of periodontitis even during treatment. Smoking affects gingival tissues, the ecology of micro-organisms, inflammatory and immunologic responses as well as healing potential of periodontal tissue (Palmer *et al.*, 2005). A calculated prevalence of periodontitis among US adult current smokers was 41.9% and that for former smokers was 10.9% (Tomar and Asma, 2000).

2.3.3 Poor Oral hygiene

The level of oral hygiene has been shown to be directly proportional to the prevalence and extent of Periodontal Disease. (Tanner *et al.*, 2005) and (Craig *et al.*, 2003a) both established a statistically significant correlation between plaque and measures of early periodontitis in individuals between 20-40years of age. (Susin and Albander, 2005) reported significantly higher levels of dental plaque and gingival bleeding in individuals with periodontitis compared with age matched controls in a Brazilian population.

2.3.4 Hormonal changes in women

A woman's health needs are unique, although daily brushing and flossing, a healthy diet and regular exercise are important for oral health throughout life. Gingival disease can also be modified by systemic factors such as sex hormones. Elevated levels of these hormones cause as exaggerated response to the dental plaque on the teeth and gums, resulting in inflammation (Mariotti, 1999). Gingival tissues respond to raised levels of progesterone and estrogen by causing vasodilatation and increasing capillary permeability. Hormonal changes during pregnancy can increase gum inflammation which worsens in the second month and gets to a peak in the eight month. Mineral density is reduced after menopause

when there is estrogen deficiency which can lead to bone loss. Certain oral contraceptives which contain synthetic progesterone increase the risk for periodontal disease. High concentrations of these hormones serve as a nutrient for growth of bacteria such as *Prevotalla intermedia* (Amar and Chung, 1994).

2.3.5 Diabetes

Diabetes is considered as a pro-inflammatory state. It is a common endocrine disorder which commonly comes in two forms. Type 1 (insulin dependent) is associated with the destruction of the beta cells of the pancreas leading to hypo production of insulin and type 2 (non insulin dependent) or insulin resistance where tissues do not respond to insulin in circulation . Both types of diabetes have been demonstrated to be associated with periodontal disease (Moore *et al.*, 1999). Duration of diabetes measured by age of onset has been found to be an important risk factor for future periodontal destruction (Thorstensson and Hugoson, 1993). Individuals with poor diabetes control have increased attachment loss compared with well controlled individuals despite similar levels of oral hygiene levels (Grossi and Genco, 1998).

2.3.6 Stress

Several studies have suggested stress, anxiety and depression as potential risk factors affecting periodontal disease (Breivik *et al.*, 1996; Genco *et al.*, 1998; Boyapati and Wang, 2007). Recent work done suggested an interesting association between occupational stress and periodontitis. A large proportion of employed adults together with smoking habits and tooth brushing frequency showed an increase periodontal pocket depth (Freeman and Goss, 1993). Mechanism by which stress affects periodontal disease progression is either through lifestyle habits such as alcohol consumption, smoking, poor nutritional diets, depression and oral hygiene or through certain physiological factors that cause increase of glucocorticoids and cathecolamides which indirectly affects inflammatory and hormonal profiles ultimately leading to a high risk of periodontal disease (da Silva *et al.*, 1998; Boyapati and Wang, 2007). Psychosocial stress may induce the neglect of oral hygiene leading to periodontal disease (Axteilus *et al.*, 1998).

2.4 METABOLIC SYNDROME (MET S)

Met S is a combination of metabolic disorders that occur together and increases the risk of developing Cardiovascular Disease (CVD) (Holt *et al.*, 2004). This condition is also known as Syndrome X, Cardio Metabolic Syndrome or Insulin Resistant Syndrome (Reaven, 1988). The Metabolic disorders include Central and Abdominal obesity, Diabetes, Hypertension and Dyslipidaemia (high triglyceride, low high density lipoprotein HDL-C, high low density lipoprotein cholesterol LDL-C) (Deedwania and Grupta, 2006). Central and abdominal obesity remain the cardinal clinical feature of this syndrome. The specific mechanism and pathophysiology of is not fully understood. Several groups have suggested different criteria for diagnosing this condition (Alberti and Zimmet, 1998; NCEP, 2001). Contributing factors to developing this condition include weight gain, aging, genetics, endocrine disorders such as polycystic ovary syndrome in women of reproductive age and sedentary lifestyle. Central obesity remains the cardinal risk indicator for this syndrome (Saito *et al.*, 2004). Changes in blood lipids associated with Met S are raised Triglycerides, high Low Density Lipoprotein and a reduced High Density Lipoprotein. Met S is associated with excess production of Reactive Oxygen Species causing oxidative stress (imbalance between pro oxidants and antioxidants) which induces insulin resistance and eventually lead to tissue damage (Katsuyuki and Toshiro, 2009). Oxidative stress plays a role in the pathophysiology of CVD (Diaz *et al.*, 1997). The biomarker commonly used as an indicator for lipid peroxidation is plasma concentrations of Malondialdehyde (MDA) a by-product of lipid peroxidation (Nielsen *et al.*, 1997). A study conducted by D'Aiuto *et al.* (2008) found an association between severe periodontitis and Met S in middle aged US citizens. Prevalence of Met S was estimated to be 18%, 34% and 37% for mild, moderate and advanced periodontitis respectively. Recently estimated prevalence of Met S in adults in the united state was approximately 24% (Ford *et al.*, 2002), with the prevalence of obesity and diabetes; the two basic components clearly increasing over the years (Mokdad *et al.*, 2003).

2.5 CRITERIA FOR DIAGNOSIS

2.5.1 IDF- international diabetes federation (2006)

Met S is diagnosed if there is Central obesity (BMI >30kg/m², waist circumference> 90 cm for men or >80 cm for women) and any two of the following:

Raised triglycerides >150mg/dl (1.7mmol/l), reduced HDL cholesterol <40mg/dl (1.03mmol/l) for men and <50mg/dl (1.29 mmol/l) for women, raised blood pressure: systolic BP>130 mmHg or Diastolic BP>85 mmHg, a raised fasting blood glucose >100mg/dl or 5.6mmol/l

2.5.2 WHO- World Health Organization (2006)

The WHO criterion requires the presence of Diabetes mellitus, impaired glucose tolerance insulin resistance and any two of the following:

Blood pressure ≥ 140/90 mmHg, Dyslipidaemia- triglyceride ≥ 1.7mmol/l, high density lipoprotein cholesterol ≤ 0.9mmol/l for men and ≥ 1.0 mmol/l for women, central obesity (BMI >30kg/m², waist to hip ratio >0.90 for men, 0.85 for women)

2.5.3 EGIR- European Group for the study of insulin Resistance(Balkau and Charles, 1999)

The European group for the study of insulin resistance requires insulin resistance and any two or more of the following: Central obesity - waist circumference >94cm for men and ≥ for women. Dyslipidaemia-TG_> 2.0mmol/l and HDL_C <1.0mmol/l. hypertension BP >140/90mmhg and a fasting blood glucose >6.1 mmol/l

2.5.4 National Cholesterol Education Programme Adult Treatment Panel III (NCEP-ATP-III, 2002)

The NCEP ATPIII requires any three of the following: central obesity- waist circumference > 102 cm or 40 inches for men, >88cm or 36inches for women, dyslipidaemia- increased TG ≥1.7mmol/l (150mg/dl), reduced HDL-C <0.90mmol/l(<40mg/dl) for men or <1.0 mmol/l or < 50mg/dl, increased blood pressure ≥130/85mmhg, increased fasting blood glucose levels >6.1 mmol/l

2.6 COMPONENTS OF MET S

2.6.1 Obesity

This is defined as abnormal or excessive fat accumulation that may impair health. It is the fifth leading risk for global death. The global estimate of obesity is about 1.5 billion (WHO, 2008). Obesity is believed to be caused by an imbalance between caloric intake and expenditure. An increased intake of energy dense foods high in fat, salts and sugars and a decrease in physical activity due to sedentary nature of many forms of work causes obesity. Common health consequences of obesity include cardiovascular disease, musculoskeletal disease (e.g. osteoarthritis), diabetes, endometrial and colon cancers. Body mass index (BMI) is commonly used to classify underweight, overweight and obesity in adults. It is defined as a person's weight in kilograms divided by a square of his height (WHO, 2008).

2.6.2 Diabetes

Diabetes is a chronic disease in which there is a high level of glucose in the blood as a result of defects in insulin secretion, insulin action or both. There are three major types of Diabetes namely: Type 1 Diabetes, Type 2 Diabetes and Gestational Diabetes. Symptoms include frequent urination and hunger, fatigue, weight loss, excess thirst and blurry vision. Laboratory test to diagnose and confirm Diabetes include Fasting Blood Glucose level, Glycated Hemoglobin (hemoglobin A1C) and Oral Glucose Tolerance test (Alemzadeh and Ali, 2011). Causes of Diabetes include genetic defects of the β cell, defects in insulin processing or action, endocrine pancreatic defects, endocrinopathies, infections and drugs

2.6.3 Hypertension

This is defined as an arterial disease in which abnormally high blood pressure is the primary symptom. Usually systolic blood pressure of 140 mmHg and a diastolic pressure of 90 mmHg. It can cause abnormal thickening of the heart muscles, kidney failure and brain damage. Although the aetiology of hypertension is not fully known it is said that genetic variations could be a cause (Luft, 1998). Other secondary factors including obesity, insulin resistance, high alcohol intake,

smoking, medication (corticosteroids and birth control pills), stress, sedentary lifestyle and aging increase blood pressure. Obesity being one of the main hypertensionogenic factor was shown to increase systolic blood pressure by 6.5 mmHg with every 10% weight gain (Ashley and Kannel, 1974). Hypertension is a known risk factor for Cardiovascular Disease morbidity and mortality (Rosemond *et al.*, 2007).

2.6.4 Dyslipidaemia

Dyslipidaemia is caused by a single or multiple gene mutation that results in overproduction or defective clearance of Triglycerides and Low Density Lipoprotein or in underproduction of High Density Lipoprotein (Bass *et al., 1993)*. The most common secondary cause is sedentary lifestyle with excessive dietary intake of saturated fat, cholesterol and trans fat. Other causes include diabetes mellitus, chronic kidney disease, hypothyroidism and drugs such as estrogens and progestins, thiazides, and high active retroviral agents. The condition is classically associated with metabolic syndrome. A follow up study from the lipids research clinics demonstrated that HDL-C and triglycerides are better predictors of coronary and cardiovascular mortality in women than total cholesterol (Bass *et al.*, 1993).

2.7 METABOLIC SYNDROME AND PERIODONTAL DISEASE

2.7.1 Diabetes and Periodontal Disease

Diabetes is a global chronic disease that currently affects about 217 million people and has been forecast to affect 366 million by 2030 (Smyth and Heron, 2006). Recent evidence regarding the biologic link between Diabetes and periodontal disease supports diabetes and persistent hyperglycemia leading to an increase in immune inflammatory response to periodontal pathogenic bacteria challenge (Southerland *et al.*, 2006; Nishimura *et al.*, 2007), resulting in a more rapid and severe periodontal tissue destruction. Persistent hyperglycemia causes non enzymatic glycation and oxidation of proteins and lipids and the subsequent formation of advanced glycated end (AGE) products resulting in more rapid and

severe periodontal tissue destruction (Ramasamy *et al.*, 2005). AGE and hyperglycemia are major causes of pathogenesis of diabetic complication (Brownlee and Lecture, 1993). In Individuals with diabetes who also have periodontitis, AGEs with accompanying markers for increased oxidant stress have been demonstrated in human gingival (Schmidt *et al.*, 1996).

The main causative agent of periodontal disease is anaerobic gram negative bacteria such as *Actinobacillus actinomycetemcomitans*, *Bacteroides forsynthus*, and *Porphyromonas gingivalis* (Zambon, 1996). Other bacteria involved in the progression of the disease include *Prevotella intermedia* and *Treponema denticular*. As dental plague increases, inflammation in the gingival is elevated causing periodontal pocket, gingival enlargement, recession, clinical attachment loss and loss of alveolar bone leading to tooth loss eventually (Haffajee and Socransky, 1994). In diabetics periodontal pathogens increases due to high glucose concentrations in saliva. Inflammatory response to the bacteria challenge and toxins produced by some bacteria results in rapid destruction of periodontal tissue. Prolonged hyperglycaemia leads to non-enzymatic glycation and oxidation of proteins and lipids subsequently forming AGEs. AGE induces oxidative stress which could be responsible for the secretion of pro-inflammatory cytokines (TNF-α, IL1β, IL 6) involved in periodontal tissue inflammation and destruction (Ramasamy *et al.*, 2005).

2.7.2 Dyslipidaemia and periodontal disease

There have been a number of reports linking lipid parameters to periodontitis. Craig *et al.* (2003b) and Furukawa *et al.* (2007) found increased total cholesterol, increased triglycerides and a reduced HDL-C to be associated with periodontitis while Katz *et al.* (2002a) and Nibali *et al.* (2007) who conducted a study in Israel and United Kingdom respectively had a strong associated with total and LDL-C cholesterol and a negative

association with HDL-C in men. However there was no association between triglyceride, LDL-C or HDL-C and periodontal status (Katz *et al.*, 2001; Montebugnoli *et al.*, 2004).

