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Association of Serum Immunoglobulin G (IgG) antibodies against Periodontal Bacteria in Type 2 Diabetes and Pre-Diabetes and Cardiovascular risk factors

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DEDICATION

I would like to dedicate my dissertation work to my parents (Mr. Ram Mohan Shrestha and Mrs. Prabha Shrestha) and all my teachers and mentors who have instilled the inner light and the life values in me and has always been my inspiration. I owe to them like the earth owes to the sun.

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ABSTRACT

Periodontal disease is an inflammatory disease caused by polybacterial infection and inflammation plays key role that associates periodontal disease with different systemic diseases including diabetes and cardiovascular diseases. The main objective of this study is to evaluate and explore the association of serum IgG antibodies in four distinct clusters that were formed empirically from species specific 19 periodontal antibody titers with T2DM and pre-diabetes adjusting for known confounders such as age, sex, race-ethnicity, income to poverty ratio, education, smoking and drinking alcohol, BMI, WC, physical activity, missing teeth, dentist visits and other nutritional factors.

From the first aim, we formed 4 clusters empirically using cluster analysis from 19 periodontal antibody titers and named it as Orange-Red, Red-Green, Yellow-Orange, and Orange-Blue based on Socransky's grouping scheme. Using the priory study model, theoretically we made 3 groups i.e. Etiologic, Putative and Health related groups from 11 periodontal bacteria antibody. On the full adjustments for these known confounders, our finding showed that Orange-Red cluster that included *P gingivalis* and *Prevotella sps* were positively associated in moderate level with diabetes but not with pre-diabetes. However, the Red-Green cluster which contained *T denticola, T forsythia, A actinomycetemcomitans and others (as shown in Flow chart 1)* were inverse but significantly associated to diabetes. The Orange-Blue cluster scores that included *A. naeslundii* and *E. Nodatum* showed an inverse relation to diabetes and pre-diabetes in the

crude analysis but that was attenuated after adjustment [diabetes: OR 0.935 (0.869-1.007), pre-diabetes OR: 0.987 (0.944-1.032) . The Yellow-Orange cluster that included majority of *Steptococcus sps* were not found to be associated with diabetes or pre-diabetes. With the same approach, we found the significant positive association of etiologic group with pre-diabetes among the association with the 3 groups.

In our second aim, we explored the possible interaction between serum IgG antibodies and clinical periodontal destruction (as assessed by clinical attachment loss and pocket depth) in association to diabetes. We found that the tertile of pocket depth modified the association of Orange-Blue cluster with diabetes upon the full adjustment for the known confounders.

In our third aim, we measured the extent of the association between empirically defined 4 clusters and theoretically defined 3 groups with each of the five key components of cardiovascular risk factors i.e. hypertension, hypertriglyceridemia, low HDL-cholesterol, central obesity, and elevated plasma glucose and also the MetS. We found no significant association with any of these components and MetS but the elevated plasma glucose. Elevated Orange-Red clusters was positively associated whereas Orange-Blue was inversely but significantly associated with elevated plasma glucose. The association became more prominent when the elevated plasma glucose greater than or equal to 110 mg/dl.

These findings show that specific periodontal antibody serological markers may be used as predictor of systemic health mainly the diabetes status.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AAP…… American Academy of Periodontology

ADA……..……..... American Diabetes Association

AHA…….American Heart Association

ASVD.....Atherosclerotic vascular disease

BMI.....Body mass index

CAL.....Clinical Attachment Loss

CDC.....Centers for Disease Control and Prevention

CHD.....Cardiovascular diseases

FPG.....Fasting plasma glucose

GDM.....Gestational diabetes mellitus

HbA1C.....Glycemic hemoglobin A1C %

HDL.....High density lipoprotein

HR.....Hazard ratio

IFG.....Impaired fasting glucose

IgA.....Immunoglobulin A

IgG.....Immunoglobulin G

IGT.....Glucose intolerance

IMT.....Intima-media thickness

INVEST.....The Oral Infections and Vascular Disease Epidemiology study

METs.....Metabolic equivalents

MetS.....Metabolic Syndrome

NCEP ATP III.....National Cholesterol Education Program Adult Treatment Panel III

NDDG.....National Diabetes Data Group

NHANES III.....The third National Health and Nutritional Examination Survey

OGTT.....Oral glucose tolerance test

OR.....Odds ratio

PD.....Periodontal disease

RCT.....Randomized controlled trials

RPG.....Random elevated plasma glucose

RR.....Relative risk

SHIP.....the study of health in Pomerania

T1DM.....Type 1 diabetes mellitus

T2DM.....Type 2 diabetes mellitus

WC.....Waist circumference

WHO.....World Health Organization

CHAPTER 1

INTRODUCTION

1.1. Statement of Problem

Periodontal disease [PD] is a persistent polybacterial infection causing chronic inflammation in periodontal tissues (Heller et al. 2012).It is present in up to 1 in 3 adults (Dye 2012) and is characterized by local and systemic inflammation, formation of deep periodontal pockets and destruction of connective tissue attachment , which may eventually lead to tooth loss (Preshaw et al. 2012). Since the onset of disease (dental carries or PD) is usually delayed for prolonged periods of time after initial colonization by pathogens and the source of infection is often endogenous bacteria and initial colonization may persist for years before the development of clinical signs and symptoms, PD is also a hidden and neglected disease. However systemic inflammation resulting from periodontitis has been found to be associated with several chronic inflammatory diseases such as diabetes, vascular disease, and metabolic syndrome [MetS] and is increasingly getting attention.

Although, PD is a polybacterial infection, majority of such association studies have used indirect method to access the periodontal destruction (such as gingivitis bleeding, tissue destruction, gum bleeding, pocket depth or attachment loss) to assess periodontal disease. The problem in using these clinical parameters to measure the periodontal destruction and study with systemic diseases is that these indirect methods cannot measure the systemic effect evoked by these periodontal bacteria challenges. In

periodontal literature, the direct microbial measures of bacterial challenges or host response to periodontitis with these systemic disease are understudied (Lockhart et al. 2012, Demmer et al. 2012, Lalla and Papapanou 2011, Desvarieux et al. 2010).

Recently, the longitudinal and cross-sectional findings from the Oral Infections and Vascular Disease Epidemiology [INVEST] study (Desvarieux et al. 2005, Desvarieux et al. 2013) have shown an association with mean carotid artery thickness by clusters of periodontal bacteria that were classified as etiologic, putative or periodontal health related. The etiologic group (*Actinobacillus actinomycetemcomitans, Tannerella forsythensis, Porphyromonas gingivalis ,and Treponema denticola*) was strongly associated with increased intima-media thickness in the carotid artery. The putative group included *Campylobacter rectus, Prevotella intermedia, Micromonas (Peptostreptococcus) micros, Eikenella corrodens, Fusobacterium nucleatum w*hile *Actinomyces naeslundii* and *Veillonella parvula* were present in the healthy periodontal group.

In the direct assessment of periodontal disease, serological markers such as elevated serum Immunoglobulin A (IgA) or Immunoglobulin G (IgG) levels against those periodontal bacteria (Darveau 2010) can measure host disruption of homeostasis. Studies have reported that elevated antibody titers to some periodontal bacteria are highly correlated with extent and severity of periodontitis while some are associated with the healthy periodontal status. Elevated IgA suggests periodontitis that is in the active phase while elevated IgG generally suggests past exposure to that specific periodontal bacteria. Numerous studies (Holmlund et al. 2011, Pussinen, Alfthan, et al. 2007, Vilkuna-Rautiainen et al. 2006, Pussinen et al. 2005, Pussinen, Jousilahti, et al. 2003) have used

these serological markers to assess periodontal disease and measure the relationship with other chronic diseases, particularly vascular diseases(Pussinen, Paju, et al. 2007, Darveau 2010)(Pussinen, Paju, et al. 2007, Darveau 2010). The clear understanding of the association of serological markers with the systemic outcomes is very important to understand the extent to which the different groups of periodontal pathogens may stimulate immune response in chronic inflammatory disease activity and how these periodontal antibody responses are related to systemic outcomes. Additionally, using serological markers to assess the periodontal relationship with systemic outcomes could be the best way because it measures the direct and cumulative life time systemic effects evoked by the periodontal bacteria.

In this dissertation, we will evaluate the association of serum IgG antibodies against periodontal bacteria with the systemic outcomes mainly type 2 diabetes mellitus (T2DM), and MetS. We used the third National Health and Nutritional Examination Survey (NHANES III 1988-1994) which is a complex, stratified, multistage probability sample designed to provide prevalence estimates describing the health and nutritional status of the civilian, non-institutionalized US population and which included people from different racial/ethnic backgrounds. NHANES III survey has the periodontal IgG antibody titers information for 19 periodontal bacteria which we linked with the periodontal examination, blood laboratory reports and socio-demographic information in 40 years or older age groups.

1.2 Purpose and Objectives

In this dissertation, we cross-sectionally evaluated serum IgG antibody titers against 19 periodontal organisms in relation to T2DM, pre-diabetes and MetS and its

components in an adult population. Periodontal antibodies were grouped by two different approaches. First, we used the data derived clustering approach (empirical approach) to create different clusters, and second, we made three different groups based on a prior study (INVEST study) by Desvarieux et al (2005) (theoretical approach). We hypothesized that antibody clusters against periodontal bacteria were associated with diabetes, pre-diabetes and MetS as PD and these systemic outcomes are interrelated.

Aim1: To cluster the 19 species- specific antibody titers against periodontal pathogens empirically and theoretically into distinct groups and measure the strength of association of these clusters in relation to T2DM, pre-diabetes, and normal plasma glucose levels.

Research question 1.1: What are the clusters obtained from species specific 19 serum IgG antibodies titers?

Research question 1.2: What are the characteristics of individuals in the tertiles of each antibody cluster?

Research question 1.3: What is the relationship between serum antibody titers against periodontal pathogens grouped by empirical and theoretical approaches and type 2 DM, pre-diabetes and normal plasma glucose levels?

Hypothesis: We expect to find out the strength of association of immune response to periodontal antibodies in relation to T2DM, pre-diabetes, and normal.

Aim 2: To investigate the joint association of severity of periodontal destruction (as assessed by clinical attachment loss (CAL) and pocket depth) and antibody clusters in relation to T2DM and normal plasma glucose.

Research question 2.1: What are the profile characteristics of individuals across tertiles of clinical periodontal measures?

Research question 2.2: What is the relationship between serum antibody clusters and T2DM, and normal glucose stratified and jointly classified by the tertiles of clinical attachment loss?

Research question 2.3: What is the relationship between serum antibody clusters and T2DMT2DM, and normal glucose stratified and jointly classified by the tertiles of pocket depth?

Hypothesis: We expect that the strength of associations varies within strata of periodontal destruction.

Aim 3: To explore the association of serum antibodies titer clusters with selected risk markers of cardiovascular disease i.e. MetS and each of its individual components.

Research question 3.1: Is there a cross-sectional association between clusters of antibody titers, MetS and each of key components of MetS (hypertension, hypertriglyceridemia, low high density lipoprotein (HDL)-cholesterol, central obesity, and elevated fasting glucose)?

Research question 3.2: Is there a cross-sectional association between etiologic, putative and health related groups of antibody titers, MetS and each of key components of MetS (hypertension, hypertriglyceridemia, low HDL-cholesterol, central obesity, and elevated fasting glucose)?

Hypothesis: We expect that the specific clusters or groups are associated with these cardiovascular risk markers.

1.3 Significance of Research

One of the main goals of this research is to determine if the clusters or groups of IgG antibody titers are associated with systemic outcomes as evidenced by T2DM and

MetS and to examine the confounding or effect modifier role of clinical periodontal measures in the association. There are numerous bacteria associated with periodontal region as periodontal bacteria coexist in a biofilm. It is therefore difficult to isolate the potential effect of a particular pathogen, and studies evaluating the effects of individual organisms are prone to type 1 error (Wang et al. 2007). To overcome this limitation, we have grouped the periodontal organisms together using both empirical and theoretical approaches. The use of cluster analysis helps to minimize the bias and makes the interpretation easier. Significant findings from both the approaches would provide the additional information on this topic.

In the first aim, we used cluster analysis to group 19 IgG periodontal antibody titers empirically and theoretically and evaluated these in relation to type 2 DM, pre-diabetes, and normal glucose. This will help in the interpretation of the relation between IgG titers of periodontal bacteria and T2DM and pre-diabetes. In the second aim, we examined the joint association between periodontal status based on CAL and Pocket Depth and antibody groups in relation to T2DM and normal glucose. The findings of this specific aim clarify the extent to which the associations between antibody titers to periodontal pathogens and DM are modified by clinical periodontal destruction. Similarly, the MetS defined as a cluster or the concurrence of abdominal obesity, dyslipidemia, hypertension and hyperglycemia according to the consensus of the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) and is associated with a very high risk for cardiovascular diseases and diabetes mellitus (Marchesini et al. 2004, Grundy et al. 2004). The knowledge of the extent of their

association with the antibodies against periodontal disease will add to the information on the systemic disease-periodontal disease relation.

1.4 Outline of the Study

Chapter 2 of this dissertation provides background information about T2DM and pre-diabetes, periodontal disease, the relation between periodontal disease and systemic disease, the relation between antibodies against periodontal disease and systemic disease, and gaps in current knowledge. Chapter 3 is the manuscript for aim 1 'The relation between Serum Immunoglobulin G (IgG) Antibodies against Periodontal Bacteria and Type 2 Diabetes and Pre-Diabetes'. Chapter 4 is the manuscript for aim 2, 'Effect modification of the relation between serological periodontal markers and type2 diabetes by periodontal pocket depth.' Chapter 5 is the manuscript for aim 3, 'Relationship between serological markers of periodontal bacteria and metabolic syndrome and its components'. Chapter 6 is an overall summary of the findings from this dissertation and includes a strengths and limitations, clinical implications and recommendation to clinician and public health professional, suggestions for future research, and conclusions.

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CHAPTER 2

LITERATURE REVIEW

2.1 Hyperglycemia: Diabetes and Pre-diabetes

Diabetes, the excess accumulation of glucose in blood has a negative impact on mortality, morbidity, and quality of life through its long term complications, which include damage and dysfunction of different major organs especially eyes, periodontal region, heart, kidneys, nerves and brain(Zarowitz 2011). It has been declared as a global public health threat to society (Yang et al. 2012, 2012).

"The American Diabetes Association (ADA) defines diabetes as a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion or its action or both" (Zarowitz 2011). Clinically, diabetes is broadly divided into four categories. Type 1 Diabetes Mellitus (T1DM) results from a B-cell destruction that leads to an absolute deficiency of insulin. T2DM which is much more prevalent, occurs when body cells cannot produce enough insulin or do not react properly to insulin. A third category is specific diabetes due to other causes, such as genetic defect of B-cells to produce insulin, genetic defects in insulin action, specific disease of pancreas, or drug/chemical-induced diabetes. The fourth category is gestational diabetes mellitus (GDM), which is diabetes diagnosed during pregnancy (Zarowitz 2011, 2012). Among those who are diabetic, T1DM accounts for approximately 5-10% and is mostly associated with children while T2DM accounts for 90-95% of diabetic cases and occurs

predominantly in adults. Other categories prevalence is in very smaller percentage compared to T1DM and T2DM.

The diagnostic criteria for diabetes developed by the ADA are based on any one of four abnormalities: glycemic hemoglobin A1C % (HbA1C) ≥ 6.5%, fasting plasma glucose (FPG) ≥ 126 mg/dl (0.7 mmol/l), random elevated plasma glucose (RPG) ≥ 200 mg/dl (11.1 mmol/l) with symptoms, or a 2 hour oral glucose tolerance test (OGTT) ≥ 200 mg/dl (11.1 mmol/l) (Zarowitz 2011).

In 1979, the National Diabetes Data Group (NDDG) first introduced the concept of a metabolic state intermediate between normal glucose homeostasis and diabetes, called glucose intolerance (IGT). The expert committee on diagnosis and classification of diabetes later extended this concept in 1997 by recognizing those individuals with impaired fasting glucose (IFG). This intermediate stage [IGT and IFG] were referred as pre-diabetes (Buysschaert and Bergman 2011). Pre-diabetes is a condition when glucose level is not in the diabetic range but is too high to be considered as normal. Individuals diagnosed with pre-diabetes are in increased risk for diabetes as well as cardiovascular and possibly, micro vascular complications (Pour and Dagogo-Jack 2011). Data suggest that 70% of patients with pre-diabetes will eventually develop diabetes (Tabak et al. 2012). According to the ADA clinical practice recommendations, current diagnostic criteria for pre-diabetes are one of these three criteria. FPG: 100 to 125 mg/dL (5.6–6.9 mmol/L), A1C between 5.7%-6.4%, or 2-hour plasma glucose values in the OGTT of 140 –199 mg/dl [7.8 –11.0 mmol/l] (Tabak et al. 2012, Buysschaert and Bergman 2011).

Globally, the prevalence of diabetes and pre-diabetes has dramatically increased with the increase in urbanization, population growth, aging, increasing prevalence of

obesity and physical inactivity. According to the World Health Organization (WHO), the prevalence of diabetes worldwide is 6.4 %, which varies from 10.2% in the Western Pacific to 3.6 % in the African region (Shaw, Sicree, and Zimmet 2010). The prevalence of diabetes has been consistently increasing since 1980 (Danaei et al. 2011). There were an estimated 30 million diabetes cases world-wide in 1985, which increased to 135 million by 1995 (King, Aubert, and Herman 1998). The latest WHO estimate is 285 million in 2010 and has been projected to increase to 438 million by 2030 (Shaw, Sicree, and Zimmet 2010). In the United States alone, 25.8 million (8.3 % of the US population) are suffering from diabetes; around 35% of US adults aged 20 & above and 50% of aged above 50 have pre-diabetes (Hhs and Ada 2003). The direct and indirect cost associated with diabetes is becoming an enormous problem, due to its chronic nature, severity of complications and the means to control them. In the United States, direct cost was $116 billion and indirect cost was $58 billion. Dietary change, insulin use, physical activity and oral medication help to control diabetes. Further control of blood pressure, blood lipids and other preventive care practices of teeth, eyes, skin, feet and kidneys helps to prevent complications from diabetes (Zekry et al. 2012, Chudyk and Petrella 2011). In order to offer better treatment, prevention and control modalities, a deeper understanding of the etiology of diabetes and pre-diabetes is needed.

2.2 Periodontal Disease

Periodontitis is a polymicrobial chronic inflammation (Papapanou 2012, Preshaw et al. 2012, Lalla and Papapanou 2011) and is the most common inflammatory disease (Dye 2012) in adults. At the same time, it is an often neglected and hidden chronic disease because of its slow progressive nature. Periodontitis is asymptomatic in early

stages, and the tissue destruction that occurs during this stage is irreversible (Preshaw et al. 2012).

There is no universally accepted case definition for periodontitis in epidemiological studies (Papapanou 2012), hence the prevalence of periodontitis varies depending upon the definition. The Centers for Disease Control and Prevention (CDC) in partnership with the American Academy of Periodontology (AAP) recently published a case definition of periodontitis. The total number of adults in the US with periodontitis (i.e. sum of mild, moderate and severe forms) is 64.7 million adults, (47.2%), (8.7% mild, 30.0% moderate, and 8.5% severe) based on full mouth data. The prevalence is 19.5 % when the two-site per tooth examination [as used by NHANES III periodontal examination] or 27.1% when the three site half-mouth protocol was used in the sample of NHANES 2009-2010 data (Papapanou 2012, Eke, Dye, et al. 2012). CDC/AAP defines periodontitis when clinical attachment loss is ≥ 6mm and pocket depth is ≥ 4mm (Eke, Page, et al. 2012). On the basis of NHANES III survey 1999–2004 data [in which the three site half-mouth protocol was used], the prevalence of moderate to severe PD in the United States was 5% among those age 35 to 49 years, 11% among those age 50 to 64 years, 14% among those age 65 to 74 years, and 20% among those age 75 years or over. Moderate forms of the disease are even more prevalent, affecting 40 -60% of adults (Dye et al. 2007). The combined expenditure for periodontal and preventive dental services in the United States was estimated at $14.3 billion in 1999, whereas approximately $4.4 billion was spent for periodontal procedures. It should be noted that the prevalence of PD varies according to socio-demographic and economic status (Papapanou 2012, Eke, Dye, et al. 2012).

With the advancement of molecular techniques (Socransky and Haffajee 2005, Colombo et al. 2009) several bacterial complexes associated with the etiology of periodontal healthy and diseased stage have been identified using the whole genome DNA probes. The sub gingival biofilm in periodontal plaque constitutes more than 700 species (Socransky and Haffajee 2002), among which around 300 cultivable species have been identified (Sugi et al. 2011). These species are not uniformly distributed in the periodontal sites as multiple factors may impact the distribution of the nature of biofilm (Haffajee, Teles, and Socransky 2006b, Socransky and Haffajee 2005, Haffajee et al. 2005) including the periodontal health or disease stage. Comparatively little research has been done in this area but evidence is strong enough to suggest that the plaque community in periodontal area could be a strong predictor of periodontal health or disease stage (Haffajee et al. 2008).

Although many different bacterial species may participate in the destruction of host periodontal homeostasis, three red complex periopathogens as described by Socransky & Haffajee (Socransky and Haffajee 2002) namely, *T forsythensis* , *P gingivalis* , *P intermedia* , *T denticola* were found to be strongly associated with disease stage. Studies have revealed that these bacteria have the ability to impair innate immune system (Rescala et al. 2010). Additionally, putative bacterial species which are predominantly the orange complex bacteria such as *C rectus, F nucleatum, M micros, Eubacterium nodatum* , and *Prevotella nigrescens* are also thought to play an important role in shifting the plaque microbial community from periodontal healthy to diseased stage(Haffajee, Patel, and Socransky 2008, Socransky and Haffajee 2002). Red and Orange complex bacterial species are found significantly higher in periodontal disease

stage (Haffajee, Teles, and Socransky 2006a). In contrast, members of Actinomyces, yellow, green, purple complex bacteria and some other species are usually found in periodontal healthy as well as diseased sites (Socransky and Haffajee 2002, 2005, Dye et al. 2009).

It is generally accepted that periodontitis infection leads to humoral immunological response which can be measured by different immunological markers including antibodies such as serum or saliva IgG and IgA antibody to periodontal bacteria. The increased IgA antibody level in saliva indicates persistent periodontal infection while its presence in serum is not fully understood (Pussinen et al. 2005). Serum IgG antibodies to periodontal bacteria are stable over time and explain that the individual was exposed to that specific bacteria (Papapanou et al. 2004).

A recent study by Pussinen and team (Pussinen et al. 2011) in a nationally representative sample of 1586 Finnish dentate subjects found that periodontal pathogen *Actinobacillus actinomycetemcomitans* and *P gingivalis* carriage rather than periodontitis determines the serum antibody level (Sugi et al. 2011). One case control study studied IgG antibody titers to 12 periodontal pathogens as a marker to predict the recurrence of periodontitis. They found that those who had higher elevated serum IgG antibody (Sugi et al. 2011) titers to *E corridens* FDC 1073, *P gingivalis* SU63 and *C rectus* ATCC 33238 resulted in recurrent periodontitis.

However, the discrepancies in findings on the correlation between elevated antibody titers to putative pathogens and severity of periodontal disease complicates the criteria to identify the antibodies IgG as immunological marker. In addition, the non-pathogenic periodontal bacteria can also elicit the serum antibodies and even people with

healthy periodontal status also harbor the serum IgG antibodies specific to perio-pathogens (Papapanou et al. 2001). Nonetheless, the intensity of serum IgG antibody to nonpathogenic periodontal bacteria is very nominal and could be due to the certain level of cross –reactivity to the common epitopes shared by periodontal bacteria (Dye et al. 2009). Further complication comes when some findings (Ozmeric 2004) support the concept that the humoral immune response plays a protective role, for instance the chronic periodontitis patients with greater pocket depths and more gingival inflammation had paradoxically lower antibody titers to suspected periopathogens. More research is needed to clarify and explore such discrepancies.

Periodontitis as well as periodontal microbiota are influenced by socio-demographic variables (Haffajee et al. 2005, Lopez et al. 2004, Dye and Thornton-Evans 2010, Dye and Selwitz 2005, Beltran-Aguilar et al. 2005, Lalla et al. 2004, Baelum et al. 2002), life style (Uzel et al. 2011, Bogren et al. 2007, Haffajee et al. 2005)(Uzel et al. 2011, Bogren et al. 2007, Haffajee et al. 2005), eating behaviors (Pussinen, Laatikainen, et al. 2003, Ervin and Dye 2009), anthropometric measures(Desvarieux et al. 2010), fitness (Haffajee and Socransky 2009, Roberts et al. 2002), diabetes mellitus and other co-morbidities(Roberts et al. 2005, Dye et al. 2005), number of teeth (Sachdeo, Haffajee, and Socransky 2008) and dental health (Dye et al. 2007). In nationally representative samples of US adults from NHANES III survey (Vlachojannis et al. 2010) found that dentate participants had higher elevated IgG antibody titers to *A actinomycetemcomitans* , and red complex species *P gingivalis* was twice as high in periodontitis than periodontal healthy individuals. Also, Mexican American and Non –Hispanic Black were more likely to have elevated serum antibodies than Whites.