2.7.3 Hypertension and periodontitis

Prevalence of high blood pressure in moderate to severe periodontitis among USA adults was 34.3% compared to periodontal healthy individuals 7.6% (Al-Emadi *et al.*, 2006). An Increase in systolic blood pressure has been found to be associated positively with periodontitis (Wakai *et al.*, 1999). Extent of periodontitis and number and depth of periodontal pocket is directly related to high blood pressure as stated by Holmlund *et al.* (2006). Moreover, severity of periodontitis and tooth loss have been found to be significantly and independently associated with increase in blood pressure (Engstrom *et al.*, 2007).

2.7.4 Oxidative Stress

Oxidative stress is defined as imbalance between the production of highly reactive molecular species (reactive oxygen and nitrogen species or free radicals) and anti oxidant defenses (Halliwell, 2007). Increase in the number of Reactive Oxygen Species (ROS) causes a state of oxidative stress which alters the intracellular signaling pathway causing number of diseases such as cardiovascular disease and diabetes (Evans *et al.*, 2003; Camera *et al.*, 2007). However in humans reactive oxygen species can be beneficial as it can be used by the immune system as a way to attack and kill pathogens (Segal, 2005). Chronic periodontitis has been shown to induce systemic inflammation and also cause changes in metabolic activity (Salzberg *et al.*, 2006). A study conducted by Basu *et al.* (2009) linked the onset of periodontitis and systemic inflammation to oxidative stress.

2.7.5 Antioxidants

Antioxidants are substances that prevent the oxidation (transfer of electron or hydrogen from a substance to an oxidizing agent) of other molecules. They are often reducing agents. Examples include vitamin C, vitamin E, and Glutathione as well as enzymes such as Catalase and Superoxide dismutase. There are two main classifications of vitamin antioxidants; water soluble and lipid soluble.

Chapter 3

MATERIALS AND METHODS

3.1 SUBJECTS

The study was conducted at the Restorative unit, Department of Oral Health, Komfo Anokye Teaching Hospital (KATH) Kumasi, Ghana from March 2011 to February 2012. Three hundred and two (302) participants were recruited with 206 being clinically diagnosed periodontal disease patients and 96 participants without PD as controls. The Committee on Human Research Publications and Ethics KATH and KNUST School of Medical Sciences approved the study protocol. Oral examination was done by a Restorative Dental surgeon using WHO Basic Periodontal Examination (BPE) / Community Periodontal Index of Treatment Needs (CPITN) to diagnose the presence of PD and to ascertain the kind of treatment required. Features for Periodontitis such as pocket depth, clinical attachment loss and radiographic bone loss were measured. A radiograph was taken for the various degrees of Periodontitis (Mild, Moderate and Advanced). All recruited participants were between the ages of 18 and 60 years. Participation in this study was voluntary and informed consent was sought from each participant.

3.2 QUESTIONNAIRE AND ANTHROPOMETRIC MEASUREMENTS

A questionnaire that captured age, sex, educational attainment, marital status, religion, frequency of tooth brushing, alcohol intake, smoking habit, frequency of exercise, family history of illness and dental history was administered. Weight, Height, waist circumference, hip circumference, arm circumference and thigh circumference.

Systolic and diastolic blood pressure was taken three times when the participant was relaxed and the average value was recorded.

3.3 INCLUSION CRITERIA

- Participants diagnosed of periodontitis with a pocket depth greater or equal to 3mm.
- Participants between the ages of 18-60 years

3.4 EXCLUSION CRITERIA

- Pregnant women
- Participants on prescribed medication for hypertension, diabetes and hypercholesterolaemia
- Participants with chronic infections e.g. Human Immuno-deficiency Virus (HIV). Hepatitis B and Pulmonary Tuberculosis.

3.5 DIAGNOSING PERIODONTITIS

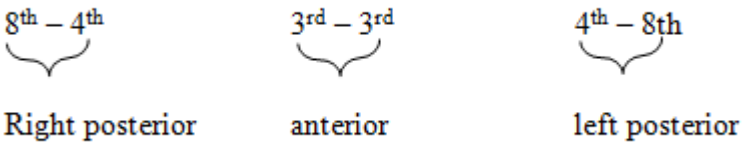
3.5.1 Materials needed

- Functional dental unit-working dental lamp, chair and unit
- Periodontal examination kit-WHO perio probe, tweezer, dental mirror, examination probe, gauze and cotton wool
- Radiographic unit-dental panoramic tomography (Orthopantogram-GENDEX-orthoralix 9200, Milan - Italy), intraoral radiography (Periapical-GENDEX-oralix, Milan Italy).

Patients who reported to the dental unit and had voluntarily agreed to be part of the research were recruited. They were taken through diagnostic procedures and classified into periodontally healthy (controls), mild, moderate and advanced periodontitis, by a qualified dental surgeon.

3.5.2 Diagnostic procedure

Patients were made to sit comfortably in a dental chair and rinse their mouth with potable water in a disposable cup. They were then made to recline in an examination position and their mouths were dried with agauze. A perio probe was then used to measure the pocket depth in six sectants.



For each patient, the teeth was divided into six sides i.e., three buccal (mesio buccal, buccal and disto buccal) and three lingual (mesio lingual, lingual and disto lingual). CPITN probe was placed into the gingival sulcus per patient and pockets depths as well as clinical attachment loss was recorded. (Advanced Periodontitis was diagnosed when one or more teeth with Probing Pocket Depth (PPD) was ≥ 7mm at any site, Moderate Periodontitis was diagnosed when one or more teeth with PPD between 5mm and 7mm was recorded at any site while mild periodontitis was diagnosed when one or more teeth had a PPD between 3mm and 5mm).

3.6 SAMPLING AND LABORATORY INVESTIGATIONS

After periodontitis was confirmed by an orthopantogram and periapical radiograph, 8ml of venous blood sample was collected from study participants after an overnight fast of at least 12 hours between 7 am and 10 am. 5 ml of blood was dispensed into a vacutainer plain tube, 1ml into fluoride oxalate tube and 2 ml into sterile Ethylene Diamine Tetracetic Acid (EDTA) tube. Serum Fasting Blood glucose (FBS) was analyzed spectrophotometrically using fortress® reagents. Serum for biochemical investigations was stored at -20^{o}C after centrifugation at an RCF of 500g for 10 minutes. The biochemical investigations include fasting lipid profile [Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL)], Uric Acid (UA) and Cardiac enzymes

(Creatine Kinase-CK, Lactate Dehydrogenase-LDH, Aspartate Aminotrasferase-AST), were measured on auto-analyzer Roche COBAS Integra® 400 plus system according to the manufacturers instructions (Roche Diagnostics, Germany, West Berlin). Friedewald equation was used to calculate low density lipoprotein cholesterol. Hematological investigations included Full Blood Count (FBC) (Sysmex Corporation Kobe, Japan) and Erythrocyte Sedimentation Rate (ESR).

Quantitative analysis of Cortisol levels was determined using an ELISA kit by Fortress ® diagnostic limited (United Kingdom). Malondialdehyde concentration was estimated using the method described by Kamal *et al.* (1989). Vitamin C (Vit C) was also determined by the micro technique described by McCormick and Green (1994).

3.6.1 Hematological Assays (Sysmex XT 2000i)

Sysmex XT 2000*i*, an automated hematology analyzer (Sysmex Corporation Kobe, Japan) designed to analyze 24 blood parameters was used for this research. Analysis of the White Blood Cell Count (WBC) with an optional detector block based on flow cytometry method using a semi conductor laser where the physiological and chemical characteristics of cells are analyzed. The blood sample was aspirated, measured and diluted to the specified ratio and stained. The sheath flow mechanism improves cell count accuracy and reproducibility. A semi conductor laser beam is emitted to the blood cells passing through the flow cell. The forward scattered light is received by the photodiode and the lateral scattered light and the lateral fluorescent light are received by the photo multiplier tube. The light is converted to electrical pulses, then making it possible to obtain blood cell information. Red Blood Cell count (RBC) and Platelet (PLT) count were analyzed by the RBC detector using hydro dynamic focusing method. Inside the detector the sample is forced from the sample nozzle into the conical chamber after dilution. Passing through the aperture centre, the cells provide nice shape of cells signals. Haemoglobin concentration (HGB) was analyzed by the HGB detector based on the sulfolyser haemoglobin detection method that takes advantage of both

cyanmethhaemoglobin and oxyhaemoglobin methods. Surfactants lyses red blood cell membrane releasing haemoglobin.

3.6.2 Erythrocyte Sedimentation Rate (ESR)

This commonly used but non-specific test measures the spread of sedimentation of red cells in plasma over a period of one hour. The spread is usually dependent on the plasma concentration of large protein e.g. Fibrinogen and immunoglobulin. The ESR is raised in a large variety of systemic inflammatory, neoplastic diseases and in pregnancy. Raised values are found in chronic infections (tuberculosis), myeloma and macroglobulinaemia, connective tissue and disseminated cancer. Raised values are also associated with increased rouleaux formation in peripheral blood film. Lower values occur in polycythaemia Vera due to increased red cell formation. ESR in useful in chronic diseases, it is inexpensive, easy to operate since no electrical power is required. Dispette® tubes made in Switzerland by Quest Scientific AG 6330 (Zug) was used.

3.6.2.1Procedure

- 1 ml of blood was added to a filling reservoir containing 0.25 ml of 3.2% trisodium citrate which was then capped
- Gentle mixing of the tube was done manually with a minimum of 8 inversions
- After ensuring that the blood returned to the bottom section of the reservoir, the cap membrane was penetrated while holding the reservoir firmly with one hand and Dispette tube with the other hand positioned at the 180mm mark.
- The dispette assembly was placed in a leveled stand at 90^{o}C
- Readings were recorded in millimeters at exactly one hour after setting upright

3.7 PROCEDURES FOR BIOCHEMICAL ASSAYS

3.7.1 Fasting Blood Glucose

3.7.1.1 Principle

An Enzymatic indicator test based on the (Barham and Trinder, 1972) quantified by the formation of a pink quinoneimine dye. In this reaction glucose was determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed is catalyzed by peroxidase and reacts with phenol and 4-aminoantipyrine to form the dye indicator.

$$Glucose + O_2 + H_2O \xrightarrow{glucose\ oxidase\ (GOD)} gluconic\ acid + H_2O_2$$

$$H_2O_2 + 4\text{-}aminoantipyrine + Phenol \xrightarrow{Peroxidase\ (POD)} quinoneimine + 4\ H_2O$$

3.7.1.2 Procedure

- 10ml of aqueous glucose standard was taken and added to 1ml glucose reagent
- The same was done for test sample
- The mixture was incubated for 10minutes at 15-25 ^{o}C or in water bath for 5 minutes at 37^{o}C.
- Absorbances of standard and samples were read against a reagent blank at a wavelength of 500nm.

3.7.1.3Calculation

$$Glucose\ concentration = \frac{Sample\ absorbance}{Standard\ absorbance} \times Standard\ concentration$$

3.7.2 Total Cholesterol- An Enzymatic Colorimetric Method

3.7.2.1 Principle

The method for this assay is based on that described by The National Institute of Health Publication number 90-2964 (1990). Cholesterol esterase hydrolyses esters to free cholesterol and fatty acids. The free cholesterol produced plus the preformed cholesterol is then oxidized in the presence of cholesterol oxidase to

cholest-4-en-3-one and hydrogen peroxide. The quinoneimine chromogen, with absorption maximum at 500 nm, is produced when phenol is oxidatively coupled with 4-aminoantipyrine the presence of peroxidase with hydrogen peroxide. The intensity of the final red color is directly proportional to the total cholesterol concentration.

$$\text{Cholesterol esters} + H_2O \xrightarrow{CE} \text{Cholesterol} + RCOOH$$

$$\text{Cholesterol} + O_2 \xrightarrow{CHOD} \text{Cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + 4AAP + \text{phenol} \xrightarrow{POD} \text{Quinoneimine dye} + 4H_2O$$

3.7.3 Triglycerides-An Enzymatic Colorimetric Test

3.7.3.1 Principle

Triglyceride is hydrolyzed by lipoprotein lipase to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide then reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red dye stuff (Barham and Trinder, 1972) that produces a fast linear end point reaction (McGowan *et al.*, 1983). At an absorbance of 500nm, the intensity of the color produced is directly proportional to the concentration of triglycerides in the sample.

$$\text{Triglycerides} + 3H_2O \xrightarrow{LPL} \text{glycerol} + 3RCOOH$$

$$\text{Glycerol} + ATP \xrightarrow{GK} \text{glycerol-3- phosphate} + ADP$$

$$\text{Glycerol-3-phosphate} + +O_2 \xrightarrow{GPO} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-aminophenazone} + \text{4-chlorophenol} \xrightarrow{POD} \text{4-(p-benzoquinone)-phenazone} + 2H_2O + HCL$$

3.7.4 HDL Cholesterol - Homogenous Enzymatic Colorimetric Assay

3.7.4.1 Principle

In the presence of magnesium sulfate and dextran sulfate, water-soluble complexes with LDL, VLDL and chylomicron are formed which are resistant to polyethylene glycol modified enzymes. The cholesterol concentration of HDL cholesterol is

determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino group (approx. 40%). Cholesterol esters are broken quantitatively into free cholesterol and fatty acids by cholesterol esterase. In the presence of oxygen cholesterol is oxidized by cholesterol oxidase to Δ4-cholesterone and hydrogen peroxide. This direct assay meets the 1995 NCEP goals of 13% total analytical error. The color intensity of the blue quinoneimine dye formed is directly proportional to the HDL cholesterol concentration at a wavelength of 600nm.

$$\text{HDL cholesterol ester} + H_2O \xrightarrow{\text{PEG-cholesterol esterase}} \text{HDL-cholesterol} + RCOOH$$

$$\text{HDL-cholesterol} + O_2 \xrightarrow{\text{PEG-cholesterol oxidase}} \Delta 4\text{-Cholesterone} + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{HSDA} + H^+ \xrightarrow{\text{POD}} \text{purple blue pigment} + H_2O$$

3.7.5 LDL Cholesterol- Homogeneous Enzymatic Colorimetric Assay

3.7.5.1 Principle

The LDL-Cholesterol concentration (LDL-C) is calculated from the total cholesterol concentration (TC), HDL-Cholesterol concentration (HDL-C) and the triglycerides concentration (TG) according to Friedewald equation (Friedewald *et al.*, 1972).

$$\text{LDL Cholesterol } (mmol\,L^{-1}) = \frac{TC\ (mmol\,l^{-1}) - TG\ (mmol\,L^{-1}) - HDL\ (mmol\,L^{-1})}{2.2}$$

3.7.6 Uric Acid

3.7.6.1 Principle

Uric acid is converted by oxidation by Uricase to Allantoin and H_2O_2, which under the catalytic influence of peroxidase, oxidizes 3, 5-dichloro-2-hydroxybenzene-sulphonic acid (chlorophenol sulphonic acid) and 4-aminophenazone (4AP) to form a red-violet quinoneimine compound, which is proportional to the amount of uric acid present (Town *et al.*, 1985). Detection of uric acid was done at a wavelength of 293nm.

$$\text{Uric acid} + H_2O + O_2 \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$2H_2O_2 + \text{chlorophenol sulphonic acid} + 4AP \xrightarrow{POD} \text{N-(4-Antipyryl)–3–chloro–5-sulphonate – P – benzo – quinoneimine}$$

3.7.7 Vitamin C

3.7.7.1 Principle

Serum Vitamin C (Vit C) was determined by a micro technique described by McCormick *et al.,* (1994) using Dinitrophenylhydrazine (DNPH). Ascorbic acid in the serum was oxidized by Cu (II) to form dehydroascorbic acid, which reacts with acidic 2, 4-dinitrophenylhydrazine (DNPH) to form a red dihydrazone, whose absorbance was measured at a wavelength of 520 nm.

3.7.7.2 Reagents used

- Ascorbic acid standard-0.1g of ascorbic acid dissolved in 100ml of distilled water
- 10% trichloroacetic acid (TCA) - 10g of TCA dissolved in 100 ml distilled water.
- Dinitrophenylhydrazine (DNPH)-2g of DNPH was added to 0.25g of thiourea and 0.03g of $CuSO_4.5H_2O$ and this was made up to 100ml with 9M H_2SO_4. 50ml of concentrated H_2SO_4 was further added and 150ml of distilled water was finally added.
- 65%H_2SO_4 – 65ml concentrated H_2SO_4 was added to 35ml of distilled water.

3.7.7.3 Procedure

- 0.4 ml of serum was added rapidly to 1.6ml of 10% TCA , mixed thoroughly and allowed to stand at room temperature for 5 minutes
- Same was done for standard
- Mixture was centrifuged at an RCF of 500g for 5-15 minutes
- 1 ml of supernatant was pippetted into a test tube and 0.4ml DNPH added

- Tubes were stoppered and incubated at 37°C for three hours.
- It was then chilled in an ice bath and 1.6ml of cold 65% H_2SO_4 added and mixed thoroughly.
- Mixture was allowed to stand for 30minutes at room temperature and the absorbance of standard and test were read against a blank (1ml 10%TCA + 0.4ml DNPH) at 520nm.

$$Conc.\ T = \frac{Abs\ T}{Abs\ S} \times Conc.\ S$$

Conc. T=*concentration of test*
Abs T=*absorbance of test*
Abs S=*absorbance of standard*
Con. S=*concentration of standard*
Con. S=*concentration of standard*

3.7.8 MALONDIALDEHYDE (MDA)

3.7.8.1 Principle

The method used for this assay was based on that of (Kamal *et al.,* 1989).

- 0.5ml of serum was treated with 2.5ml of 20% Trichloroacetic Acid (TCA) and then 1ml of 0.67% Thiobaturitic Acid (TBA).
- The mixture was incubated at 100°C for 30 minutes
- After cooling the sample was extracted with 4ml n-butanol and centrifuged at 500g for 10 minutes.
- The absorbance of supernatant were measured at 535nm and the results expressed as µmol L^{-1}, using the extinction coefficient of 1.56×10^5 L $mmolcm^{-1}$

Abs = CεL

Abs=*absorbance of the test sample*

C=*concentration of test sample*

ε= *extinction coefficient*

L= *light path (1cm)*

3.7.9 Aspartate Aminotransferase (AST)

3.7.9.1 Principle

ALT catalyses the reaction between L- alanine and 2-oxyglutarate. The pyruvate formed is reduced by NADH in a reaction catalyzed by Lactate Dehydrogenase (LDH) to form L-lactate and NAD^+. Pyridoxal phosphate serves as a coenzyme in the amino transfer reaction. It ensures full enzyme activation.

$$L\text{-}Aspartate + 2\text{–}oxoglutarate \xrightarrow{AST} Oxaloacetate + L-glutamate$$

$$Oxaloacetate + NADH + H^+ \longrightarrow MDH + L-Malate + NAD^+$$

Rate of NADH oxidation is directly proportional to the catalytic AST activity (Bergmeyer *et al.*, 1986). AST is measured at a wavelength of 510nm.

3.7.10 Lactate Dehydrogenase (LDH)

3.7.10.1 Principle

Lactate dehydrogenase catalyses the conversion of L – lactate to pyruvate; NAD+ is reduced to NADH in the process.

$$L-Lactate + NAD^+ \xrightarrow{LDH} Pyruvate + NADH + H+$$

The initial rate formation is directly proportional to the catalytic LDH activity (Van der Heiden *et al.*, 1994). The absorbance was measured at a wavelength of 450nm.

3.7.11 Creatine Kinase (CK)

3.7.11.1 Principle

$$\text{Creatine phosphate} + ADP \xrightarrow{CK} \text{Creatine} + ATP$$

$$ATP + D-\text{glucose} \xrightarrow{HK} ADP + G6P$$

$$G6P + NADP^{+} \longrightarrow D-6-\text{Phosphogluconate} + NADPH + H$$

Rate of NADPH formation is directly proportional to the catalytic CK activity (Horder *et al.*, 1989). The absorbance was measured at 340nm.

3.7.12 Cortisol-Competitive Enzyme Immunoassay- Competitive Immune Assay

3.7.12.1 Principle

The essential reagent required for an enzyme immunoassay include antibody, enzyme antigen conjugate and native antigen. Upon mixing biotinylated antibody, enzyme antigen conjugate and a serum containing the native antigen, a competitive reaction results between the native antigen and the enzyme antigen conjugate for a limited number of antibody binding sites. The interaction is illustrated by the following reaction.

$$^{ENZ}Ag + Ag + Ab_{Btn} \rightleftharpoons AgAb_{Btn} + {}^{ENZ}AgAb_{Btn}$$

Ab_{Btn} – Biotinylated Antibody (Constant quantity)

Ag – Native antigen (variable quantity)

^{ENZ}Ag – Enzyme-antigen conjugate (Constant Quantity)

$AgAb_{Btn}$ – Antigen-Antibody Complex

$^{ENZ}AgAb_{Btn}$ – Enzyme antigen Conjugate- Antibody complex

k_a =Rate constant of association

k_{-a} = Rate constant of disassociation

$k = k_a/k_{-a}$ = Equilibrium constant

The simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration

$AgAb_{Btn} + {}^{ENZ}AgAb_{Btn} + streptavidin_{cw} \longrightarrow$ immobilized complex

$Streptavidin_{cw}$ = streptavidin immobilized on wells

Immobilized complex = sandwich complex bound to the solid surface

The enzyme activity in the antibody- bound fraction is inversely proportional to the native antigen concentration. Several different serum references of known antigen concentration were used to draw a calibration curve from which the unknown antigen concentration was ascertained (Fortress Diagnostic limited [UK]). Absorbance of cortisol was measured at 450nm.

3.8 DATA ANALYSIS

Data from the study was expressed as either mean ± SD (for continous data) or proportions (for nominal data) and expressed in tables or bar graphs. For continous data, comparison of various diseased groups with control group was done using unpaired t-test. Fishcer's exact test was used to compare proportions of disease groups with control group. For all tests, a p-value < 0.05 was considered statistically significant at 95% confidence interval. Statistical analysis was done using Microsoft Excel Spreadsheet (Microsoft, Califonia, USA, www.microsoft.com) and GraphPad Prism version 5 (San Deigo, Califonia, USA www.graphpad.com).

Chapter 4

RESULTS

4.1 DEMOGRAPHICS, ORAL HYGIENE AND DENTAL TREATMENT VARIABLES FOR EACH OF THE STUDIED POPULATIONS

From the study a higher proportion of the case (consisting of mild, moderate and advanced periodontitis) (50.97%) were married as compared to the control group (23.96%). There was a statistical significance among cases with high educational status with a p value < 0.001. The proportion of periodontal disease patients with high education status (33.01%) was low and statistically significant ($p<0.001$) compared to the control group (51.04%). Generally the proportion of married people was increased among the cases with an increased statistical significance as the disease advances. Being married posed a risk for developing periodontitis (Mild - $p<0.001$, Moderate - $p<0.0001$ and Advanced - $p<0.001$). Tooth extraction and tooth filling did not confer any level of protection or risk of developing periodontitis. Only 2.9% of cases had undergone tooth polishing or scaling which was significant as compared to the control group (10.42%) ($p\leq0.05$). Gum inflammation defined as swelling, pain, bleeding of gum and sensitivity was highest and statistically significant as the disease progressed (mild – 92.44%, moderate – 100%, advanced – 100%) compared to control group (88.33%) (Table 1).

Table 4-1 Demographics, Oral Hygiene and Dental Treatment variables in each of the studied populations

	Total N = (302)	**Control N = (96)**	**Case N = (206)**	**Mild N = (119)**	**Moderate N = (60)**	**Advanced N = (27)**
Socio-demographic parameters						
Married	128/302(42.38)	23/96(23.96)	105/206(50.97)***	49/119(41.18)**	39/60(65.0)***	17/27(62.96)***
Christians	287/302(95.03)	93/96(96.87)	194/206(94.17)	116/119(97.48)	55/60(91.67)	23/27(85.19)*
High Education	117/302(38.74)	49/96(51.04)	68/206(33.01)**	49/119(41.18)	12/60(20.0)***	7/27(25.93)*
Alcoholics	143/302(47.35)	43/96(44.79)	100/206(48.54)	61/119(51.26)	33/60(55.0)	6/27(22.22)*
Smokers	12/302(3.97)	1/96(1.04)	11/206(5.34)	6/119(5.04)	4/60(6.67)	1/27(3.70)
Exercise	184/302(60.93)	60/96(62.5)	124/206(60.19)	79/119(66.38)	49/60(81.67)*	12/27(44.44)
Oral hygiene and treatment						
T B	194/302(64.24)	56/96(58.33)	138/206(66.99)	79/119(66.39)	41/60(68.33)	18/27(66.67)
R D V	120/302(39.74)	34/96(35.42)	86/206(41.75)	33/119(27.73)	32/60(53.33)*	21/27(77.78)***
T E	102/302(33.77)	27/96(28.13)	75/206(36.41)	27/119(22.69)	32/60(53.33)	16/27(59.26)
T F	36/302(11.92)	14/96(14.58)	22/206(10.68)	8/119(6.72)	10/60(16.67)	4/27(14.81)
T P	16/302(5.30)	10/96(10.42)	6/206(2.91)*	2/119(1.68)**	2/60(3.33)	2/27(7.41)
G I	277/302(91.72)	80/96(83.33)	197/206(95.63)***	110/119(92.44)	60/60(100)***	27/27(100)*

Socio demographic data and dental variables presented as proportion with percentages in parenthesis. TB- Tooth Brushing, RDV- Regular Dental Visits, TE- Tooth Extraction, TF- Tooth Filling, TP-Tooth Polishing, GI- Gum Inflammation; *-p<0.05, **p<0.001, ***p<0.0001. The proportions in disease group were compared with controls using Fischer's exact test.