Antibiotic use affects the oral microbiota (Haffajee, Patel, and Socransky 2008, Haffajee, Torresyap, and Socransky 2007, Socransky and Haffajee 2002) but its impact on serum immunoglobulin has not been explored adequately. Nevertheless, a few research findings (Papapanou et al. 2004, Darby, Mooney, and Kinane 2001, Alfakry et al. 2011) have shown that there is long stability of serum IgG antibody to periodontal bacteria even after successful periodontal therapy with antibiotics or scaling, the presence of which markedly suggests past history of periodontal infection.

2.3 Correlation of Periodontal disease with other diseases

The contribution of periodontitis to a state of systemic inflammation is getting considerable attention in recent times (Lalla and Papapanou 2011). Periodontal diseases have long been associated with major chronic diseases such as diabetes and vascular diseases.

2.3.1 Periodontal Disease and Diabetes/ Pre-diabetes

The relation between chronic periodontal diseases and diabetes has been studied intensively since 1947 (Glickman 1947). Both the diabetes and periodontal disease are multifactorial diseases, and the association is bidirectional i.e. the presence of one disease tends to boost the other disease and precise management of either disease tends to assist in the treatment of the other (Lalla and Papapanou 2011, Preshaw et al. 2012, Pradhan and Goel 2011). Loe was the first to propose periodontal disease as the sixth complication of diabetes in 1993 (Loe 1993) and ADA acknowledged that periodontal disease is often found in diabetic cases in 2003 (Preshaw et al. 2012).

In the US NHANES III population, adults with an HbA1c level of $\geq 9\%$ were found to have a significantly higher prevalence of severe periodontitis than those without

diabetes (OR 2.9; 95% CI 1.40-6.03) after controlling for age, ethnicity, education, sex and smoking (Tsai, Hayes, and Taylor 2002). A recent 5 year prospective longitudinal study in 2,973 non-diabetic participants from the study of health in Pomerania (SHIP), revealed that participants with advanced periodontitis at baseline showed an approximately fivefold higher increase in HbA1c after 5 years when compared to those with no periodontitis at baseline, which suggest the impact of periodontitis on diabetes (Demmer et al. 2010).

The outcome of a meta-analysis study from 49 cross-sectional and eight longitudinal studies of the 2440 identified studies published between 1980 to 2007 found that periodontitis is a risk factor of T2DM while more studies are needed to confirm the harmful effect to T1DM (Chavarry et al. 2009). This is probably due to T1DM being more prevalent in young children while middle aged men and women have increased risk for periodontitis and T2DM (Merchant et al. 2011).

Glycemic control with decreased HbA1c levels has been an important marker of decreased complications from diabetes. Several different meta-analysis (Teeuw, Gerdes, and Loos 2010, Darre et al. 2008, Janket et al. 2005, Jones et al. 2007, Simpson et al. 2010) outcomes have indicated the reduction of HbA1c% (T1DM or T2DM) around 0.4% after effective periodontal treatment for at least 3 months. One meta-analysis (Janket et al. 2005) of glycemic control after mechanical treatment of periodontal diseases showed the weighted average 0.38% decrease in actual HbA1c level for all studies, 0.66% when restricted to type 2 diabetic patients, and 0.71% if antibiotics were given to them. Although none were statistically significant, clinical significance of these results cannot be underestimated. The 2010 systemic review (Simpson et al. 2010) which

included seven randomized clinical trials exclusively with type 2 diabetes was similar [0.4% reduction of HbA1c; 95% confidence interval (CI) fixed effect -0.78% to -0.01%)] to earlier meta-analysis results. Although these randomized clinical trials had heterogeneity of data and low power for the statistical analysis, the 0.4 % HbA1c reduction value has substantial clinical significance. Less potent classes of oral glucose – lowering agents reduce HbA1c % level by 0.5-1% and other classes of oral agents (i.e. insulin secretagogues) combined with other methods such as nutritional therapy and physical activity increases HbA1c by 1-2% (Santos Tunes, Foss-Freitas, and Nogueira-Filho Gda 2010).

Furthermore, many studies have revealed that presence of diabetes and periodontal disease combined aggravating the complications of macrovascular and microvascular diseases. The recent prospective study of the Gila River Indian community with 529 participants unfolded the findings that cardio-renal mortality (combination of ischemic heart disease and diabetic nephropathy) was three times more likely in diabetics with severe periodontitis compared to diabetics without severe periodontitis after adjusting for age, sex, diabetes duration, HbA1c, macroalbuminuria, BMI, cholesterol, hypertension, electrocardiogram abnormalities, and smoking (Shultis et al. 2007). Prospective diabetes studies (Stratton et al. 2000, Khaw et al. 2004) have revealed the finding that every 1% reduction in HbA1c resulted in a 21% reduction in the risk for any end point related to diabetes, 21% reduction in deaths related to diabetes, 14% reduction in myocardial infarction and a 35% decreased risk of microvascular complications.

There is a very limited knowledge about the periodontal bacterial profiles or antibody response patterns that are distinctly associated with T1DM or T2DM cases and

no large epidemiological studies have focused on this area. In the few studies involving culture of oral bacteria, *P gingivalis, P intermedia* and *Capnocytopha* species were found to be significantly higher in those with diabetes than non-diabetic participants (Zambon et al. 1988, Mealey 1999, Sbordone et al. 1998). Among children with T1DM, it was found that level of *E nodatum* antibody was significantly higher in diabetic patients while no difference was seen in serum IgG levels to 11 other bacteria (Lalla et al. 2006). These findings suggest apparent lack of significant differences in potential pathogens in diabetic and non-diabetic participants; however it should be noted that these studies are limited with respect to sample size and diversity of bacteria assessed (Lalla and Papapanou 2011). More detail literature review related to epidemiological studies evaluating the relation between periodontal microorganisms with diabetes and periodontitis are shown in Table 2.1.

Inflammation is believed to be the common link which affects glycemic control, insulin resistance and periodontal damage. The mechanism is not clearly understood, but it includes immune functioning, cytokine and neutrophil activities. More research are needed to understand the biological pathway that links between the periodontitis and diabetes (Preshaw et al. 2012). The association of these two chronic diseases provides a perfect example of a cyclic association in which systemic diseases predisposes an individual to oral infection and once the oral perio-pathogens are established, the systemic disease is exacerbated (Pradhan and Goel 2011). Figure 2.1 shows the bidirectional relationship of periodontal infection and diabetes contributing to insulin resistance. The detail pathological mechanism of periodontitis and diabetes association

can be found elsewhere (Lalla and Papapanou 2011, Santos Tunes, Foss-Freitas, and Nogueira-Filho Gda 2010, Pradhan and Goel 2011).

2.3.2 Periodontal Disease and Cardiovascular markers including MetS

Independent of diabetes, increasing epidemiological evidence suggests an association of periodontal disease and atherosclerotic vascular disease (ASVD) due to its systemic inflammation. The American Heart Association (AHA) (Lockhart et al. 2012) recently investigated the independent association of periodontal disease and ASVD reviewing all the available data from 1950 to 2011. The AHA Scientific committee reviewed 473 out of 537 studies that met the inclusion criteria of the association between periodontal disease and any cerebrovascular, peripheral vascular or cardiovascular disease. Observational studies supported an association between periodontal disease and ASVD independent of known confounders (age, diabetes mellitus, and smoking); however causation was not supported. Periodontal intervention helps in a reduction in systemic inflammation and endothelial dysfunction but there is no evidence to suggest intervention prevents ASVD or modify its outcomes.

Out of hundreds of studies that have investigated the association with cardiovascular diseases, very few studies have explored the direct linkage with periodontal microbes or host immune response to these periodontal microbes. Periodontal pathogens such as *P gingivalis, A actinomycetemcomitans, P intermedia, T forsythia,* and *Streptococcus mutans have* been found to be associated with atherosclerotic plaques. Desverieux et al (Desvarieux et al. 2005) assessed 11 known periodontal microorganisms from 657 dentate subjects enrolled in INVEST and found that overall bacterial burden was related to carotid artery intima-media thickness (IMT) after adjusting for age, race,

gender, education, BMI, smoking, diabetes, blood pressure, and Low density lipoprotein (LDL) and HDL cholesterol. Adjusted mean IMT values of etiologic bacterial burden with four organisms, *P gingivalis, A actinomycetemcomitans, T forsythia* and *T denticola* were found to have significant increasing trend across tertiles. Spahr et al, 2006 investigated an association of combined colonization of five periodontal bacteria [*P gingivalis, A actinomycetemcomitans, P intermedia, T forsythia and T. denticola* to Cardiovascular diseases (CHD) and revealed that for each 1 unit log increase in total burden, the OR increased by 1.83 times (95% CI 1.23-2.71).

Pussinen et al in Finland investigated an association of combined serum IgG antibody titers of *P gingivalis* and *A actinomycetemcomitans* to ASVD and found significantly higher titers [Hazard Ratio (HR):1.87 (1.13-3.08)]. In a separate study (Pussinen, Jousilahti, et al. 2003), the same team investigated serum IgG antibody titers of *P gingivalis* and *A actinomycetemcomitans* individually to CHD. The association was significant with each of high titers of bacteria [Relative risk (RR) for high *A actinomycetemcomitans*: 2.0 (1.2-3.3) and RR for *P gingivalis*: 2.1 (1.3-3.4)] after adjusting for potential confounders. Currently, attention has been paid to the applicability of anthropometric markers to measure diabetes complications, cardiovascular risks and their control.

MetS is associated with a very high risk for cardiovascular diseases and diabetes mellitus (Marchesini et al. 2004, Grundy et al. 2004). In the NHANES III study in US population, participants older than 45 suffering from severe periodontitis were 2.31 times (95% CI 1.13-4.73) more likely to have MetS than unaffected individuals after adjusting for confounders (D'Aiuto et al. 2008). Poor periodontal health conditions were found in

patients with diabetes. Periodontal disease association with MetS was found true in other study populations independent of other risk factors (Li et al. 2009, Han et al. 2012, Kwon et al. 2011, Yu et al. 2012); however no recent studies have investigated the role of periodontal microbiota with MetS.

Waist circumference (WC) is recognized as one of key component of MetS and also provides a better measurement of cardiovascular risk and obesity. WC represents central adiposity and is measured using a steel measuring tape to the nearest 0.1 cm at the high point of the iliac crest at minimal respiration when the participant was in a standing position (Wood, Johnson, and Streckfus 2003). A recent meta-analysis revealed a significant association between periodontitis and obesity (OR 1.35; 95% CI 1.23, 1.47), suggesting that abdominal obesity is independently associated with periodontitis(Chaffee and Weston 2010). Evidence is increasing for an association of gut microbiota with obesity (Le Chatelier et al. 2013) while no study has explored the association with periodontal microbiota.

There is evidence that supports a correlation of periodontitis with other markers of MetS such as hypertension(Desvarieux et al. 2010, Bonato et al. 2012, Vidal et al. 2009), LDL (Itabe 2012), HDL (Pussinen, Jauhiainen, et al. 2004, Wu et al. 2000), Total Cholesterol (TC) (Wu et al. 2000), and Triglycerides (TG) (Banihashemrad, Moeintaghavi, and Rafighdoost 2008, Cutler and Iacopino 2003, Morita et al. 2004) individually but none of epidemiological studies have investigated the role of periodontal microbiota or the host response with these metabolic complication markers.
Since both the periodontitis and MetS are associated with systemic inflammation and insulin resistance, these two diseases may be linked through a common

pathophysiological pathway (D'Aiuto et al. 2008). Oxidative stress may act as a potential common link to explain relationships between each component of MetS and periodontitis. Both conditions show increased serum levels of products such as elevation of C-reactive protein and fibrinogen derived from oxidative damage, with a pro-inflammatory state likely influencing each other bidirectionally (Bullon et al. 2009).

2.4 Gap in the Knowledge

The possible association between periodontal disease and other diseases has been studied extensively assessing periodontitis with self-reported periodontal disease or clinical periodontal examination, but periodontal microflora associated with periodontitis as a marker of periodontal disease is understudied. There are very few large studies (Pussinen et al. 2011, Papapanou et al. 2000) which have combined clinical, bacterial and immunological data which could elucidate the possible role of these periodontal bacteria in systemic immune response and periodontitis. The need to understand the periodontal microbiota profile and its immunological marker is important primarily to measure direct systemic activity, but also to comprehend increasing evidence of periodontal disease being a potential modulator of diabetes mellitus(Dye and Genco 2012, Lalla and Papapanou 2011), obesity (Chaffee and Weston 2010, de Castilhos et al. 2012, Jagannathachary and Kamaraj 2010), atherosclerosis (Papapanou and Trevisan 2012, Lockhart et al. 2012, Behle and Papapanou 2006, Desvarieux et al. 2005, Beck et al. 2001), other vascular diseases (Pussinen, Alfthan, Tuomilehto, et al. 2004, Pussinen, Alfthan, Rissanen, et al. 2004, Pussinen, Jousilahti, et al. 2003, Papapanou 2009, Beck et al. 2005), preterm labor (Pussinen, Paju, et al. 2007, Albert et al. 2011, Michalowicz, Novak, et al. 2009, Michalowicz, Hodges, et al. 2009, Jarjoura et al. 2005) and different

other systemic diseases(Demmer et al. 2012, Michalowicz et al. 2011, Desvarieux et al. 2010, Behle et al. 2009, Beck et al. 2005, Zoppini et al. 2012).

In this dissertation, we analyzed serum IgG antibodies against 19 periodontal bacteria from the NHANES III survey dataset. The NHANES III dataset provided a unique opportunity to explore these relationships because this survey has data on periodontal examination, laboratory assessment of serum IgG antibodies to 19 different periodontal bacteria, socio-demographic, anthropometric and nutritional information in large number of US adult's participants. The variables that were used from these different NHANES III sub datasets are shown in Table 2.2. Figure 2.2 depicts the phyla (and groups as defined by *Socransky and Haffajee (Socransky and Haffajee 2002, 2005)* of 19 periodontal bacteria species to which serum IgG antibodies were identified and measured by checker board DNA-DNA hybridization in NHANES III survey (Papapanou et al. 2001).

Table 2.1- Epidemiological studies evaluating the relation between periodontal microorganisms in individuals with diabetes or pre-diabetes

No	**Author , Year title and publication Study design**	**Purpose of the study**	**Characteristics of the population**	**Method of detecting microorganisms & Statistical Analysis**	**Findings**	**Inferences & Limitations**
1	Zhou et al (2013). Investigation of the Effect of Type 2 Diabetes Mellitus on Subgingival Plaque Microbiota by High-Throughput 16S rDNA Pyrosequencing. PLoS One.; 8(4): e61516. Cross-sectional study	Subginival biofilm in different groups of periodontitis and diabetes are compared within different groups. They included non-diabetic subjects without periodontitis (P-D-), non-diabetic subjects with periodontitis (P+D-), type 2 diabetic patients without periodontitis (P-D+), and type 2 diabetic patients with periodontitis (P+D+).	31 **Chinese subjects,** 30-65 years old had at least 20 teeth without any clinical signs of oral mucosal disease or root caries	16s rDNA Pyrosequencing Wilcoxon rank-sum test and Fisher's exact test	The sequencing depth was similar among different sample groups although a slightly smaller amount of sequences were generated from the P+D- group. The samples in the periodontitis-positive groups (i.e., P+D- or P+D+) are well separated from those in the periodontitis-negative groups (i.e., P-D- or P-D+) based on the unweighted UniFrac distances	These findings reflect that the bacterial compositions in periodontitis and healthy samples were distinct. Furthermore, compositional shifts in the subgingival plaque bacterial community associated with periodontitis also exist in diabetic patients. small sample size

					measured at the OTU level In the subjects with healthy periodontium, the abundances of three genera (*Prevotella, Pseudomonas, and Tannerella*) and nine OTUs were significantly different between diabetic patients and their non-diabetic counterparts. In the subjects carrying periodontitis, the abundances of three phyla (*Actinobacteria, Proteobacteria*, and *Bacteriodetes*), two genera (*Actinomyces* and *Aggregatibacter*), and six OTUs were	

					also significantly different between diabetics and non-diabetics.	
2	Casarin et al. (2012) Sub gingival biodiversity in subjects with uncontrolled type-2 diabetes and chronic periodontitis. J Periodont Res 2013; 48: 30–36. Case Only study [Severe and generalized periodontitis]	Compare the sub gingival diversity in deep periodontal pockets of diabetes or non diabetic subjects with chronic periodontitis.	Severe and generalized periodontitis with uncontrolled type 2D (T2DM) [Hba1c >8% , n=12 subjects] and non-diabetic [n=11 subjects] were consecutively recruited from the population referred to **the Periodontal clinic of Guarulhos University**	Subgingival biofilm were identified and quantified using 16s Rrna Gene cloning and quantified by sequencing a finite number of clones from each sample. The microbiological data were transformed using variance-stabilizing transformation and proportion of each species in the periodontal biofilm community was calculated. Two tailed student t test and chi – square test	Among the top 15 clones, subjects with uncontrolled T2DM presented higher percentages of total clones of *TM7*, *Aggregatibacter*, *Neisseria*, *Gemella*, *Eikenella*, *Selenomonas*, *Actinomyces*, *Capnocytophaga*, *Fusobacterium*, *Veillonella and Streptococcus genera*, whereas higher percentages of *P. gingivalis*, *T. forsythia*, *Filifactor alocis* and Synergistetes clone BH017 were found	Significant differences were observed in subgingival microbiota between uncontrolled diabetic and nondiabetic subjects. small sample size

			and Piracicaba Dental school, Brazil from July 2007 to Feb 2010. Mean age group were slightly higher in diabetes group [51.8 (40-70)] compared to non diabetic group [47.8 (41-62)]. Female were in higher proportion in diabetic group than non diabetic.		in the periodontal pockets of Non-DM subjects ($p < 0.05$). Moreover, some phylotypes, such as Fusobacterium nucleatum, Veillonella parvula, V. dispar and Eikenella corrodens were detected significantly more often in diabetic subjects than in nondiabetic subjects ($p < 0.05$).	
3	Field et al. (2012) Investigatio	Detect the quantitative differences	48 subjects who	Cultivation (*A actinomycete*	*A actinomycete mcomitans, F*	No significant difference

	n and quantification of key periodontal pathogens in patients with type 2 Diabetes. J Periodont Res 2012; 47: 470–478 Matched Case - control study	in selected periodontopathogens in the sub gingival plaque of diabetes patients using TaqMan quantitative PCR	have confirmed T2DM with (n=9) or without periodontitis (n=15) were recruited from **New Castle Dentist Hospita, UK.** Age, sex, and smoking status-matched control non-diabetic subjects (with periodontitis (n=12) and without periodontitis (n=12) were recruited from from same hospital.	*mcomitans, F nucleatum* and *P gingivalis)* and quantification by qPCR One way ANOVA Bonferroni post hoc tests Mann whitney U-test	*nucleatum and P gingivalis* were present in significantly different quantities and proportion according to periodontal disease status but there was no clear distinction between three microbiota in diabetes and non diabetes group. However, *P ginivalis* were higher in periodontitis (diabetic and non diabetic group) than periodontal healthy subjects. *A actinomycete mcomitans were found in lower quantities in all subgroups while F nucleatum* were abundant in all subgroups	were identified between the sub gingival flora between T2DM and non –diabetic patients. Limited no of microorganism. Control Patients with T2DM were recruited who currently deemed stable with regard to diabetes symptoms by their medical practioner.

			Majority of study participants were Whites male and Ex or non smokers, over 18 years of age and a minimum of 20 teeth.		with no clear distinction.	
4	da Cruz et al. (2008) Clinical and laboratory evaluation of non-surgical periodontal treatments in type 2 Diabetes patients. J periodont, 79 :1150-1157 Longitudinal follow-up study (3 months) following non-surgical periodontal therapy	to evaluate the clinical and laboratory changes 3 months after full-mouth scaling and root planing in subjects with and without diabetes mellitus	20 adults with periodontitis, of whom 10 were type 2 diabetes mellitus who required insulin therapy (DM) and 10 healthy adult control subjects (NDM) with generalized chronic	PCR analysis of subgingival plaque samples for *A. actinomycetemcomitans, P. gingivalis and T. forsythia* two-way ANOVA x2 test	Periodontal status improved following treatment, but no changes in HbA1c Occurred. Reductions in the frequency of bacterial recovery occurred over the 3 months, but there were no significant differences between the two groups	Clinical and laboratory responses were similar in T2DM and NDM groups 3 months after full-mouth scaling and root planing. Small numbers Uncontrolled diabetes subjects who required insulin therapy

			periodontal disease were recruited from dental clinic, University of Campinas, Brazil Age ranged from 30-70 years and had at least 20 teeth. Subjects were evaluated 3 months (Oct-Dec 2006)			
5	Ebersole et al. 2008 Microbiologic and Immunologic Characteristics of Periodontal Disease in Hispanic Americans With Type 2 Diabetes	comparison of sub gingival plaque samples and serum antibody levels to selected oral microorganisms in T2DM suffering Hispanic population.	39 **Hispanic Americans** with T2DM, and 24 non-diabetic controls were recruited from Brady Green	DNA checkerboard hybridization of plaque samples, and ELISA analysis of serum IgG levels were performed two-way ANOVA x2 test	Similar pathogens were found in periodontitis sites in subjects with and without T2DM, though *P.gingivalis, A. actinomycetemcomitans and Campylobact*	poor oral hygiene, site-specific samples should be considered

	J Periodontol Vol74 (4) Cross-sectional		clinic, Texas, US in 1994 Mean age of diabetic groups 54.2 (39-72) while non diabetes group was 47.4 (33-68) years. Female were in higher proportion in diabetes groups. In T2DM group, non smokers were 27 out of 39 and in non diabetic non smokers were 20 out of 24.		*er species* were found more frequently ($p < 0.05$) in subjects with T2DM compared to those without. Also, serum IgG responses were broadly similar between the two groups, apart from a significant elevation in antibody to *C rectus* in the diabetic subjects	
6	Makiura et al. 2008 Relationship of	assess the relationship between serum	30 **Japanese adults**	Used polymerase chain reaction	It was observed that post-treatment, P.	The authors postulated that glycemic control in

	Porphyromonas gingivalis with glycemic level in patients with type 2 diabetes following periodontal treatment. Oral Microbiol Immunol 2008: 23: 348–351. Prospective longitudinal study	glycemic levels and subgingival microbial profile alteration following periodontal treatment in patients with type 2 diabetes mellitus.	(14 male and 16 female) suffering from chronic periodontitis and T2DM were recruited. The age of the subjects ranged from 41 to 80 years (mean 63.9 ± 9.4 years) and duration of DM was between 3 and 13 years (8.5 ± 5.6 years), with HbA1c values ranging from 6.0% to 10% (7.11 ±	[PCR] method, The target microorganisms were *P gingivalis, A actinomycetemcomitans, T forsythensis, T denticola, and P intermedia.* t-test	gingivalis was detected more frequently in those who had increased HbA1c values compared to those with decreased values relative to baseline, and more specifically, *P. gingivalis* with type II fimbriae was detected only in patients with an increase in HbA1c.	patients with periodontitis and diabetes is potentially influenced by the persistence of *P. gingivalis*, particularly clones with type II fimbriae, following treatment Small sample size

			1.16%).			
7	Hintao et al. 2007 The microbiological profiles of saliva, supragingival and subgingival plaque and dental caries in adults with and without type 2 diabetes mellitus. Oral Microbiol Immunol 22: 175–181 Statified Cross-sectional study	determine the effect of T2DM on coronal and root surface caries and to investigate some factors suspected of being related to or interacting with DM, that may be associated with coronal and root surface caries	Data were obtained from 105 patients with T2DM and 103 non-diabetic subjects in **Southern Thailand.** Among the T2DMs, the mean duration of diabetes was 8.7 years (SD 5.7). The mean HbA1c value in the group was 8.5% (SD 2.1) and ranged from 5.6 to15.8 %.	17 bacterial species using the Checkerboard DNA–DNA hybridization method. The chi-squared test (Bonferroni correction) The Mantel–Haenszel odds ratio	Significantly more diabetic subjects had higher levels of T denticola, P nigrescens, S sanguinis, S oralis and S intermedius in their supragingival plaque than non-diabetic subjects. None of the bacterial species tested in the subgingival plaque samples demonstrated an association with T2DM No significant difference was found for the organisms in saliva, oral rinse and subgingival plaque between the two groups.	Possible association of T2DM with some specific bacteria Mixture of insulin and non-insulin diabetes patients. When using the checkerboard method in patients with poor oral hygiene, site-specific samples should be considered.