4.2 ANTHROPOMETRIC AND BIOCHEMICAL CHARACTERISTICS OF STUDIED POPULATION (TABLE 2)

Mean waist circumference WC (83.67±0.82) cm as a marker of central obesity was significantly higher in the mild and moderate periodontal disease patients compared to the control group (79.13±1.22) cm $p<0.001$. In addition to WC, moderate periodontitis cases exhibited a wider mid arm circumference ($p<0.05$). Even though the Thigh Circumference and BMI of cases (53.86±0.48 and 25.05±0.32) were slightly higher in the case group than in the control group (53.66±0.72 and 24.44±0.56 respectively) it was not statistically significant. The mean systolic (131.90±1.23mmHg) and diastolic (82.82±0.68mmHg) blood pressure was significantly higher in comparison to the control group (124.80±1.23, 80.19±0.99mmHg respectively) ($p<0.0001$ and $p<0.05$ respectively).

Considering the biochemical assays, the mean Triglyceride, Very Low Density Lipoprotein, Uric Acid and Fasting Blood Glucose were significantly higher in the cases as compared to the controls. Mean MDA levels increased and significantly higher with disease progression compared to controls. However in the mean VLDL and Uric acid for mild to moderate periodontitis were significantly raised ($p<0.0001$). Mean AST was significant ($p<0.05$) in moderate and advanced periodontitis compared to controls while mean CK and LDH levels were significantly high ($p<0.05$) in only moderate periodontitis (210.90±18.07 and 208.80±6.40) compared to the controls (169.30±10.44 and 192.20±4.41 respectively)

4.3 PREVALENCE OF METABOLIC RISK FACTORS STRATIFIED BY EXTENT OF PERIODONTAL DISEASE

Table 3 is presented as a proportion with corresponding percentages in parenthesis. Fischer's exact test was used to compare the proportions. Using NCEP ATP III and IDF the prevalence of abdominal obesity in cases (17.46 and 45.63 respectively) were higher compared to the control group (11.46% and 34.37% respectively). Prevalence of raised blood pressure was high in cases using NCEP ATPIII (50%), WHO (14.56%) and IDF (28.64%) but this trend was not statistically significant except for NCEP ATPIII cases ($p<0.05$) and moderate periodontitis

($p<0.001$). Using the WHO criteria, prevalence of hypertriglyceridaemia was significantly raised in only advanced periodontitis ($p<0.05$). In the IDF criteria mild PD showed the highest prevalence of abdominal obesity(47.06%) and the lowest among advanced PD (25.93%), however prevalence of raised fasting blood glucose was highest in the advanced PD (18.52%) and slightly significant ($p<0.05$). Raised triglycerides and raised blood pressure showed a higher prevalence among the case (4.85% and 28.64%) compared with the control (4.17% and 21.88% respectively) but no significance was seen. Prevalence of low HDL Cholesterol in periodontal disease patients as determined by NCEP ATP III, WHO and IDF showed no statistical significance likewise in the prevalence of raised fasting blood glucose using NCEP ATP III and WHO criteria.

Table 4-2 Anthropometric and biochemical characteristics of studied population stratified by severity of Periodontal Disease

Parameters	Total n=(302)	Control n=(96)	Case n=(206)	Mild n=(119)	Moderate n=(60)	Advanced n=(27)
Anthropometric parameters						
WC(cm)	82.21±12.01	79.13±10.04	83.67±11.75**	83.66±12.23**	84.92±11.24**	80.72±10.41
TC(cm)	53.8±6.97	53.66±7.12	53.86±6.92	54.05±6.55	54.52±6.95	51.38±8.23
MAC(cm)	29.01±3.75	28.48±3.41	29.26±3.88	29.33±4.10	29.67±3.58*	27.90±3.26
WHR	1.07±2.19	1.32±3.41	0.87±0.07	0.86±0.07	0.88±0.06	0.88±0.07
BMI(kg/m2)	24.78±5.07	24.44±5.55	25.05±4.53	25.13±4.4	25.45±4.79	23.72±4.48
SBP(mmHg)	129.6±16.31	124.80±12.09	131.90±17.54***	129.7±15.65*	134.20±20.65***	137.10±16.87***
DBP(mmHg)	81.97±9.85	80.19±9.84	82.82±9.77*	81.98±9.04	84.57±10.33**	82.68±11.54
Biochemical parameters						
TC(mmol/l)	4.77±1.05	4.66±1.03	4.81±1.05	4.76±1.04	4.91±1.07	4.85±1.10
TG(mmol/L	0.92±0.38	0.85±0.36	0.95±0.38*	0.92±0.37	0.93±0.33	1.11±0.50**
HDL-C(mmol/L)	1.45±0.40	1.50±0.42	1.43±0.39	1.42±0.41	1.44±0.34	1.42±0.42
LDL-C(mmol/L)	2.91±0.90	2.81±0.93	2.96±0.88	2.91±0.87	3.05±0.96	3.02±0.75
VLDL	1.61±1.67	0.97±1.16	1.92±1.79***	1.92±1.88***	2.21±1.70***	1.20±1.29
CR	3.49±1.24	3.30±1.10	3.58±1.29	3.60±1.51	3.53±0.88	3.58±0.98
FBS(mmol/L)	4.74±0.86	4.58±0.80	4.81±0.87*	4.74±0.78	4.74±0.79	5.30±1.30***
UA(µmol/L)	269.30±92.93	223.50±86.91	291.00±87.82***	292.5±80.38***	294.20±91.93***	276.90±11.90*
CK(U/L)	182±127.7	169.30±102.8	183.40±120.0	176.70±113.7	210.90±140.0*	149.70±83.36
LDH(U/L)	199.20±47.24	192.20±43.39	202.50±48.72	198.40±46.67	208.80±49.54*	207.20±55.98
AST(U/L)	23.30±9.58	21.74±6.55	24.03±10.66	22.84±7.1	25.75±15.57*	25.65±9.92*

Data is presented as mean ±SD. The means in each study group was compared with controls using unpaired t-test .WC-Waist Circumference, TC-Thigh Circumference, MAC- Mid Arm Circumference, WHR-Waist to Hip Ratio, BMI-Body Mass Index, SBP-Systolic Blood Pressure, and DBP-Diastolic Blood Pressure. *p≤0.05,**p≤0.001,p≤0.0001 indicates the level of significance.***

Table 4-3 Prevalence of Metabolic risk factors stratified by severity of Periodontal Disease

Parameter	Total N=(302)	control N=(96)	Case N=(206)	Mild N=(119)	Moderate N=(60)	Advanced N=(27)
NCEP-ATPIII						
Abdominal obesity	47(15.56%)	11(11.46%)	36(17.46%)	20(16.81%)	14(23.33%)	2(7.41%)
Raised Fasting glucose	12(3.97%)	3(3.13%)	9(4.37%)	2(1.68%)	4(6.67%)	3(11.11%)
Raised Triglycerides	17(5.63%)	4(4.17%)	13(6.31%)	7(5.88%)	3(5.0%)	3(11.11%)
Raised Blood pressure	139(46.03%)	36(37.5%)	103(50%)*	57(47.90%)	36(60%)**	10(37.04)
Reduced HDL-C	16(5.30%)	5(5.21%)	11(5.34%)	5(4.20%)	3(5.0%)	3(11.11%)
WHO						
Central Obesity	117(38.74%)	29(30.21%)	88(42.72%)	59(49.58%)	21(35.0%)	8(29.63%)
Raised fasting glucose	12(3.97%)	3(3.13%)	9(4.37%)	2(1.68%)	4(6.67%)	3(11.11%)
Raised Triglycerides	17(5.63%)	3(3.13%)	14(6.80%)	7(5.88%)	3(5.0%)	4(14.81%)*
Reduced HDL-C	24(7.95%)	8(8.33%)	16(7.77)	9(7.56%)	4(6.67%)	3(11.11%)
Raised blood pressure	38(12.58%)	8(8.33%)	30(14.56%)	18(15.13%)	10(16.67%)	2(7.41%)
IDF						
Abdominal obesity	117(38.74%)	33(34.37%)	94(45.63%)	56(47.06%)	21(35.0%)	7(25.93%)
Raised Fasting glucose	16(5.3%)	3(3.13%)	13(6.31%)	4(3.36%)	4(6.67%)	5(18.52%)*
Raised Triglycerides	14(4.64%)	4(4.17%)	10(4.85%)	6(5.04%)	2(3.33%)	2(7.41%)
Raised Blood pressure	80(26.49%)	21(21.88%)	59(28.64%)	31(26.05%)	20(33.33%)	8(29.63%)
Reduced HDL-C	78(25.83%)	29(30.21%)	49(23.79%)	29(24.37%)	12(20.0%)	8(29.63)

Data presented as percentages in parenthesis. *=P value < 0.05. **p value < 0.001 and *** p value < 0.0001. The proportions in disease group were compared with controls using Fischer's exact test.

Table 4-4 Prevalence of Obesity, Hypertension, Diabetes and Dyslipidaemia stratified by extent of Periodontitis

Parameter	Total n=(302)	Control n=(96)	Case n=(206)	Mild n=(119)	Moderate n=(60)	Advanced =(27)
BMI						
Underweight	28(9.3)	11(11.5)	17(8.3)	10(8.4)	2(3.3)	5(18.5)
Normal	149(49.3)	49(51.0)	100(48.5)	55(46.2)	31(51.7)	14(51.9)
Overweight	77(25.5)	22(22.9)	55(26.7)	36(30.3)	16(26.7)**	3(11.1)
Obese	46(15.2)	14(14.6)	32(15.5)	18(15.1)	11(18.3)	3(11.1)
WHR						
Normal	151(50.0)	55(57.3)	96(46.6)	62(52.1)	24(40.0)*	10(37.0)
Overweight	74(24.5)	20(20.8)	54(26.2)	27(22.7)	19(31.7)	8(29.6)
Obese	75(24.8)	20(20.8)	55(26.6)	29(24.4)	17(28.3)	9(33.3)
FBS						
Hyperglycemia	12(3.9)	3(3.1)	9(4.4)	2(1.7)	3(5.0)	4(14.8)*
Impaired glucose	6(1.9)	2(2.1)	4(1.9)	0(0.0)	2(3.3)	2(7.4)
Diabetes	6(1.9)	1(1.0)	5(2.4)	2(1.7)	1(1.7)	2(7.4)
Hypertension	35(13.2)	1(1.0)	34(16.5)***	12(10.1)**	17(28.3)***	5(18.5)**
Dyslipidaemia	2(0.7)	1(1.0)	2(0.9)	0(0.0)	1(1.7)	1(3.7)

WHO recommendation for categorizing BMI was used.BMI < 20kg/m2 as underweight, BMI 20-24.9 as healthy weight, BMI 25-29.9 as overweight and BMI ≥ 30 as obese. Men with WHR < 0.9, 0.9-0.99 and ≥ 1.0 were classified as normal weight, overweight or obese respectively while women were classified in the same category on the basis of WHR of < 0.80.0.80-0.84 and ≥ 0.85. Hyperglycemia = fasting blood glucose ≥ 6.1mmol/l, impaired glucose = fasting blood glucose between 6.1-6.9mmol/l, Diabetes = fasting blood glucose greater or equal to 7.0mmol/l and Dyslipidaemia = TG > 1.7mmol/l and HDL< 0.9mmol/l. Data presented as proportion with corresponding percentages in parenthesis *=P value < 0.05. **p value < 0.001 and *** p value < 0.0001. The proportions in disease group were compared with controls using Fischer's exact test.

4.4 PREVALENCE OF OBESITY, HYPERTENSION, DIABETES AND DYSLIPIDAEMIA

The prevalence of BMI underweight and normal were higher in the control group (11.5% and 51.0%) than the cases(8.3% and 48.5%) while the BMI overweight and obese were higher in the cases (26.7% and 15.5%) compared to the control group (22.9% and 14.6% respectively). However only moderate periodontitis cases who were overweight showed statistical significance ($p<0.001$) compared to controls. Prevalence of normal WHR among the moderate periodontal disease cases was statistically significant ($p<0.05$) compared to the controls. Disease progression correlated with an increase in the prevalence of WHR obese group with no statistical significance shown. Prevalence of WHR is the overweight group increased drastically from mild (22.7%) to moderate (31.7%) periodontitis cases with no statistical significance. Prevalence of hyperglycemia among the advanced periodontal cases was significantly higher (14.8%, $p<0.05$) compared to the controls (3.1%). Among the periodontal disease cases (16.5%), prevalence of hypertension was about sixteen times that of the control group (1.0%) with a statistical significance of $p<0.0001$ (Table 4-4).

4.5 HAEMATOLOGICAL PROFILE OF MALES

Table 4-5 presents the haematological profile of males vs. controls stratified by severity of periodontal disease as mean values ± SD. The mean haemoglobin level and red blood cell count were slightly higher in the cases than in control showing no statistical significance. The mean neutrophil count of cases was higher and statistically significant (48.83±14.38, $p<0.001$) compared to controls (41.4±9.94). The moderate and advanced periodontal disease patients are more likely to have a raised neutrophil count compared to the control group ($p<0.0001$).

Mean monocyte count among the cases (7.42±3.32) was lower than in control group (8.95±2.59). Moderate periodontal disease showed a lower statistical significance ($p<0.001$). Mean ESR values increased with increase in the severity of periodontitis. Advanced stage of periodontitis predisposes an individual to more gum

inflammation followed by moderate and then mild. A high statistical significance was associated with periodontal disease (9.19±8.56) ($p<0.001$).