			85% of the patients were receiving oral anti-diabetic treatment or dietary controls only, 7.5% had insulin treatment and 7.5% were treated with a combination of insulin and oral antidiabetic agents. Mean age (54 in T2DM and 53 in Non diabtetes group) and sex in both groups were similar proportion			
8	Yuan et al. (2001)	the detection	105 **Taiwan**	PCR analysis detection	No significant	No difference

	Detection of putative periodontal pathogens in non-insulin-dependent diabetes mellitus (NIDDM) and non-diabetes mellitus by polymerase chain reaction. J Periodont Res 2001; 36: 18±24. Case-Control comparison	rates of 5 putative periodontal pathogens: between NIDDM and non-DMadults were compared.	**ese adults** with noninsulin-dependent diabetes and 141 age- and sex matched non-diabetic individuals ages ranging from 28 to 78 yr in the NIDDM group and 21 to 80 yr in the non-DM group. The ratio of male to female in non-DM and NIDDM groups were 1.39 and 1.33, respectively.	rates of *A. actinomycetemcomitans, P. gingivalis, E. corrodens, T. denticola and C. albicans* Student's t-test The chi-squared test	differences in the prevalence rates of the 5 microorganisms between the diabetic and non-diabetic individuals was found	between subgingival micro-organisms in T2DM and Non diabetic group Limited set of microorganisms Age and sex were not adjusted

9	Castrillon et al (2013) Occurrence of red complex microorganisms and *Aggregatibacter actinomycetemcomitans* in patients with diabetes Journal of Investigative and Clinical Dentistry, 4, 1–7 Cross-sectional study.	Analyze the occurrence of red complex micro-organisms ***Aggregatibacter actinomycetemcomitans*** in patients with diabetes (type 1 or type 2)	60 patients confirmed diagnosis (fasting glucose ≥126 mg/dL or glycated hemo-globin ≥6.5%) of type I or II DM with a previous (>2 years) and from the Hospital Universitario San Vicente de Paul (Medellin, Colombia), and 62 patients without diabetes from the School of Dentistr	Using PCR, the prevalence of red complex microorganisms (*P. gingivalis*, *T. forsythia*, and *T. denticola)* and *A. actinomycetemcomitans* were determinedPeriodontitis Patients were classified according to the American Academy of Periodontology and Page and Eke's classica-tions Kruskal–Wallis, Mann–Whitney *U*-test, and Fisher's exact test	Periodontitis patients presented with more Bleeding on Probing (BOP) and probing depth (PD) than systemically-healthy patients without periodontitis ($P < 0.05$). The CAL was increased in DM periodontitis patients compared to ND periodontitis patients, and this difference was statistically significant ($P < 0.05$). In addition, patients with diabetes and periodontitis had lost more teeth than ND periodontitis patients $P < 0.05$) Red complex microorganisms were	Possible association of the subgingival microbiota between diabetic and non-diabetic patients. In addition, *P. gingivalis* and *A. actinomycetemcomitans* were associated with periodontitis in patients without diabetes and patients with diabetes, respectively Small sample size Limited micro-organisms

			y at the Universidad del Valle (Cali, Colombia) They participated in this study from February 2011 to March 2012, Patients had a mean age of 47 years, and there were more females than males.		detected in lower frequencies in patients with diabetes. The detection of *A. actinomycetemcomitans* was higher in patients with diabetes and periodontitis compared to systemically-healthy patients without periodontitis ($P < 0.05$). *P. gingivalis* was associated with periodontitis in non-diabetic patients ($P < 0.05$), whereas *A. actinomycetemcomitans* was associated with periodontitis in diabetic patients ($P < 0.05$).	
10	Vlachojannis et al. (20100 Determinants of serum	assess the distribution of elevated antibody titres to	>=40-year-old participants	checkerboard immunoblotting to assess serum IgG levels to 19	Edentulous individuals showed lower antibody	Demographic, behavioural, and oral- and general

	IgG responses to periodontal bacteria in a nationally representative sample of US adults. J Clin Periodontol 2010; 37: 685–696 Cross-sectional study.	multiple periodontal bacteria, including established/ putative pathogens and health-related species, by selected demographic, behavioural, and oral- and general health-related characteristics.	from the third National Health and Nutrition Examination Survey (NHANES III) were used, including 1588 edentulous individuals.	periodontal species two-sided t-tests ($p<0.05$) Multivariable logistic regression modelling	responses than dentate participants, notably for titres to ‘‘red complex’’ species and *A actinomycetemcomitans*. Elevated *P gingivalis antibody IgG titers* was significantly different in diabetes including type 1 or type 2 than having no diabetes	health-related characteristics were strong determinants of systemic antibody responses to periodontal bacteria in a nationally representative sample of US adults. Diabetes as an exact diagnosis on the type of diabetes was not available in the present analysis

Table2.2. Covariates from different data sources of NHANES III

Household Adult Interview Questionnaires data file	NHANES III Examination Data File	laboratory examination data file	NHANES III Antibodies to Periodontal Pathogens (SPSDEPPX)	Dietary recall and vitamin supplement dataset
Age, Sex, Race/ethnicity income-poverty ratio, education, smoking, Physical activity, self-reported diabetes, hypertension and frequency of dental visits	BMI, Waist circumference, Blood pressure, number of missing teeth, Clinical attachment loss (CAL), Pocket depth (PD)	Plasma HbA1c%, Plasma Blood glucose, Serum Tri-glycerides, Low Density Lipoprotein, High Density Lipoprotein, Systolic Blood Pressure, Diastolic Blood Pressure	*Aggregatibacter actinomycetemcomitans mix (*ATCC strains #43718, #29523 and #33384); *Porphyromonas gingivalis mix (*ATCC #33277 and #53978); *Tannerella forsythia* ATCC#43037; *Treponema denticola* OMGS#3271; *Campylobacter rectus* ATCC#33238; *Eubacterium nodatum* ATCC#33099; *Prevotella intermedia* ATCC#25611; *Prevotella nigrescens* ATCC#33563; *Prevotella melaninogenica* ATCC#25845; *Fusobacterium nucleatum* ATCC#10953; *Micromonas micros* ATCC #33270; *Selenomonas noxia* ATCC#43541; *Eikenella corrodens* ATCC#23834; *Capnocytophaga ochracea* ATCC#33624; *Streptococcus intermedius* ATCC#27335; *Streptococcus oralis* ATCC#35037;	total fibers intake, Protein intake, Carbohydrate Intake, Fat intake

			Streptococcus mutans ATCC#25175; *Veillonella parvula* ATCC#10790; and *Actinomyces naeslundii* ATCC#49340	

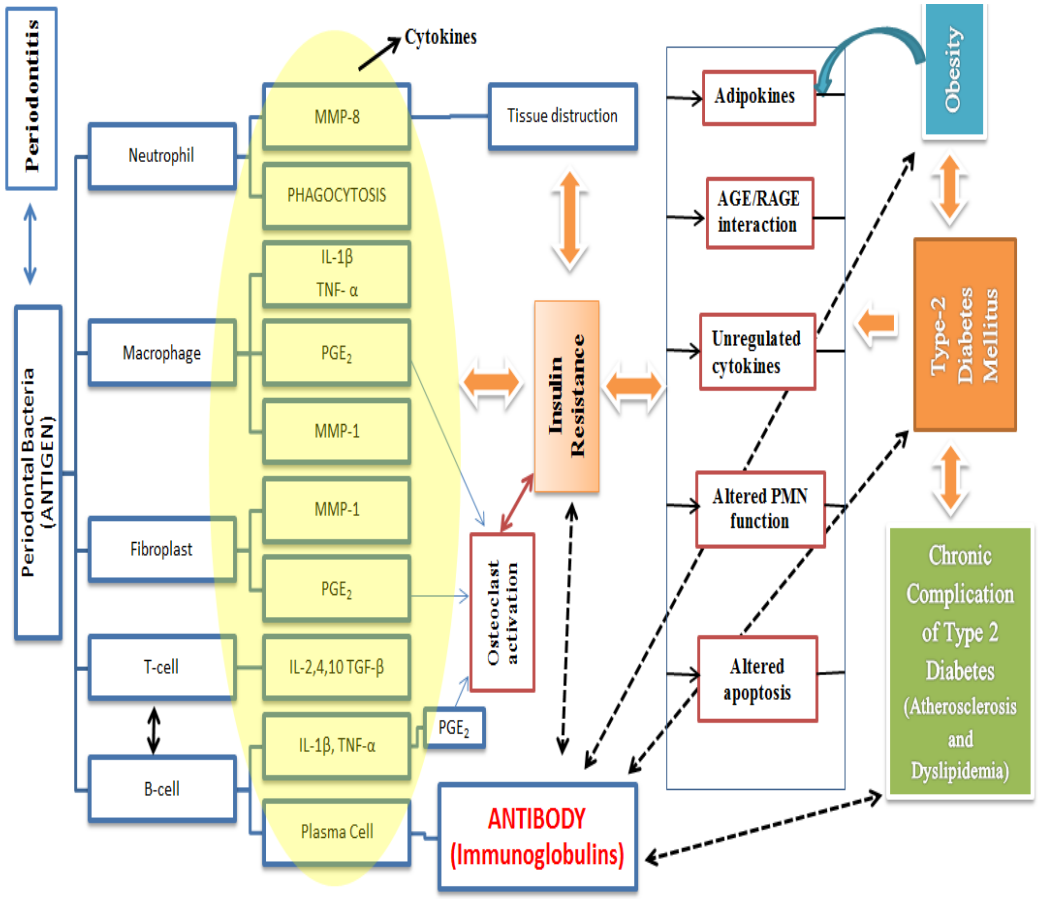

Figure 2.1: Model for bidirectional relationship between periodontal infection and diabetes, contributing to insulin resistance. (Ozmeric, 2004; Preshaw et al., 2012; Santos Tunes, Foss-Freitas, & Nogueira-Filho Gda, 2010) (Santos Tunes, Foss-Freitas, and Nogueira-Filho Gda 2010, Ozmeric 2004, Preshaw et al. 2012) (Santos Tunes, Foss-Freitas, and Nogueira-Filho Gda 2010, Ozmeric 2004, Preshaw et al. 2012) (14, 35, 68) (14, 35, 68) (14, 35, 68) (Santos Tunes, Foss-Freitas, and Nogueira-Filho Gda 2010, Ozmeric 2004, Preshaw et al. 2012) MMP: Matrix metalloproteinase, IL: Interleukin, TGF-β: Transforming growth factor β, TNF-α: Tumor necrosis factor α, PGE_2: Prostaglandin E_2, PMN: Poly morphonuclear leucocyte, AGE: Advanced glycation end products, RAGE: Receptor for AGE

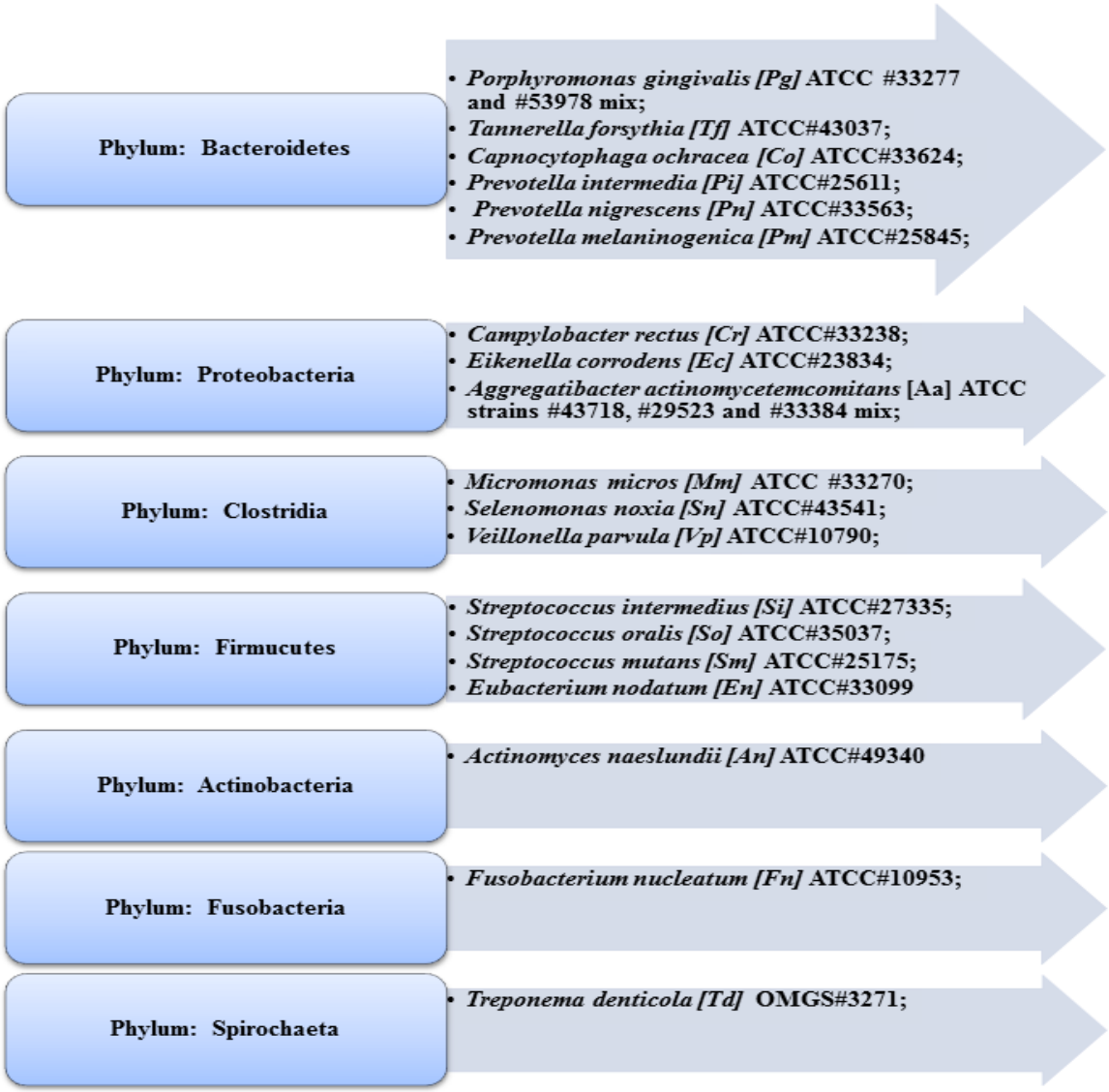

Figure 2.2: Serum Immunoglobulin (IgG) to 19 periodontal bacteria species and their respective phyla identified by Checkerboard hybridization in NHANES III study.

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CHAPTER 3

MANUSCRIPT 1

The relation between Serum Immunoglobulin G (IgG) Antibodies against Periodontal Bacteria and Type 2 Diabetes and Pre-Diabetes

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ABSTRACT

Aim: To investigate the association between antibodies against oral microorganisms and hyperglycemia in a nationally representative sample.

Methods: Participants in the NHANES III (1988-1994), aged ≥40 years, who provided blood samples that were assessed for serum IgG titers against 19 periodontal bacteria (*n* = 8153) were included. Antibody titer z-scores were used to get four clusters ("Orange-Red", "Red-Green", "Orange-Blue" and "Yellow-Orange") using cluster analysis. Logistic regression weighted for complex survey design was used to analyze the relationship between the clusters and hyperglycemia.

Results: The Orange-Red cluster score, that included *P. gingivalis* and *Prevotella sps,* was positively associated with diabetes [OR 1.08 (1.03-1.13)] while Red-Green score (which included *A. actinomycetemcomitans mix, S. noxia, E. corrodens, V. parvula, T. forsythia, T. denticola, C. rectus)* was inversely related to diabetes [OR 0.96 (0.912 - 0.999)] in multivariable analyses. The Orange-Blue cluster (*A. naeslundii and E. nodatum)* was inversely associated with diabetes and pre-diabetes but the relations were attenuated after adjustment. The Yellow-Orange cluster, that included predominantly *Streptococcus sps,* was not associated with hyperglycemia.

Conclusion: Clusters of serum IgG antibodies against periodontal bacteria are both positively and negatively associated with diabetes. The relation between periodontal bacteria and hyperglycemia is complex and deserves further study.

Key words: *Type 2 diabetes; pre-diabetes; periodontal bacteria; serum IgG antibody; NHANES III*

Introduction

Periodontitis is associated with diabetes and its related outcomes in observational studies (Borgnakke et al. 2013). In prospective studies, periodontitis predicted the development of type 2 diabetes (Demmer, Jacobs, and Desvarieux 2008), and type 2 diabetes mellitus at baseline predicted increased risk of incident self -reported periodontitis and tooth loss (Jimenez et al. 2012), suggesting that the relation between diabetes and periodontal diseases is bi-directional. However, the question whether periodontal treatment improves glycemic control in individuals with diabetes remains unanswered with randomized controlled trials (RCT) reporting discrepant results. Engebretson et al reported no effect of periodontal treatment on glycemic control (Engebretson et al. 2013), but Sun et al found that periodontal treatment improved glycemic control, lipid profile, inflammatory cytokines among individuals with type 2 diabetes, and a systematic review of all RCTs found periodontal treatment to have a net benefit (Engebretson and Kocher 2013, Sun et al. 2011). A possible drawback of these studies was that none evaluated immunological changes evoked by periodontal pathogens (Holmlund et al. 2011), which may explain the poorly understood mechanism linking periodontal disease with glycemic control (Lalla and Papapanou 2011).

The periodontal environment harbors more than 700 different species of periodontal microbiota (Socransky and Haffajee 2002, 2005) making it a potentially important site for host microbe interaction (Rams, Listgarten, and Slots 2006). Several periodontal bacteria are capable of invading the gingival tissues and initiating robust serum antibody responses that are capable of interfering with host defense mechanisms and stimulating local and systemic inflammations (Kinane and Lappin 2001). Recent

studies (Teles et al. 2012, Socransky et al. 2013, Socransky and Haffajee 2005, 2002) showed that periodontitis is associated with higher prevalence of *Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Actinobacillus actinomycetemcomitans, Prevotella sps.*; whereas *Actinomyces* groups are predominantly associated with healthy periodontal status. Furthermore, Dye et al (2009) (Dye et al. 2009) in a large nationally representative US population study from the third National Health and Nutritional Examination survey (NHANES III) found that elevated serum immunoglobulin (IgG) titers against *P. gingivalis* are associated with periodontitis while elevated IgG titers against against *Eubacterium nodatum* are associated with healthy periodontal status. There is growing evidence that the relationship between periodontal disease and systemic diseases is affected by the profile of periodontal bacteria. For instance, Desvarieux et al., in cross-sectional (Desvarieux et al. 2005) and longitudinal (Desvarieux et al. 2013) studies reported that the etiologic group of periodontal bacteria which included *P. gingivalis, A. actinomycetemcomitans, T. forsythia and T. denticola* was associated with increased carotid intima media thickness, while the group that included *V. parvula and A. naeslundii* was related with less carotid intima media thickness. The humoral response of elevated *P. gingivalis and, A. actinomycetemcomitans* titers has been linked to cardiovascular outcomes (Pussinen, Alfthan, et al. 2007, Pussinen, Alfthan, Tuomilehto, et al. 2004, Pussinen et al. 2003, Pussinen et al. 2005, Pussinen, Paju, et al. 2007).

A recent study (Le Chatelier et al. 2013) that linked selected gut microbiota with adverse metabolic control markers reinforced our thinking about the possible role of periodontal microbiota in hyperglycemia. The altered bacterial composition during

periodontitis in reference to diabetes is well studied (Lalla et al. 2006, Li et al. 2013, Field et al. 2012, Zhou et al. 2013) but the host response role in hyperglycemia is understudied (Vlachojannis et al. 2010, Takahashi et al. 2001). In this study, our main aim was to characterize the extent to which serological markers against oral microorganisms were associated with diabetes and pre-diabetes. We hypothesized that selected oral microorganisms would be positively, while others inversely associated with hyperglycemia, based on what is known about the relation between the periodontal biofilm clusters and health and disease (Socransky and Haffajee 2005, 2002, Desvarieux et al. 2013), This is a small, but important, first step in our understanding of this question, and sets the stage for further studies aimed at better understanding of mechanisms. If it is discovered that oral micro-organism affect metabolic parameters, it may be possible to impact metabolic parameters by altering the microbial profile.

Materials and Methods

Study Population

The NHANES III used a multi-stage, probability cluster sampling design to provide nationally representative data on a variety of health risks and behaviors, including both self-reported and objective estimates of diabetes and clinical and antibody information related to periodontal disease. We included participants of the NHANES III 1988-1994 survey, aged 40 years or above who had complete information on serum IgG antibodies titers against periodontal bacteria (Ezzati et al. 1992). Serum antibody titers were assessed on stored blood samples and released for the first time in 2008.

We linked the following datasets from NHANES III public release data files: NHANES III Household Adult Data File, NHANES III Examination Data File,

NHANES III Laboratory Data File, NHANES III Dietary Recall Data Files, and Antibodies to Periodontal Pathogens using a unique de-identified respondent identification number provided to each participant. All data for this report are available at: http://www.cdc.gov/nchs/nhanes/nh3data.htm

Out of 33,994 individuals in the NHANES III sample in the six year period (1988-1994) 14,464 were aged 40 years or older, of which 11,448 were interviewed, 9379 were examined and 8153 provided sera that were analyzed for IgG titers against 19 periodontal bacteria (Vlachojannis et al. 2010). Of the eligible 8153 samples, we excluded individuals who reported taking insulin or who had gestational diabetes leaving 7848 participants in the final sample for analysis.

IgG assay for periodontal bacteria

All available sera from NHANES III participants (1988-1994) 40+ years were tested for the presence and level of IgG antibodies against a broad panel of periodontal bacteria. The following 19 bacterial strains were used to prepare whole cell antigenic extracts by checkerboard immunoassay: *Aggregatibacter actinomycetemcomitans (*ATCC strains #43718, #29523 and #33384); *Porphyromonas gingivalis (*ATCC #33277 and #53978); *Tannerella forsythia (*ATCC#43037); *Treponema denticola (*OMGS#3271); *Campylobacter rectus (*ATCC#33238); *Eubacterium nodatum (*ATCC#33099); *Prevotella intermedia (*ATCC#25611); *Prevotella nigrescens (*ATCC#33563); *Prevotella melaninogenica (*ATCC#25845); *Fusobacterium nucleatum (*ATCC#10953); *Micromonas micros (*ATCC #33270); *Selenomonas noxia (*ATCC#43541); *Eikenella corrodens (*ATCC#23834); *Capnocytophaga ochracea (*ATCC#33624); *Streptococcus intermedius (*ATCC#27335); *Streptococcus oralis (*ATCC#35037); *Streptococcus mutans*

*(*ATCC#25175); *Veillonella parvula (*ATCC#10790); and *Actinomyces naeslundii (*ATCC#49340).. The laboratory tests were performed at Columbia University College of Dental Medicine, using the ‘‘checkerboard’’ immunoassay technique measured in gravimetric units (Papapanou et al. 2000)(31)(Papapanou et al. 2000)(Papapanou et al. 2000). The detailed procedure of assessing IgG by checkerboard immunoassay is described in NHANES III documentation: ftp://ftp.cdc.gov/pub/Health_Statistics/NCHS/nhanes/nhanes3/30a/spsdeppx.pdf

Cluster Formation and Naming the Clusters

For each of 19 bacterial species, serum IgG antibody titer values were natural log transformed and then standardized by dividing these values by the log-transformed population standard deviation (Desvarieux et al. 2005). Standardized z-scores of serum IgG of diabetes and pre-diabetes subgroups were used in cluster analysis to derive four mutually distinct groups of periodontal bacteria. The standardized z-scores of IgG against those periodontal bacteria in the respective clusters were summed to obtain cluster scores for each group. These four cluster scores were the main exposure variables used in this analysis.

We used the color scheme of microbial complexes given by Socransky and Haffajee (Socransky and Haffajee 2005, 2002) to name these clusters. In that scheme, organisms in the Red and Orange clusters are related to periodontal disease; organisms in the Yellow and Purple clusters are associated with a healthy periodontal condition; the Blue cluster includes Actinomyces species which is found in both periodontal disease and in the healthy state; organisms in the Green cluster may be weakly related to periodontal disease (Socransky et al. 1998, Socransky and Haffajee 2005, 2002). In naming the

clusters, we put the dominant color of periodontal bacteria first. For example, if cluster scores included 4 organisms of which 3 are from the Orange group and one from Red, we named that cluster as Orange-Red. If there is a tie (i.e. same numbers of Red and Orange cluster organisms then we started with Red). Further details are shown in figure 3.1. This way we have four clusters named as “Orange-Red”, “Red-Green”, “Yellow- Orange” and “Orange-Blue”.