Table 4-5 Hematological profile of men vs. control stratified by severity of Periodontitis

Parameter	Total n= 143	Control n=33	Case n= 110	Mild n=61	Moderate n=36	Advanced n=13
HBG (g/dL)	15.24±1.05	15.1±0.83	15.28±1.10	15.35±1.09	15.26±1.11	15.02±1.14
RBC (10^6/µL)	5.55±0.55	5.54±0.58	5.55±0.55	5.62±0.58	5.45±0.51	5.51±0.44
PCV (%)	45.49±3.11	44.62±2.77	45.75±3.16	46.21±3.33*	45.29±3.09	44.81±2.15
MCV (fl)	82.53±7.36	81.2±7.30	82.93±7.36	82.78±7.68	83.61±7.29	81.74±6.22
MCH (pg)	27.65±2.35	27.46±2.37	27.71±2.36	27.53±2.48	28.13±2.18	27.36±2.25
MCHC (g/dL)	33.54±1.49	33.89±1.79	33.44±1.38	33.27±1.29	33.69±1.27	33.51±2.01
PLT (10^3/µL)	227.9±64.72	230.6±69.77	227.1±63.45	222.0±67.38	225.7±54.23	225.2±65.23
WBC (10^3/µL)	5.02±1.30	5.18±1.50	4.97±1.24	4.72±1.12	5.33±1.41	5.18±0.99
NEUT (%)	47.13±13.82	41.4±9.94	48.83±14.38**	43.2±9.63	55.58±15.88***	56.98±17.76***
LYMPH (%)	41.97±11.04	44.76±9.35	41.14±11.40	41.8±11.71	41.16±11.22	37.92±10.69*
MONO (%)	7.77±3.22	8.95±2.59	7.42±3.32*	7.46±3.10*	6.76±3.26**	9.05±4.05
EO (%)	3.51±3.56	3.88±3.16	3.4±3.67	3.37±3.35	3.0±3.65	4.68±5.02
BASO (%)	0.77±0.36	0.75±0.30	0.78±0.37	0.82±0.39	0.79±0.35	0.59±0.32
ESR (mm Fall/Hr)	8.17±8.19	4.73±5.64	9.19±8.56**	7.87±7.71*	10.08±8.66**	13.0±11.14**

Continuous Data is presented as mean ± SD. The means in each study group was compared with controls using unpaired t-test. HGB-Haemoglobin, RBC- Red Blood Cell count, PCV-Packed Cell Volume, MCV- Mean Cell Volume, MCH- Mean Cell Haemoglobin, MCHC- Mean Cell Haemoglobin Concentration, PLT- Platelet, WBC- Total White Blood Cell Count, NEUT- Neutrophils, LYMPH- Lymphocytes, MONO-Monocyte, EO- Eocinophil, BASO- Basophil, ESR- Erythrocyte Sedimentation Rate *=*P value* < *0.05. **p value* < *0.001 and *** p value* < *0.0001*

Table 4-6 Hematological profile of females vs. controls stratified by extent periodontal disease

Parameter	Total n=159	Control n= 63	Case n= 96	Mild n= 58	Moderate n= 24	Advanced n= 14
HBG (g/dL)	12.79±1.25	12.58±1.17	12.93±1.28	12.93±1.12	13.27±1.02*	12.33±2.07
RBC (10^6/µL)	4.68±0.52	4.53±0.44	4.77±0.55**	4.76±0.58*	4.79±0.50*	4.81±0.59
PCV (%)	39.15±3.43	38.37±3.13	39.67±3.54*	39.82±3.22*	40.24±2.93*	37.96±5.33
MCV (fl)	83.78±7.52	84.99±6.59	82.97±8.02	83.06±6.40	84.47±7.49	79.82±13.65
MCH (pg)	27.29±2.75	27.84±2.28	26.92±2.98*	26.79±2.42*	27.78±2.32	25.95±5.36
MCHC (g/dL)	32.68±1.47	32.81±1.59	32.6±1.39	32.5±1.34	32.99±1.23	32.32±1.83
PLT (10^3/µL)	258.4±77.41	256.3±89.32	259.8±68.86	264.1±67.99	252.7±48.54	253.4±102.1
WBC (10^3/µL)	5.42±1.48	5.35±1.40	5.46±1.53	5.32±1.39	5.56±1.44	5.88±2.22
NEUT (%)	46.08±11.04	45.43±9.94	46.51±11.74	43.62±9.18	50.94±13.76*	51.19±14.61
LYMPH (%)	42.39±9.31	41.4±9.28	43.04±9.32	43.39±9.05	43.3±10.71	41.02±8.16
MONO (%)	8.05±2.60	8.53±2.40	7.73±2.69	7.69±2.66	6.91±2.35**	9.39±2.85
EO (%)	3.02±2.86	3.38±3.58	2.77±2.25	2.58±1.85	2.47±2.23	4.18±3.41
BASO (%)	0.72±0.39	0.63±0.35	0.78±0.41*	0.79±0.42*	0.85±0.40*	0.58±0.31
ESR (mm Fall/Hr)	18.67±13.45	13.35±11.26	22.2±13.67***	18.05±11.65*	25.71±13.53***	34.23±14.24

*Continuous Data is presented as mean ± SD. The means in each study group was compared with controls using unpaired t-test *=P value < 0.05. **p value < 0.001 and *** p value < 0.0001*

4.6 HAEMATOLOGICAL PROFILE FOR FEMALES

Evaluation of the mean haematological profile of the female population is presented in table 6. From the study females with periodontitis are more prone to inflammation with a mean ESR (22.2±13.67) $p<0.0001$ even though mean ESR for advanced periodontitis was highest no statistical significance was observed. Neutrophil count which gives an indication of a bacterial infection was only significantly high in the moderate periodontitis (50.94±13.76, $p<0.05$) though the mean Neutrophil count among the mild and advanced periodontitis was high as compared to the controls, no significance was observed. The red cell indices had RBC count and PCV level being significantly higher (4.77±0.55, $p<0.001$ and 39.67±3.54, $p<0.05$) with a lower statistical significance for MCH (26.92±2.98, $p<0.05$) among cases compared with the control. Mean MCV (82.97±8.02) and MCHC (32.6±1.39) were slightly lower compared to controls (84.99±6.59, 32.81±1.59 respectively) but showed no statistical significance.

4.7 PREVALENCE OF MET S AND ITS SCORE

The prevalences in percentage of Met S (Table 4-7) were 0.99%, 10.60% and 6.29% using WHO, NCEPATPIII and IDF criteria respectively for the total population. The prevalence was generally higher among the advanced periodontitis group (3.70%, 18.52% and 14.81% for WHO, NCEPATPIII and IDF criteria respectively) compared to the control group (1.04%, 11.46% and 4.17%).the prevalence was about 3 times higher in the advanced cases group than in the control using WHO and IDF. A higher prevalence of Met S was also observed among the moderate group (1.67%, 13.33% and 13.33% for WHO NCEPATPIII and IDF respectively) compared to the control group. However using the WHO, NCEPATPIII and IDF criteria, the mild periodontitis group showed a lower prevalence (0.00%, 6.72% and 2.52% respectively) compared to controls

Individuals without any Met S risk factors (i.e. zero metabolic score) had a lower proportion compared to controls using WHO and NCEPATPIII except for IDF which presented with a higher proportion in only the mild and moderate

individuals. Some individuals who exhibit three or more met S risk factors yet do not have Met S was high in the cases using WHO criteria but shoed no significance. However only advanced periodontitis had a higher proportion of three or more Met S risk factors without Met S which was also not significant.

Table 4-7 Prevalence of Metabolic Syndrome and Metabolic Score among the population stratified by extent of periodontal disease

Parameter		Total N=(302)	Control N=(96)	Case N=(206)	Mild N=(119)	Moderate N=(60)	Advanced N=(27)
Prevalence of Metabolic Syndrome							
WHO		3(0.99)	1(1.04)	2(0.97)	0(0.0)	1(1.67)	1(3.70)
NCEP ATPIII		32(10.60)	11(11.46)	21(10.19)	8(6.72)	8(13.33)	5(18.52)
IDF		19(6.29)	4(4.17)	15(7.28)	3(2.52)	8(13.33)	4(14.81)
Prevalence of clustering of one or two or more components of Metabolic Syndrome							
WHO							
	0	146(48.34)	52(54.17)	94(45.63)	62(52.10)	26(43.33)	6(22.22)**
	1	90(29.80)	26(27.08)	64(31.07)	37(31.09)	14(23.33)	13(48.15)
	2	45(14.90)	15(15.63)	30(14.56)	14(11.76)	12(20.0)	4(14.81)
>2 without Met S		18(5.96)	3(3.13)	15(7.28)	5(4.20)	7(11.67)	3(11.11)
NCEP ATPIII							
	0	123(40.73)	42(43.75)	81(39.32)	49(41.17)	22(36.67)	10(37.04)
	1	77(25.50)	28(29.17)	49(23.79)	34(28.57)	10(16.67)	5(18.52)
	2	69(22.85)	15(15.63)	54(26.21)	28(23.53)	20(33.33)	6(22.22)
>2 without Met S		0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
IDF							
	0	120(39.74)	37(38.54)	83(40.29)	50(42.02)	26(43.33)	7(25.93)
	1	91(30.13)	29(30.21)	62(30.10)	38(31.93)	13(21.67)	11(40.74)
	2	66(21.85)	23(23.96)	43(20.87)	27(22.69)	13(21.67)	3(11.11)
>2 without Met S		3(0.99)	1(1.04)	2(0.97)	0(0.0)	0(0.0)	2(7.41)

Data are presented as proportion with corresponding percentages. The proportions in each disease group were compared with controls using Fischer's exact test

Table 4-8 Prevalence of Metabolic Syndrome stratified by age

Diagnostic criteria	18-24(yrs)	25-34(yrs)	35-44(yrs)	45-54(yrs)	≥55(yrs)
		Control			
NCEP ATP III	0/24(0.0)	4/52(7.69)	4/15(26.67)	2/4(50.0)	1/3(33.33)
WHO	0/24(0.0)	1/52(1.92)	0/15(0.0)	0/4(0.0)	0/3(0.0)
IDF	0/24(0.0)	2/52(3.85)	1/15(6.67)	1/4(25.0)	1/3(33.33)
		Case			
NCEP ATP III	0/35(0.0)	2/65(3.08)	6/56(10.71)	6/39(15.38)	3/11(27.27)
WHO	0/35(0.0)	0/65(0.0)	1/56(1.79)	1/39(2.56)	0/11(0.0)
IDF	0/35(0.0)	0/65(0.0)	5/56(8.93)	5/39(12.82)	4/11(36.36)
		Mild			
NCEP ATP III	0/26(0.0)	1/44(2.27)	2/30(6.67)	2/18(11.11)	0/1(0.0)
WHO	0/26(0.0)	0/44(0.0)	0/30(0.0)	0/18(0.0)	0/1(0.0)
IDF	0/26(0.0)	0/44(0.0)	0/30(0.0)	3/18(16.67)	0/1(0.0)
		Moderate			
NCEP ATP III	0/2(0.0)	1/16(6.25)	1/18(5.56)	3/17(17.65)	3/7(42.86)
WHO	0/2(0.0)	0/16(0.0)	0/18(0.0)	1/17(5.88)	0/7(0.0)
IDF	0/2(0.0)	0/16(0.0)	3/18(16.67)	1/17(5.88)	3/7(42.86)
		Advanced			
NCEP ATP III	1/7(12.50)	0/5(0.0)	3/8(37.5)	1/4(25.0)	0/3(0.0)
WHO	0/7(0.0)	0/5(0.0)	1/8(12.5)	0/4(0.0)	0/3(0.0)
IDF	0/7(0.0)	0/5(0.0)	2/8(25.0)	1/4(25.0)	1/3(33.33)

Data are presented as proportion with corresponding percentages.

4.8 PREVALENCE OF MET S STRATIFIED BY AGE

In fig. 1, using NCEPATPIII prevalence of met S increased from 0.0% among adolescent to 8.2% among young adults to 24.7% in middle adults. WHO Met S prevalence also increased from 0.0% through 0.9% in young adults to 1.2% in middle adults. Similarly in the IDF Met S prevalence rose from 0.0% through 4.1% to 17.3% in middle adults. Distribution of Met S with respect to age was generally higher in females than in males. Highest prevalence was found in young adult men (4.9%) using NCEPATPIII and in middle adult using IDF. The highest prevalence for females with Met S was found to be 47.5% in middle adult group using WHO criteria. No male had Met S using WHO criteria from the study population. Generally prevalence of Met S increased with age.