Definition of Type 2 Diabetes and pre-diabetes

We defined pre-diabetes as having HbA_{1c} 5.7 to <6.5% or fasting plasma glucose (FPG) 100 to <126 mg/dl (5.6-6.9 mmol/L). Individuals with $HbA_{1c} \geq 6.5\%$ or FPG ≥ 126 mg/dl [7 mmol/L] or those who self-reported diagnosed diabetes were classified as having diabetes (Bullard et al. 2013).

Covariate Information

Demographic, lifestyle and anthropometric measures such as gender, age, race/ethnicity, education, income-poverty ratio, smoking and alcohol drinking habits, physical activity, fiber intake, frequency of regular dentist visits, waist circumference (WC) and body mass index (BMI) are used as potential confounders. Race/ethnicity was self-reported and was divided into four groups: non-Hispanic white, non-Hispanic black, Mexican American and other. Income was grouped into three groups of the income-to-poverty ratio (≤1.5, >1.5 to ≤3.0, and >3.0), education level into three groups ≤6 years; 7 to 12 years; and ≥13 years).

Smokers were defined into three groups (current, past and never smokers). A never smoker was defined as an individuals who had smoked <100 cigarettes in his or her lifetime. The alcohol drinkers were divided into three groups (current, past and never

alcohol drinkers). Individuals who reported consuming less than 12 drinks in their lives were “never drinkers”. Physical activity was divided into three groups based on metabolic equivalents (METs) (active, ≥6; moderate, ≥4 and <6; and less active, <4).

People who had regular dental checkups were defined as those who had visited a dental clinic at least once in the past year. WC to assess central obesity was measured using a steel measuring tape to the nearest 0.1 cm at the high point of the iliac crest at minimal respiration when the participant was in a standing position. Central adiposity was said to be present if waist circumference was ≥101.6 cm for male subjects and ≥88.9 cm for female subjects. Missing teeth was categorized into four groups: none, 1-5, 6-10, > 10 missing teeth (Choi et al. 2011). Dietary Fibers, carbohydrate, protein, fat, total calories intake was assessed based on estimates from the 24-hour dietary recall.

Statistical Methods

SAS, version 9.3 (SAS Institute, Cary, NC) was used for data management and statistical analyses. SAS survey procedures were used to take into account the complex weighted sampling design and yielded unbiased parameter estimates and standard errors. Sample weights, cluster, and strata variables provided by CDC were used. A statistically significant α level was considered at 0.05.

Descriptive statistics were estimated using procedures for complex surveys in SAS (proc surveymeans and proc surveyfreq). The distribution of socio-demographic, lifestyle, anthropometric measures, and other potential risk factors across tertiles of each of four cluster scores were estimated. The mean values of four clusters scores and z scores of species –specific serum IgG were evaluated across the diabetes status.

The multivariable survey logistic regression model was used. In the diabetes status (diabetes, pre-diabetes and normal blood glucose), normal blood glucose group was the reference in all analysis. The first model included the four clusters which were regressed across the outcomes; in second model, we additionally adjusted for age (continuous), sex, race and income-to-poverty ratio. In the third model, we adjusted further for smoking and drinking alcohol and in the fourth model we included WC, physical activity, BMI (continuous), and dentist visits. Finally, we then adjusted further for missing teeth, dietary fibers, total energy, carbohydrate, protein and fat intake.

We repeated the same analysis by using the standardized z-scores of serum IgG antibodies of 11 periodontal bacteria to obtain the 3 groups (etiologic, putative and health related) as defined in a priori study by Desvarieux et al. (Desvarieux et al. 2005).

Results

The four mutually exclusive groups; “Orange-Red”, “Red-Green”, “Yellow-Orange” and “Orange-Blue” of periodontal bacteria antibodies obtained from cluster analysis and their naming pattern are shown in Flow chart 1. In our study population of 7848 individuals, 9.6% had diabetes, 36.6% pre-diabetes, and 69.7% normal blood glucose. The distribution of profile characteristics across the diabetes status are shown in Table 3.1.There were higher numbers of Non-Hispanic Whites, females, high school graduates, never smokers or past smokers but current or former alcohol drinkers, overweight, with sedentary lifestyle and those who didn’t visit dentist at least once a year. The general characteristics of study population by tertiles of cluster scores (sum of standardized z- scores in periodontal bacteria antibodies in each respective cluster) are shown in Table 3.2. There were a higher proportion of individuals with diabetes in the

top versus bottom tertiles (11.2 vs 8.1) in Orange-Red cluster (which included *P. gingivalis and prevotella species)*, however the opposite trend (8.6 vs 11.1) was observed for Orange-Blue (which included *A. naeslundii and E. nodatum)* and no such difference was seen in other clusters and pre-diabetes also showed similar pattern. Whereas distribution of participants with normal blood glucose showed an opposite pattern of Orange-Red (56.1 vs 50.3) and Orange-Blue clusters (49.0 vs 56.4) when compared in top vs bottom tertiles (Table 3.2). Age of the participants was similar across tertiles of all clusters (on average 57years) but Orange-Blue had a higher proportion of older participants compared to other clusters. BMI and fiber intake were relatively higher in top tertiles across all four clusters (Table 3.2).

The mean z-scores of species specific serum IgG against 19 bacteria and their respective four clusters are shown in Table 3.3. Significantly higher trend of z-scores of Orange-Red were seen in diabetes compared to pre-diabetes and normal blood glucose while decreasing trend of Orange-Blue clusters scores were observed in diabetes groups.

In this study, we found a one unit higher- Orange-Red cluster score was associated with 11% (p value <0.0001) higher odds of type 2 diabetes when other clusters scores were held constant. With further full adjustment for age, sex, race and income-to-poverty ratio, cigarette smoking, alcohol drinking, physical activity, BMI, WC, dentist visit, missing teeth, and different nutrients intake, the odds attenuated to 8% (p value <0.05) (Table 3.4). With pre-diabetes, there was a 3% increased odds but after adjusting for confounders, the association was not statistically significant. In Orange-Blue cluster score, one unit increase was associated with 11% (p=<0.001) lowering odds of diabetes and 7% (<0.05) lower odds of pre-diabetes holding all other clusters constant. Overall,

there was an inverse but insignificant association between this cluster score and diabetes and pre-diabetes after adjusting for all the measured confounders evaluated. Similarly, a unit increase in Red-Green cluster score (which included *A. actinomycetemcomitans mix, S. noxia, E. corrodens, V. parvula, T. forsythia, T. denticola, C. rectus)* showed an inverse relation) with diabetes but positive association with pre-diabetes, however statistically not significant. Yellow Orange cluster score (which included *S. intermedius, S. oralis, S. mutans, F. nucleatum, M. micros, C. ochracea)* showed no association with pre-diabetes and diabetes (Table 3.4). (In a sensitivity analysis comparing dentate (n=5458) and edentulous participants (n=2390) Orange-Red scores significantly increased the odds of having diabetes by 15% [OR=1.15 (1.046-1.263) in edentulous participants whereas Orange-Blue scores decreased the odds by 8% [OR=0.083 (0.80-0974) in dentate participants after adjusting for all confounders in the full model.

The relation between periodontal bacteria antibodies grouped in etiologic, putative and health related categories (Desvarieux et al. 2005) with diabetes and pre-diabetes were described in Table 3.5. A one unit increase in etiologic group (which included *P. gingivalis, A. actinomycetemcomitans, T. denticola, T. forsythia*) score was associated with 7% and 4% (both p values <0.05) higher odds of diabetes and pre-diabetes respectively. The health related group that comprised *V. parvula and A. naeslundii* was inversely related to diabetes (13% lower odds, p value <0.001) and pre-diabetes (7% lower odds, p value <0.05). After adjustment for measured confounders, the etiologic and health related group association remained insignificant for both diabetes and pre-diabetes. The putative group that included *E. corrodens, C. rectus, F. nucleatum, M. micros, and P. intermedia* was not associated with diabetes and pre-diabetes.

Discussion

The association between clusters of serum IgG antibodies against periodontal bacteria and hyperglycemia varied qualitatively in a large, representative sample of US adults. Increased Orange-Red cluster titers were associated with 8% higher odds of diabetes while increased Red-Green cluster titers were associated with 4% lower odds after adjustment for potential confounders. The Orange-Red cluster included periodontopathic bacteria that are linked to local inflammatory response, hard and soft tissue breakdown leading to clinical signs of local periodontitis, and systemic inflammation.

There is a strong biological rationale behind using cluster analysis in this study. Since periodontal bacteria coexist naturally in a biofilm, it is therefore to isolate the potential effects of particular pathogens; moreover, studies evaluating the effects of individual organisms are prone to type 1 error(Wang et al. 2007). To overcome this limitation some investigators have grouped the periodontal organisms together either using empirical or theoretical approaches (Socransky and Haffajee 2002, Desvarieux et al. 2005). Specific clusters of periodontal bacteria profiles for type 2 diabetes have not been reported in the literatures. In the current study, we used both empirical as well as theoretical approaches to group antibodies against oral pathogens among individuals with hyperglycemia and measured the extent of association of these bacteria with diabetes and pre-diabetes. We did this because we were interested in evaluating the relative (rather than absolute) contributions of the titers to the overall score.

The immunological role of some periodontal bacteria such as *P. ginigvalis, and A. actinomycetemcomitans* in relation to periodontitis and other systemic disease has been

reported in recent studies. Elevated serum anti- *P. gingivalis* antibody has been associated with ishemic stroke (Pussinen, Alfthan, Rissanen, et al. 2004), myocardial infarction (Holmlund et al. 2011, Pussinen, Alfthan, Tuomilehto, et al. 2004) and coronary heart disease (Pussinen et al. 2003) but *A. actinomycetemcomitans* showed inconsistent relationships in different populations (Mantyla et al. 2013, Hosomi et al. 2012, Pussinen et al. 2005).

There are few studies evaluating serum antibody titers against periodontal bacteria and diabetes. Ebersole et al (Ebersole et al. 2008) compared serum antibody levels of *P gingivalis, A actinomycetemcomitans,T. denticola, T. forsythia, C. rectus,F. nucleatum, E. corrodens, and P.intermedia* using standard enzyme-linked immunosorbent assay methodology in type 2 diabetes and non-diabetes group. Antibodies against *P gingivalis* were significantly elevated in the diabetes group while serum IgG responses to other microbes were broadly similar between the two groups. However, these studies had small sample sizes, differences in characteristics of the study populations, and methods of detecting of periodontal microbiota, making it difficult to compare the findings. Another study (Vlachojannis et al. 2010) included both type 1and type 2 diabetes cases found elevated titers of *P. gingivalis* associated with diabetes.

In our study, the Orange-Red cluster scores and the etiologic group that included *P. gingivalis* were positively associated with hyperglycemia (Table 3.4 and Table 3.5). Interestingly, the cluster Red-Green that included antibodies against three organisms that have been associated with periodontitis and systemic disease (Desvarieux et al. 2005) was inversely related with diabetes in this study (Table 3.4). This may be because this cluster also included the purple complex organisms, *V. parvula*, which has been linked to

healthy periodontal status (Desvarieux et al. 2005), and could have shadowed the effect of the Red-cluster organisms. There are no specific similar studies related to diabetes to compare our findings but a study evaluating serum IgG antibodies against 12 periodontal pathogens among Japanese children with type-1 diabetes mellitus found significantly higher antibody titers to *P. gingivalis, P intermedia* and *T denticola* compared to other periodontal bacteria (Takahashi et al. 2001). In a separate recent study (Hosomi et al. 2012), serum IgG antibody against *P. gingivalis* was significantly associated with atrial fibrillation, and antibody against *P. intermedia* was associated with carotid artery atherosclerosis and atherothrombotic stroke whereas no such association was seen with anti- *A. actinomycetemcomitans* serum antibody. Our findings support the hypothesis that *P. gingivalis* may have a major role in systemic conditions including diabetes compared to other periodontal pathogens (Jeong et al. 2012).