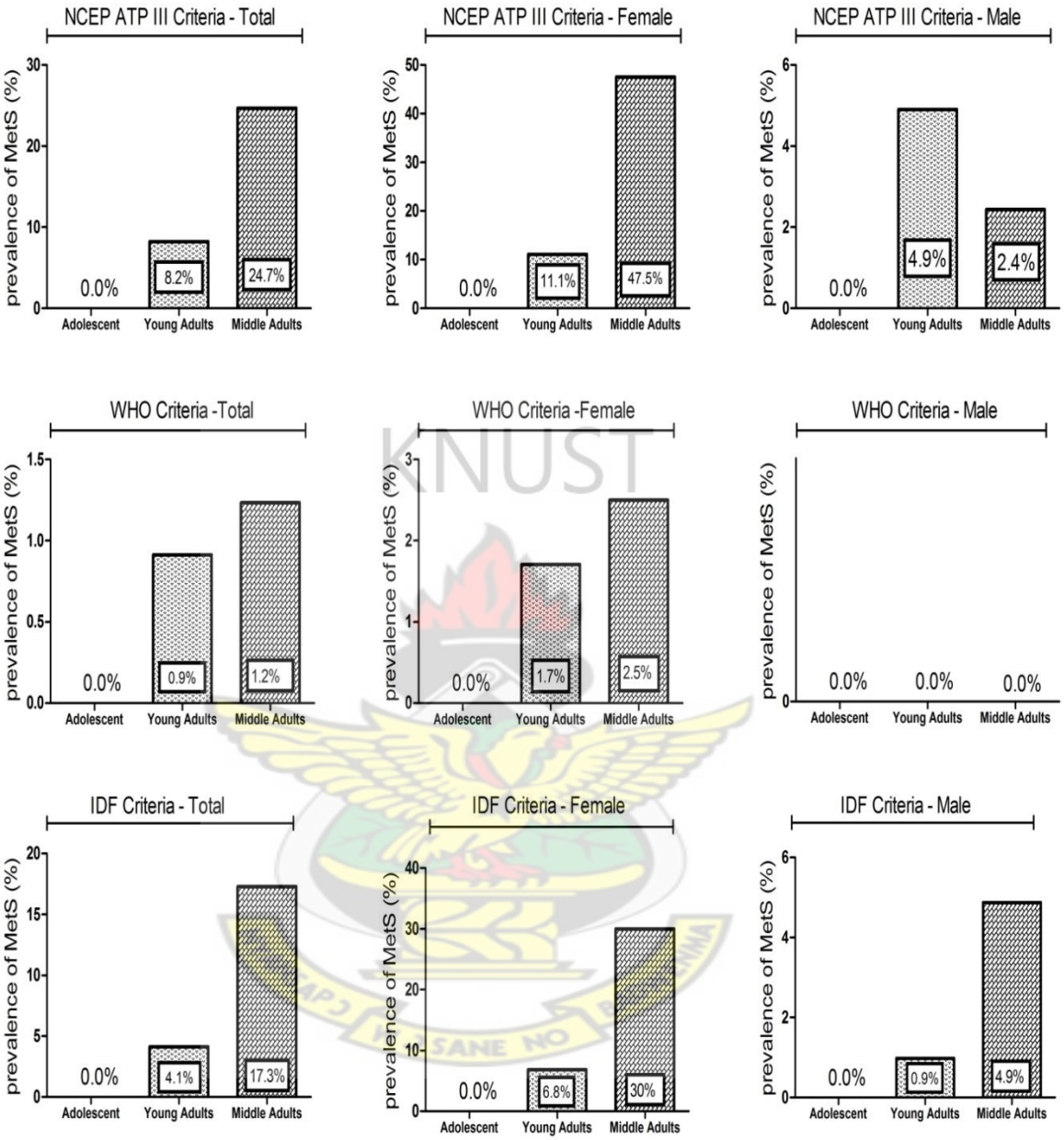


Figure 4-1 Prevalence of Met S using NCEPATP III, WHO and IDF criteria stratified by gender and age group. Adolescent=18-20years, Young adult=21-40, Middle adult >40years

Table 4-9 Oxidative stress markers among individuals with periodontal disease

Parameter	Total N=302	Control N=96	Case N=206	Mild N=119	Moderate N=60	Advanced N=27
MDA (µmol/L)	0.87±0.69	0.65±0.64	0.97±0.69***	0.78±0.66*	0.86±0.58**	1.78±0.16***
VIT C (mg/dL)	2.78±1.91	3.05±1.92	2.65±1.90	2.89±2.26	2.48±1.15	2.04±1.34

Data is presented as mean ± SD. The means in each study group was compared with controls using unpaired t-test. MDA- Malondialdehyde, VIT C vitamin C *p<0.05, **p<0.001, ***p<0.0001

Table 4-10 Cortisol levels among study population stratified by extent of periodontitis

Parameter	Total N=302	Control N=96	Case N=206	Mild N=119	Moderate N=60	Advanced N=27
Cortisol (µg/dL)	10.44±4.81	9.48±3.69	10.89±5.20*	9.72±3.57	12.24±7.22**	13.09±4.68***

Data presented as mean ± SD. The means in each study group was compared with controls using unpaired t-test. *p<0.05, **p<0.001, ***p<0.0001

4.8.1 Oxidative stress and cortisol levels in study population

In table 9 MDA an oxidative stress marker was significantly higher in the cases (mild - 0.78±0.66, p<0.05, moderate-0.86±0.58 p<0.001 and advanced - 1.78±0.16 p<0.0001) than in the controls (0.65±0.64). Mean oxidative stress increased with disease progression. The mean vitamin C was generally lower in the cases compared to the controls and showed no significance which reduced with disease progression.

Cortisol was used as a marker for stress and presented with a higher mean value in the cases especially in the moderate (12.24±7.22) and advanced (13.09±4.68) individuals with a statistical significance (p<0.01 and p<0.001 respectively) when

compared to the control group (9.48±3.69). In effects this study suggests that stress predisposes an individual into developing periodontitis.

Table 4-11 Cardiovascular risk factors for periodontal disease men vs. Control

PARAMETER	TOTAL n=143	CONTROL n=33	CASE n=110	MILD n=61	MODERATE n=36	ADVANCED n=13
CK (U/L)	232.2±156.1	246.9±128.9	227.9±163.6	232.6±177.4	243.9±159.9	161.2±71.31*
LDH (U/L)	202.9±48.84	205.6±56.03	202.1±46.73	199.5±45.29	205.5±43.22	205.4±63.73
TC (mmol/L)	4.652±1.04	4.94±1.15	4.56±0.99	4.42±0.91*	4.83±1.06	4.50±1.04
TRIG (mmol/L)	0.917±0.35	0.963±0.39	0.90±0.33	0.92±0.36	0.86±0.33	0.88±0.13
HDL-C (mmol/L)	1.4±0.37	1.423±0.36	1.39±0.37	1.33±0.36	1.48±0.36	1.38±0.38
LDL-C (mmol/L)	2.852±0.89	3.07±1.16	2.79±0.78	2.71±0.71	2.95±0.91	2.68±0.74
VLDL(mmol/L)	1.934±1.79	0.83±1.21	2.26±0.81***	2.27±2.02***	2.41±1.41***	1.74±1.60*
CR	3.507±1.14	3.70±1.31	3.45±1.09	3.52±1.32	3.35±0.72	3.36±0.71
AST (IU/L)	25.48±11.65	24.34±7.78	25.82±12.59	25.31±8.27	27.44±18.98	23.79±6.24
GLU (mmol/L)	4.754±0.73	4.60±0.73	4.79±0.73	4.77±0.62	4.68±0.76	5.24±0.97*
WT (kg)	68.71±12.16	69.45±13.87	68.49±11.67	68.81±10.62	69.67±13.30	63.71±11.40
HT (cm)	168±17.68	166.90±10.65	168.30±19.31	170.3±16.14	164.8±26.18	168.8±6.32
W C(cm)	79.15±10.63	77.35±14.29	79.69±9.28	79.06±8.90	81.58±9.78	77.46±9.47
H C (cm)	91±9.73	89.41±14.28	91.47±7.97	901.01±8.00	93.21±7.44	88.85±8.81
MAC (cm	28.63±3.10	28.59±3.37	28.65±3.03	28.50±2.83	29.25±3.35	27.69±2.96
T C (cm)	51.51±6.80	50.83±8.25	51.71±6.33	51.31±4.82	53.17±7.47	49.54±8.60
WST/R	0.8707±0.07	0.86±0.10	0.87±0.05	0.86±0.04	0.87±0.06	0.87±0.06
WGT/R	0.7611±1.16	0.81±0.32	0.74±0.08	0.75±0.08	0.73±0.08	0.72±0.08
BMI (kg/m²)	23.58±3.71	24.41±4.40	23.40±3.46	23.26±3.28	24.01±3.65	22.34±3.71
SBP (mmHg)	132.1±14.5	131.10±10.22	132.4±15.58	130.6±11.35	134.4±21.10	135.4±15.25
DBP (mmHg)	81.1±8.31	81.91±6.83	80.86±8.71**	79.68±6.98	83.19±10.37	80.00±10.50

Data presented as mean ± SD. CK- Creatine Kinase, LDH –Lactate Dehydrogenase. TC- Total Cholesterol, TRIG- Triglyceride, HDL-C- High Density Lipoprotein Cholesterol, LDL-C- Low Density Lipoprotein Cholesterol, VLDL- Very Low Density Lipoprotein, CR- Coronary Risk, AST- Aspartate Transaminase, GLU- Glucose WT-Weight, HT-Height, WC- Waist Circumference, HC-Hip Cirmference, MAC- Mid Arm Circumference, TC- Thigh Circumference, WST/HP- Waist to Hip Ratio, WGT/HP- Weight to Hip Ratio, BMI-Body Mass Index, SBP- Systolic Blood Pressure, DBP- Diastolic Blood Pressure.

Table 4-12 Cardiovascular parameters for periodontal disease females vs. Control

Parameter	Total n=158	Control n=63	Case n=95	Mild n=58	Moderate n=24	Advanced n=13
CK (U/L)	139.3±77.79	135.9±83.38	141.6±74.22	132.2±62.56	170.8±84.76*	125.3±91.96
LDH (U/L)	195.8±45.64	186.2±35.30	202.20±50.53	197.1±6.24	208.3±50.25	213.5±62.65*
TC (mmol/L)	4.87±1.05	4.55±0.91	5.08±1.09**	5.04±1.11**	5.14±1.09*	5.13±1.04*
TRIG (mmol/L)	0.91±0.40	0.78±0.32	1.00±0.43***	0.92±39*	1.06±0.39***	1.22±0.60***
HDL-C (mmol/L)	1.50±0.43	1.56±0.44	1.45±0.42	1.48±0.45	1.35±0.31*	1.51±0.47
LDL-C (mmol/L)	2.97±0.91	2.63±0.79	3.18±0.92***	3.12±0.96**	3.30±0.99**	3.26±0.60**
VLDL(mmol/L)	1.32±1.49	1.04±1.15	1.50±0.67	1.54±1.66	1.97±1.90*	0.56±0.27
CR	3.48±1.32	3.08±0.93	3.73±1.47**	3.70±1.70*	3.91±0.03***	3.58±1.12
AST (IU/L)	21.30±6.64	20.29±5.38	21.98±7.31	20.17±4.41	22.03±5.06	29.62±14.07***
GLU (mmol/L)	4.71±0.96	4.55±0.85	4.82±1.02	4.70±0.93	4.73±0.70	5.52±1.60**
WT (kg)	67.44±12.99	63.27±12.18	70.21±12.83***	70.61±12.13**	73.39±13.61**	64.58±12.32
HT (cm)	160.90±6.07	159.6±6.27	160.4±5.95	160.8±6.05	160.50±6.51	158.3±4.13
W C(cm)	85.09±12.47	79.37±10.48	88.88±12.29***	88.84±13.01***	92.04±10.28***	82.23±10.99
H C (cm)	100.30±9.57	97.61±9.24	102.00±9.42**	103.5±9.56***	102.1±7.67*	95.38±9.44
MAC (cm	29.39±4.22	28.02±3.43	30.29±4.47***	30.47±4.88**	30.94±3.41***	28.27±3.82
T C (cm)	55.92±6.46	54.88±6.13	56.61±6.61	57.15±6.77	56.747±5.12	53.88±8.02
WST/R	1.14±2.67	1.55±4.22	0.87±0.08	0.85±0.09	0.90±0.07	0.87±0.07
WGT/R	0.67±0.08	0.65±0.07	0.68±0.08*	0.67±0.0.07*	0.71±0.09**	0.64±0.07
BMI (kg/m²)	29.99±5.50	24.07±6.15	27.26±4.64***	27.29±4.43**	28.41±4.62**	25.03±5.15
SBP (mmHg)	127.60±17.52	121.70±11.94	131.4±19.51***	128.1±17.74*	137.3±23.11***	135.5±17.99***
DBP (mmHg)	82.77±11.05	79.60±11.07	84.86±10.58**	83.66±10.28*	87.88±10.31**	84.69±12.04

Data presented as mean ±SD.*P<0.05, **P<0.001, ***P<0.0001. The means in each study group was compared with controls using unpaired t-test

4.8.2 Cardiovascular parameters of study population

Tables 4-11 and 4-12 presents the cardiovascular parameters among periodontal disease individuals for males and females respectively. Creatine kinase (CK) and lactate dehydrogenase (LDH) were higher in the female case group but was not statistically significance. However mean CK (U/L) among moderate periodontitis in females (170.8±84.76) was statistically significant ($p<0.05$). The remaining cardiac enzyme AST had a mean values higher in the case group compared to controls but the values were not significant for both male and female study population.

Mean total cholesterol for male periodontal disease was lower compared to the control group. However mean cholesterol for females increased with severity of periodontitis without any significance. Among the males triglycerides decreased with severity of the disease compared to the controls. The converse was the case among the females with mean triglycerides (1.00±0.43) mmol/L being significantly higher ($p<0.001$) compared to the controls (0.78±0.32) mmol/L. High density lipoprotein was high among the males in only moderate periodontitis compared to the control but not in the females. Low density lipoprotein was highly significant ($p<0.0001$) among periodontal disease cases (3.18±0.92) mmol/L compared to the control group (2.63±0.79)mmol/L in the female population unlike the male population. Among men with periodontitis, mean very low density lipoprotein (VLDL) was higher and statistically significant in all extent of periodontitis compared to the control. The female population however showed no significance. Waist circumference in the female population was statistically significant in the mild and moderate periodontitis ($p<0.0001$). Mid Arm Circumference (MAC) as well as Body Mass Index (BMI) among the females with periodontitis were significantly higher (30.29±4.47 cm, $p<0.0001$ and 27.26±4.64 kg/m^2, $p<0.0001$) respectively compared to the controls group (28.02±3.43 cm and 24.07±6.15 kg/m^2 respectively). In the male population MAC showed no significance but BMI was significance ($p<0.05$) among the case compared to controls.

Chapter 5

DISCUSSION

5.1 SOCIO-DEMOGRAPHIC AND ORAL HYGIENE CHARACTERISTICS OF PERIODONTAL PATIENTS

Recent studies suggest that every day events such as brushing of teeth and chewing contribute significantly to the cumulative exposure of the vascular system to oral bacteria (Forner *et al.*, 2006). This assertion was in agreement with the findings of this study which established that tooth polishing is a risk factor for periodontitis. D'Aiuto *et al.* (2008) concluded in their study that periodontitis was associated with metabolic syndrome in middle aged individuals which is consistent with the findings of this study (fig. 1).

Considering the outcome from the studies of the relationship between the host and the microbiota of the oral cavity, periodontal disease progression has been strongly linked with important modifying factors such as systemic disease and medication, environmental, sociodemographic and even behavioural conditions (Paulander *et al.*, 2003).

A number of epidemiological studies have assessed the association between educational attainment and periodontal disease (PD), with most, like a study by Nikias *et al.* (1977) establishing that poor educational status are associated with poor oral hygiene. Under such circumstances, the poor oral hygiene status is the most probable cause of the underlying periodontal disease observed. Moreover, studies investigating tooth loss have observed a high tooth loss rate among poorly educated people (Hansen and Johansen, 1977; Berge and Fylkesnes, 1991; Eklund and Burt, 1994; Ahlqwist *et al.*, 1999). Persons with periodontal disease in this study were significantly less educated than those without the disease. Other studies focused on the symptoms of periodontal disease like gingivitis, attachment loss level (Oliver *et al.*, 1998) and pocket depth (Oliver *et al.*, 1991) and found an association with level of education.

Reports on the role played by marital status on the development and progression of PD uvsually support one of two theories. Under one theory regarding 'social network', various studies have established that people low on the social hierarchy regardless of material affluence could suffer more PD just like any other chronic disease (Sheiham and Nicolau, 2005). Under this theory, marriage is considered higher up the hierarchy than not being married which means that married individuals are expected to be at a lower risk. One study to assess the relationship between social network as well as support and periodontal disease among older American adults observed that married and cohabiting individuals had a lower PD prevalence than widowed and divorced individuals (Sabbah *et al.*, 2011), with other studies also supporting this theory (da Silva *et al.*, 1995; Marcenes and Sheiham, 1996; Hugoson *et al.*, 2002; Klinge and Norlund, 2005). Results from this study seems to conflict with this theory however since a significantly higher proportion of Periodontal disease patients were married compared to those without Periodontal Disease. This observation may be explained better by the alternate theory about marital status and PD which proposes that, stress in marriage could predispose married people to PD as against their single counterparts. Stress and depression may induce the neglect of oral hygiene leading to periodontal disease (Axteilus *et al.*, 1998). Furthermore a study by Sabbah *et al.* (2011) among the older American adults reported a low PD risk for individuals with more close friends, and considering that single persons are more likely to dedicate time to making and indulging friends compared to married persons, then the observations made in this study could be put into perspective.

Well documented facts as well as a host of studies have reported smoking to be a fundamental risk factor for PD (Bergstrom *et al.*, 2000; Kinane and Chestnutt, 2000; Ojima *et al.*, 2006). Risk calculations which suggests an odds ratio close to 6.0 for smokers presents a clear evidence for this assertion (Brothwell, 2001). In a ten year study by Hugoson and Rolandsson (2011), non-smokers in the study were found to have, better oral hygiene, less gingivitis, higher alveolar bone

level and lower incidence of severe periodontal disease than smokers. There even appears to be a dose-effect relationship between smoking and the severity of PD (Albandar, 2002). Definite conclusions on smoking and PD from this study cannot be made due to the extremely low number of smokers among the periodontal disease individuals as well as those without PD. The questionnaire also did not capture dosage and length of time of smoking which are risk factors for developing periodontitis. The prevalence of smoking was nonetheless higher among PD patients than controls as expected, probably suggesting that smoking had something to do with the PD observed in the smoking group. The potential mechanism by which smoking may predispose a person to periodontal disease has been proposed in a variety of studies. Available data seems to suggest that individuals who smoke harbour a host of more 'pathogenic' microbial flora compared to non-smokers, which could be the genesis of the smoking-induced PD (Kinane and Chestnutt, 2000). Nicotine has been shown to play a major role in the mechanism. On one side, it has the ability to cause vasoconstriction of blood vessels, hence reducing the clinical signs of gingivitis. On the other side, nicotine has been shown to affect various aspects of the immune system like fibroblast function, chemotaxis and phagocytosis by neutrophils, immunoglobulin production, and even influence cytokine levels (Kinane and Chestnutt, 2000).

The number of individuals who took alcohol and had PD in this study were more than those without PD, though the difference was not statistically significant. A study conducted by Okamoto *et al.* (2006) found no significant difference between alcohol intake and PD but found an association between high alcohol consumption and high tooth loss in the younger age group. However conclusions cannot be made from this study since dosage, frequency of intake and length of time of alcohol consumption was not assessed. In this study, periodontal disease patients indulged in less physical exercise compared to controls, though the difference was not deemed significant. Physical activity and exercising have been proposed to be associated with lower risks of PD.

This link is rather indirect as it exists due to the link physical activity has with chronic diseases like cardiovascular disease, diabetes mellitus, hypertension and obesity. The inversely-related relationship with these chronic diseases, each of which has a direct association with PD could imply that low physical activity is associated with higher PD risks (Bawadi *et al.*, 2011).

5.2 DENTAL CARE AND PERIODONTAL DISEASE

The prevalence of periodontal disease in individuals who brushed their teeth once a day was high compared to persons who brushed twice daily. In effect frequency of tooth brushing has an influence on developing periodontal disease. This is consistent with the study conducted by (Vysniaskaite and Vehkalahi, 2009) confirming that twice daily brushing is a better contributor to periodontal health. Oral cleanliness, which is important for the preservation of oral health encompasses both daily brushing of teeth and regular dental visits, both ensuring that microbial plagues that could accumulate on the teeth and gingivae which could ultimately result in PD is removed (Van Der Weijden and Slot, 2011). A host of other studies have also outlined the important roles played by tooth brushing and regular dental visits in preventing PD (Anaise, 1978; Axelsson and Lindhe, 1978; Anaise, 1979; Burt *et al.*, 1985; Loos *et al.*, 1988; Thornton *et al.*, 1989; Commisso *et al.*, 2011; Crocombe *et al.*, 2011).

The proportion of periodontal disease Patients who had ever had one or more tooth extractions in their lifetime was higher than those without PD. This proportion was nonetheless not significant implying that, tooth extraction had little to do with the PD observed among the cohort of study participant. Tooth extraction however has been postulated to set the tone for future occurrence of PD especially with poor oral hygiene practices after the tooth is extracted (Machtei *et al.*, 1999). The relationship between the effects of tooth filling, polishing and tooth scaling with periodontal health has come under intense debate by experts in the field and various conflicting reports exist. Beirne *et al.* (2000) in their review of nine studies investigating tooth scaling/polishing and PD reported that some of the studies found a significant difference in the

periodontal health of 'scale and polish' participants against control participants, whereas others report no difference between the two groups. Other studies investigated the periodontal health of participants who received scaling and polishing at designated time intervals against those who never received any and those that received scaling and polishing only once. In one study, participants were made to follow oral hygiene instructions after tooth scaling and polishing and their periodontal health status were compared with those without any scaling or polishing. The authors suggested a 5 year (or more) clinical trial to clarify the issue but such a study is yet to be undertaken. The results obtained from this study seems to suggest that tooth polishing is associated with PD, but may require further studies to fully clarify the association. It is important to mention however that some studies suggest that every day events such as brushing of teeth and chewing as well as events like tooth polishing and scaling contribute significantly to the cumulative exposure of the vascular system to oral bacteria (Forner *et al.*, 2006).

Gum inflammation is considered as a symptom for the presence of PD, and gingivitis which is a variant and probably a precursor of PD is also characterized by inflammation of the gum. It comes as no surprise therefore that majority of periodontal disease patients in this study reported ever having a gum inflammation compared to controls. The statistical significance of the observation stresses the pivotal role that gum inflammation had on the PD. It is also important to mention the observation that gum inflammation increased with disease progression. In periodontal disease, the gum pulls away from the teeth forming pockets that become infected. The body's immune system fights the bacteria as the plague spreads and grows below the gum line. The bacteria toxins and the body's natural response to infection start to break down the bone and connective tissues that supports the gum (Pischon *et al.*, 2007).

5.3 ANTHROPOMETRICS AND PERIODONTAL DISEASE

The link between PD and the various indices of overweight and obesity, that is, Waist Circumference (WC), Thigh Circumference (TC), Mid-Arm Circumference (MAC), Waist to Hip Ratio (WHR) and Body Mass Index (BMI) have been extensively studied. Enjoying a direct association with PD, overweight and obesity has been suggested as the second strongest risk factor for PD development and subsequent progression (Pischon *et al.*, 2007). The exact biological mechanism for this association is not well-known, but has been postulated to be mediated by adipose-tissue-derived cytokines as well as hormonal mechanisms (Kershaw and Flier, 2004).

PD risk assessments where obesity is concerned mainly exploits two of the obesity indices: BMI and WC, though WHR may also be included. By W.H.O. criteria, most periodontal disease cases in this study were classified as overweight and hence as expected, their BMIs were higher than that of controls even though the difference was not significant. Several studies have postulated that measures of abdominal obesity like WC and WHR may be better related to PD prevalence than BMI (Al-Zahrani *et al.*, 2003; Kim *et al.*, 2010). Results from this study points in the direction of such postulation since WC was significantly high among Periodontal disease patients than controls, even showing higher proportions among both those with mild and the moderate forms of the disease. These results, together with that obtained for BMI conforms with the findings in a number of studies including that by (Ritchie, 2007); (Pischon *et al.*, 2007); (Morita *et al.*, 2011); (Khader *et al.*, 2009); (Gorman *et al.*, 2012); (Dickie de Castilhos *et al.*, 2012).

Just like BMI, the link between PD and high blood pressure has been extensively studied. The actual mechanism behind the link is unclear and various propositions have been put forward. Chapple *et al.* (2000) and Serne *et al.* (2001) proposed that PD is possible because the microcirculation dysfunction associated with hypertension could affect periodontal tissues. With regards to this postulation, Castelli *et al.* (1978) proposed that hypertension is associated

with vessel intimae and elastic layer thickening that ultimately decreases the lumen of vessels irrigating the periodontal membrane. Furthermore, others propose that the link could be as a result of the side effects that come with the various anti-hypertensive drugs (Nederfors, 1996). In line with the reasoning above, periodontal disease patients in this study were expected to have higher blood pressure levels than controls. This was exactly the trend observed, with periodontal disease patients exhibiting higher systolic blood pressure (SBP) and diastolic blood pressure (DBP) levels than controls. The strong statistical significance associated with the difference in the SBP of the two groups confirms the strength of the association, which compares with a study by Borges-Yáñez *et al.* (2006) who also reported a strong positive associations between SBP and PD.

5.4 DYSLIPIDAEMIA AND PERIODONTAL DISEASE

The commonly observed lipid abnormalities usually related to metabolic diseases include increased levels of Very Low Density Lipoprotein (VLDL), Total Cholesterol (TC) and Triglycerides (TG), and a decreased level of High Density Lipoprotein (HDL) cholesterol levels (Tiejian *et al.*, 2000). Of these metabolic diseases, Cardiovascular Disease (CVD) is the condition most directly associated with these abnormalities or risk factors, and the effect of poor periodontal health status on these risk factors may explain partly the link between PD and increased risk of CVD reported in some studies. A confirmation of this association was made in this study as VLDL, TC and TG levels in periodontal disease patients were higher than controls, and HDL cholesterol levels were lower in the cases. Various mechanisms that have been proposed to explain the PD-CVD association include: pathogen endotoxin effects in the circulation; perturbations in lipid profile as a result of infection; and formation of acute-phase reactants (Tiejian *et al.*, 2000). Association of oral bacteria with platelet aggregation is an important event for thrombosis and presence of oral bacteria in artheromatous plague (GENCO *et al.*, 2002).

5.5 CARDIAC PROFILE AND PERIODONTAL DISEASE

Host responses to periodontal disease include the production of different enzymes that are released by stromal, epithelial or inflammatory cells (Todorovic *et al.*, 2006). The enzymes include Aspartate and Alanine Aminotransferase (AST, ALT), Lactate Dehydrogenase (LDH), Creatine Kinase (CK), Alkaline and Acidic Phosphatase (ALP, ACP), Gamma Glutamyl Transferase (GGT). Changes in the activity of these enzymes reflect metabolic changes in the gingiva and periodontium in inflammation, and therefore their estimation may be useful in both diagnosis and following disease progression in periodontitis. CK, LDH and AST, however are more useful as cardiac enzymes and hence could be indicative of cardiovascular disease. The levels of these enzymes were higher among periodontal disease cases in this study compared to the controls though the differences were not significant except among the patients with moderate periodontitis. There was no observed level of significance as far as the advanced PD group was concerned, probably due to the small number of advanced PD patients in the study.

Other biochemical indices that were higher in the PD patients compared to the controls included Fasting Blood Sugar (FBS) levels, which was highly significant among those presenting with the advanced form of PD. Uric Acid (UA), was significant at all stages of PD, and so was Malondialdehyde (MDA). Vitamin C (Vit C) on the other hand was reduced in the cases compared to the control group and decreased with disease progression with no statistical significance.

5.6 METABOLIC SYNDROME AND PERIODONTAL DISEASE

The primary objective of this study was to evaluate the prevalence of Met S among individuals presenting with Periodontal Disease. It also assessed the association between the various extent of periodontal disease and Met S with its individual components. Prevalence of the Met S among various Ghanaian populations has been reported (Titty *et al.*, 2008; Turpin *et al.*, 2008; Owiredu *et al.*, 2011; Owiredu *et al.*, 2012). Met S was present in PD individuals in this

study when all three criteria for its classification were used, and it increased as the disease progressed through its various stages. Using WHO criteria, the prevalence of Met S was 3.70% in the advanced periodontitis group, whereas the same group gave prevalence rates of 18.52% and 14.81% using the NCEP ATP III and IDF criteria respectively. Similar studies, through cross-sectional surveys have made reports that are consistent with the findings in this study. A few of such studies have assessed a nationally representative sample such as one by D'Aiuto *et al.* (2008) among 13,994 US men and women aged 17 years and above. Another study in Finland by Timonen *et al.* (2010) used 8028 people aged 30 years and above, and a more recent study was conducted among 7178 Korean males and females (Kwon *et al.*, 2011). All of these studies reported various prevalence rates of the Met S among Periodontal disease individuals, with the syndrome increasing with severity of PD. With increasing age, the Met S prevalence rates in this study increased irrespective of the criteria used and were highest among the middle adult age group (> 40 years). These findings are supported by studies by Han *et al.* (2010), Morita *et al.* (2009) and the study by D'Aiuto *et al.* (2008), all of whom reported the Met S prevalence to be highest at ages above 45 years, and also the study by Kwon *et al.* (2011) reported PD to be associated with Met S in people aged above 40 years. Among the middle aged adults with periodontal disease in this study, the Met S was more prevalent in females than male patients. Reports concerning the gender differences as far as Met S and PD are concerned are conflicting, as some studies like that by Han *et al*. (2010) and Morita *et al*. (2009) reports PD to be more strongly associated with Met S in males. Notwithstanding this however, other studies like that by Kwon *et al*. (2011) and Shimazaki *et al.* (2007) have reported the association to be strongest in females, as reported in this study.

The link between PD and Met S has been proposed to exist through a possible common pathophysiological pathway, as both conditions are associated with systemic inflammation and insulin resistance (D'Aiuto *et al.*, 2008). As a result, the associations between PD and the various components of the Met S such as

large WC or obesity, high TG levels and low HDL cholesterol levels (dyslipidaemia), high BP (hypertension), and high FBS levels either separately or in combination have been widely studied. It is evident from this study that the metabolic risk factors assessed namely abdominal obesity, central obesity, raised FBS, raised TG and raised BP, were higher among periodontal disease patients than the controls. Also, as expected, the levels of HDL cholesterol were lower in the diseased population than the control population. Raised blood pressure emerged the most prevalent risk factor using the NCEP-ATP III criteria, and the second most prevalent using both the IDF and WHO criteria. As a result a highly significant number of periodontal cases were reported to have hypertension, which was also prevalent in all stages of the disease. Obesity was the most prevalent risk factor using the WHO and IDF criteria and the second most prevalent using the NCEP-ATP III criteria. The number of periodontal disease patients who were classified as overweight were significantly higher in the moderate form of the disease compared to the control population. The less prevalent risk factors when all three criteria were used were FBS and dyslipidaemia. A high proportion of PD cases presenting with the advanced form of the disease were however hyperglycemic.

5.7 PERIODONTAL DISEASE, PSYCHOSOCIAL STRESS AND OXIDATIVE STRESS

Both psychosocial and oxidative stress have been documented to have critical roles to play in both the development and progression of periodontitis, as well as act as the link between PD and the Met S and cardiovascular disease (Nagappa *et al.*, 2011; Owiredu *et al.*, 2012).

Psychosocial stress and depression have both been proven to be associated with an increased risk of PD (Clarke and Hirsch, 1995; da Silva *et al.*, 1995; Rosania *et al.*, 2009). This is possible since the down-regulation of the host defense mechanisms that protect against PD as a result an impaired immune system caused by the various stress and depression hormones, could facilitate the growth of more opportunistic pathogens in the gingival sulcus (Nagappa *et*

al., 2011). In addition, stress and depression could negatively impact oral hygiene behaviours, such as tooth brushing and flossing which, in turn, can multiply the chain of events that may lead to the severity of, and/or susceptibility to the disease (Rosania *et al.,* 2009). Periodontal disease cases in this study were associated with more stress as indicated by their higher cortisol levels compared to the controls. The difference in cortisol levels between the two groups became highly significant with disease progression as those with the advanced form of the disease had very high cortisol levels. Various studies that have reported similar high cortisol results in Periodontal disease patients include that by Ishisaka *et al.* (2008) among older Japanese adults, Nagappa *et al*. (2011) among an Indian population, Hilgert *et al.* (2006) among a Brazilian population and Genco *et al.* (1998).

It has been established that periodontitis manifests itself as a multifactor phenomenon, one which includes the generation of reactive oxygen species. Oxidative stress in periodontal tissues could thus be the link between PD and the various components of the metabolic syndrome. In this regard, several studies have demonstrated that a pro-oxidant state, characterized by an increase in the products of oxidative damage is associated with persons with periodontitis compared with controls (Battino *et al.,* 2001; Baltacioglu *et al.*, 2006). Other studies have also reported a decreased antioxidant capacity in subjects with periodontal disease (Brock *et al.*, 2004; Chapple *et al.*, 2007). Reasoning can then be made that the increased pro-oxidant state coupled with the decreased antioxidant state in periodontitis could steer a decrease in insulin sensitivity that could affect lipid metabolism especially coupled with a high fat diet that ultimately could lead to cardiovascular disease and Met S (Pischon *et al.*, 2007). It is important to mention however that the whole oxidative stress, Met S, PD and cardiovascular disease form a vicious cycle whose onset could come from any of the four conditions, especially since oxidative stress is also associated with each component of the metabolic syndrome. Hence the fact that the development of a component of the Met S by a previously periodontally

healthy individual could lead to a pro-oxidant state which could in turn diminish the anti-oxidant capacity of periodontal tissues ultimately leading to PD, cannot be ruled out.

In this study, two oxidative stress markers were used: vitamin C, an antioxidant and malondialdehyde, a pro-oxidant that is indicative of lipid peroxidation were assayed. Vitamin C levels were lower among periodontal disease cases in this study compared to controls, and the levels of the antioxidant decreased with disease progression as seen when a look at the mild and moderate against the advanced form of the diseases is made. These results are confirmed by a host of studies that have assessed the antioxidant capacity of periodontal tissues of periodontal disease individuals. A study by Abou Sulaiman and Shehadeh (2010) reported a similar finding, and a very recent follow-up study by Iwasaki *et al.* (2012) among a Japanese older population reported that individuals who took in a dietary antioxidant supplements which included vitamin C were in the subsequent follow up, at a lower risk of periodontitis. The decrease in antioxidant capacity must mean an increase in pro-oxidant levels and the measurement of MDA in both controls vs. diseased individuals confirmed this. As expected, the levels of MDA were significantly higher in the diseased population compared to the control group. The MDA levels were significantly higher as the diseased progressed to the advanced stages.

Uric acid is the end product of purine metabolism in humans. It has antioxidant properties that may be protective but can also be pro-oxidant, depending on its chemical microenvironment. Hyperuricemia predisposes to disease through the formation of urate crystals that cause gout, but hyperuricemia, independent of crystal formation, has also been linked with hypertension, atherosclerosis, insulin resistance, and diabetes (So and Thorens, 2010).

5.8 HEMATOLOGICAL PROFILE IN PERIODONTAL DISEASE

Some studies have hypothesized that chronic periodontitis could lead to reduced hemoglobin levels and erythrocyte count and an improvement in periodontal therapy improves anaemic status in patients (Pradeep and Anuj, 2011; Yamamoto *et al.,* 2011). However the outcome of this study was contrary to these findings. Further studies should be done to confirm this finding.

Neutrophil which is an important first line defense and innate immunity against bacterial infection was raised and highly significant in this study and this is consistent with the findings of Scott and Krauss (2012).

Al Saad Thafeed (2009) found a positive association between periodontitis and high levels of erythrocyte sedimentation rate. This is consistent with the findings in this study where inflammation increased with disease progression estimated by ESR levels (Tables 4-5 and 4-6,).

Chapter 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

From this study it can be proposed that periodontitis could possibly predispose an individual into developing metabolic syndrome. Although the prevalence was quiet low, metabolic syndrome among the study population increased with periodontal disease progression. Using the WHO, NCEP ATP III and IDF, the prevalence of Met S were 0.97%, 10.19% and 7.28% respectively. Based on the findings from this study the possible risk factor for developing periodontal disease was marriage. Also lower educational status and a poor oral hygyiene emerged as possible risk factors for periodontal disease development. The cardinal metabolic syndrome possible risk factors from this study were raised blood pressure, raised Very Low Density Lipoprotein and hypertriglyceridemia. Females in their middle age tend to have a higher prevalence of Met S compared with their male counterparts. This study also supports a relationship between stress and periodontitis.

6.2 RECOMMENDATIONS

- It is recommended that further studies should be done in a larger population of periodontal disease patients in the country. Healthy lifestyle changes such as increased physical activity, low caloric intake and smoking cessation is highly recommended for the management of Met S. Strengthening antioxidant defenses would be a prudent channel in protecting periodontal disease patients against oxidative stress and any other metabolic abnormality.

- It is also necessary to educate patients visiting the dental units in hospitals about the relationships periodontal disease has with Met S which eventually leads to cardiovascular disease hence the need for regular checkups. A good oral hygiene is essential to minimize the

effect of periodontal pathogens in the tooth supporting tissues. Oral hygiene status of participants should be assessed to ascertain whether it has a bearing with the presence of PD and Met S. furthermore patients with PD should seek laboratory investigations to ascertain the presence or absence of Met S to enable corrective measures to be put in place early enough.

- For further studies, microbiological investigations such as identification of periodontal pathogens associated with PD in the Ghanaian population should be assessed.

- Specific inflammatory markers such as IL 1, IL 6, IL 10 and FC-gamma receptor should be evaluated since its expression is known to play a role in the pathogenesis of PD.

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Questionnaire

Prevalence of Metabolic Syndrome and its association with stress among periodontal disease patients visiting Komfo Anokye Teaching Hospital (KATH)

(Tick appropriately)

Study ID: [ ] [ ] [ ]
Laboratory ID: [ ] [ ] [ ]

Patient Name:

Gender: Male - [1]
Female - [2]

Age (*years*):
Blood pressure:

Anthropometry
Height:
Weight;
Waist circumference;
Hip circumference;
Arm circumference;
Thigh circumference;

Family history of chronic disease

Hypertension	[ ]
Diabetes	[ ]
Obesity	[ ]
Others	[ ]

If others specify:

Educational attainment

None	[ ]
Basic	[ ]
Secondary	[ ]
Tertiary	[ ]
Postgraduate	[ ]

Marital status

single	[ ]
married	[ ]
Divorced	[ ]
Widowed	[ ]

Frequency of exercise

Daily	[ ]
weekly	[ ]
monthly	[ ]

(Tick appropriately)

Alcohol consumption;

Occasional	[ ]
Daily	[ ]
Never	[ ]

Smoking habits;

Never	[ ]
Occasional	[ ]
Daily	[ ]
Past	[ ]

Religion;

Christian [ ] Islamic [ ] Traditional [ ]

Oral hygiene variables
(Tick appropriately)

Frequency of tooth brushing

Hardly ever	[ ]
Once daily	[ ]
Twice daily	[ ]
After each meal	[ ]

Regular dental visits

never	[ ]
regular	[ ]
symptom based	[ ]

Tooth polishing	[ ]
Tooth extraction	[ ]
Tooth filling	[ ]
Gum inflammation	[ ]