The Orange-Blue cluster scores that included *A. naeslundii* and *E. Nodatum* showed an inverse relation to diabetes and pre-diabetes in the crude analysis but that was attenuated after adjustment [diabetes: OR 0.935 (0.869-1.007), pre-diabetes OR: 0.987 (0.944-1.032) (Table 3.4). Several studies have shown that the higher counts of *A. naeslundii* are associated with periodontal healthy status (Socransky and Haffajee 2002, Desvarieux et al. 2005, Socransky and Haffajee 2005, Darveau 2010, Papapanou et al. 2000) but a case-control study (Haffajee, Teles, and Socransky 2006) reported higher level of *E. nodatum* to be associated with periodontitis and another (Lalla et al. 2006) reported an association with type 1 diabetes. The health related group that included *A. naeslundii* and *V. parvula* was inversely related to pre-diabetes and diabetes (Table 3.4) which supports the evidence that long term predominance of *A. naeslundii* in the

periodontal area may be related to healthy clinical outcomes and may play a protective role in systemic conditions.

Yellow-Orange cluster scores which included health-related bacteria, Streptococcus species and *C. ochracea* showed no distinct association with either of the outcomes. This might be due to the presence of serum IgG of other periodontal bacteria such as *F. nucleatum, M. micros* in this cluster scores which had the putative role (Haffajee, Teles, and Socransky 2006, Shin et al. 2013).

Our study has some drawbacks. As this was a cross-sectional study, we could not verify the temporal sequence of the associations. As is the case with all observational studies unmeasured confounding could potentially impact the results. However, we adjusted for a number of known potential confounders in our analyses. We did not adjust for clinical measures of periodontal disease (such as clinical attachment loss or probing depth) because these are on the causal pathway (periodontal microorganisms cause attachment loss and periodontal pockets), and adjusting for them would likely introduce bias (Cole and Hernan 2002). This dataset included information from 1988-1994. Even though the prevalence of periodontal disease, diabetes and other covariates such as smoking have changed over this time, this would not impact our results because change in prevalence does not impact the relation between the exposure and outcome (Szklo and Nieto 2007). In this dataset, there are information on only 19 periodontal bacteria, hence a new sophisticated study that combines the immunological, microbiological and clinical information of periodontal disease are needed to provide further insights in systemic association and further study of undisputed hypothesis that oral health could be important source of chronic inflammation.

The study also had some strength. The CDC conducted assays for an expanded panel of antibodies against periodontal microorganisms on stored blood samples from the NHANES III and released the results in 2008. This was timely, because there is renewed interest in studying the relation periodontal microorganisms and the microbiome in relation to systemic outcomes. The NHANES III dataset has good quality information for periodontal disease, serum IgG antibodies against 19 different periodontal bacteria, diabetes, and other covariates of interest in a large, representative number of US adults. Serum IgG against *A. actinomycetemcomitans* and *P. gingivalis* are stable over at least 15 years (Lakio et al. 2009), and represent a measure of cumulative lifetime infection of periodontal disease and host response towards it. For this reason we included edentulous individuals in the main analyses, however, the results did not change materially when missing were adjusted (Table 3.4 and Table 3.5). These data have not been reported on extensively and hence provide new information.

The association between antibodies against oral microorganisms and hyperglycemia moderately varied by the type of periodontal organisms that are involved in a large, representative sample of US adults. Monitoring the type of periodontal bacteria in plaque may be important in individuals with diabetes and pre-diabetes.

Table 3.1− Profile characteristics of study population NHANES III (1988-1994)

	Normal(n=3424)	Pre-diabetes(n=3334)	Diabetes(n=1090)
Age, years, mean (se)	53.85 (0.3)	59.5(0.6)	61.7(0.6)
Sex: %			
Male	42.7	50.2	46.8
Female	57.3	49.8	53.2
Race/ Ethnicity %			
Non-Hispanic Whites	85.3	76.9	71.6
Non –Hispanic Blacks	6.1	11.3	14.6
Mexican American	2.6	3.9	6.0
Others	5.9	7.9	7.7
Education %			
≤ 6 years	4.7	8.5	12.3
7 – 12 years	50.4	57.6	62.2
≥ 13 years	44.9	33.9	25.4
Income to poverty ratio %			
Lower (≤1.5)	15.0	22.0	30.2
Middle (≤3.0)	28.6	30.5	31.5
Higher (>3.0)	56.4	47.4	38.3
Smoking %			
Current	41.6	35.1	37.2
Past	41.7	46.4	47.3
Never	16.7	18.5	15.5
Alcohol intake %			
Current	13.9	15.3	20.0
Past	34.0	42.2	48.9
Never	52.0	42.5	31.1
Physical activity %			
Sedentary	54.8	49.3	45.6

Moderately active	12.3	13.4	11.1
Vigorously active	8.0	7.4	6.7
Missing	24.9	29.8	36.6
Missing Teeth, %			
0	53.1	51.7	50.5
1-5	37.1	34.7	29.7
6-10	7.7	9.3	12.1
>10	2.1	4.3	7.8
Annual visits to dentist %			
Yes	59.1	43.9	38.4
No	40.9	56.1	61.6
Waist circumference %			
Normal	60.0	45.2	23.8
Elevated	40.0	54.8	76.2
Dietary Fibers, g/day, mean (se)	17.2(0.3)	16.4(0.3)	16.2(0.5)
Total Carbohydrate, g/day, mean (se)	250.5(4.1)	243.9(4.1)	222.7(5.1)
Proteins, g/day, mean (se)	76.6(1.0)	77.8(1.3)	75.0(2.2)
Total fats, g/day,mean (se)	77.0(1.3)	75.7(1.6)	70.4(2.8)
BMI, kg/m^2, mean (se)	26.4(0.1)	27.8(0.2)	30.1(0.3)
Orange-Red score, mean (se)	-0.5 (0.1)	-0.3(0.1)	0.1(0.2)
Red-Green score, mean (se)	-0.1 (0.3)	0.02(0.3)	-0.2(0.5)
Yellow-Orange score, mean (se)	-0.01(0.2)	0.004(0.2)	0.06(0.3)
Orange-Blue score, mean (se)	0.2 (0.1)	0.07(0.1)	-0.04(0.1)

Table 3.2− Distribution of general characteristic of study population (n=7848) in the top vs bottom tertiles of four different clusters scores

Variables	[a]n	Orange-Red		Red-Green		Yellow-Orange		Orange-Blue	
		Tertile1	Tertile3	Tertile1	Tertile3	Tertile1	Tertile3	Tertile1	Tertile3
Sex (%)									
Male	3677	40.1	50.3	41.3	49.8	42.9	47.0	45.9	46.7
Female	4171	59.9	49.7	58.7	50.2	57.1	52.4	54.1	53.3
Education (%)									
≤ 6 years	1403	5.5	9.2	5.7	7.3	7.0	7.1	7.9	5.1
7 – 12 years	4344	57.2	52.1	58.5	50.5	58.6	52.0	58.7	49.3
≥ 13 years	2046	37.3	38.7	35.8	42.3	34.4	40.9	33.4	45.6
[b]**Income to poverty ratio** (%)									
Lower (≤1.5)	2387	18.5	20.6	19.3	17.7	22.4	18.4	22.8	15.0
Middle (≤3.0)	2214	28.8	28.8	30.6	30.4	28.8	30.8	33.0	27.2
High (>3.0)	2496	52.7	50.6	50.1	51.9	48.8	50.8	44.1	57.8
Race/ethnicity (%)									
Non-Hispanic Whites	4012	87.6	71.9	85.8	76.2	84.0	76.7	80.4	82.6
Non-Hispanic Blacks	1817	5.6	13.4	6.8	10.8	7.4	11.2	9.8	8.3
Mexican Americans	1699	2.2	4.9	2.8	4.1	2.9	3.8	3.1	3.6

Other	320	4.6	9.8	4.6	8.9	5.7	8.3	6.7	5.5
Cigarette Smoker (%)									
Never smoker	3562	39.5	46.3	37.5	45.9	38.7	45.7	38.6	46.5
Past smoker	2521	33.2	35.5	34.2	36.7	33.3	35.3	32.7	35.4
Current Smoker	1765	27.3	18.2	28.3	17.4	28.0	19.0	28.7	18.1
Alcohol (%)									
Never drinker	1480	15.6	15.7	14.7	16.1	15.2	16.2	17.2	13.6
Past drinker	3185	38.3	37.4	38.5	38.9	38.7	37.6	39.6	38.3
Current drinker	3031	46.1	46.9	46.7	45.0	46.1	46.2	43.2	48.1
Dentist visit once in year (%)									
Yes	3010	51.7	52.0	49.4	52.2	48.1	54.6	47.9	55.3
No	4588	48.2	8.1	50.6	47.8	51.9	45.4	52.0	44.7
Physical Activity (%)									
Sedentary	3550	53.6	51.7	54.4	50.3	51.6	50.5	51.3	54.5
Active	927	12.3	11.4	11.1	12.3	12.0	11.8	12.1	12.4
Vigorously active	500	6.8	7.8	7.2	7.8	7.6	8.2	6.8	8.1
[c]**Central Obesity** (%)									
Normal	3602	52.1	49.4	50.5	49.4	51.9	51.2	50.9	52.0
Elevated	3904	47.9	50.6	49.5	50.6	48.2	48.8	49.1	48.0

Diabetes Status (%)									
Diabetes	1090	8.1	11.2	9.2	9.8	10.4	10.5	11.1	8.6
Pre-diabetes	3334	35.9	38.5	37.1	37.6	38.1	36.2	39.9	35.1
Normal	3424	56.1	50.3	53.7	52.6	51.5	53.3	49.0	56.4
					Mean ± SD (n)				
AGE (years)		56.9±0.5	56.6±0.6	56.8±0.5	56.5±0.6	57.8±0.6	56.2±0.6	58.4±0.6	57.1±0.1
BMI (kg/m^2)		26.9±0.2	27.8±0.2	27.0.9±0.2	27.7±0.2	26.9±0.2	27.5±0.2	26.9±0.2	27.3±0.2
Fiber Intake (g/day)		16.1±0.3	17.0±0.3	15.8±0.3	17.3±0.3	16.0±0.3	16.9±0.2	16.3±0.3	16.5±0.3
HbA1c (%)		5.5±0.0	5.6±0.0	5.5±0.03	5.5±0.0	5.6±0.0	5.6±0.0	5.6±0.0	5.6±0.0
Fasting Plasma Glucose (mg/dl)		99.8±0.6	103.5±1.1	101.7±0.8	101.8±0.9	102.7±0.9	102.1±1.1	103.9±1.1	101.3±0.9

[a]Totals may be different due to missing data
[b]Income-to-poverty ratio: (midpoint family income)/(poverty threshold values based on calendar years and inflation)
[c]Central adiposity present if waist for male subjects ≥101.6 cm and for female subjects ≥88.9 cm

Table 3.3− Mean z-score values of four cluster scores and the species-specific IgG antibody titers across diabetes status

Clusters/Periodontal Bacteria	Normal blood glucose (N= 3424)	Pre-diabetes (N= 3334)	Diabetes (N= 1090)	P-trend
	Clusters z-scores mean (SEM)			
Orange-Red	-0.45 (0.1)	-0.26 (0.1)	0.11 (0.2)	**0.0008**
Red-Green	-0.11 (0.4)	0.02 (0.3)	-0.15 (0.5)	0.7753
Yellow- Orange	-0.01 (0.2)	0.004 (0.2)	0.06 (0.3)	0.7443
Orange-Blue	0.24 (0.1)	0.08 (0.1)	-0.04 (0.1)	**0.0009**
	Species specific z scores mean (SEM)			
Porphyromonas gingivalis	-0.22 (0.03)	-0.12 (0.03)	-0.05 (0.05)	**0.0014**
Prevotella intermedia	-0.12 (0.02)	-0.09 (0.02)	-0.02 (0.04)	**0.0248**
Prevotella nigrescens	-0.06 (0.03)	-0.03 (0.03)	0.06 (0.04)	**0.0338**
Prevotella melaninogenica	-0.05 (0.03)	-0.02 (0.03)	0.11 (0.1)	**0.0051**
Tannerella forsythia	0.01 (0.1)	0.01 (0.1)	0.03 (0.1)	0.6760
Veillonella parvula	-0.01 (0.04)	0.01 (0.04)	0.003 (0.1)	0.5443

Selenomonas noxia	-0.004 (0.1)	0.03 (0.04)	-0.02 (0.1)	0.7147
Campylobacter rectus	-0.08 (0.1)	-0.05 (0.04)	-0.01 (0.1)	0.1231
Treponema denticola	0.02 (0.1)	0.001 (0.04)	-0.02 (0.1)	0.4837
Aggregatibacter actinomycetemcomitans	-0.04 (0.1)	0.004 (0.1)	-0.04 (0.1)	0.4167
Eikenella corrodens	-0.01 (0.1)	0.01 (0.1)	-0.10 (0.1)	0.3792
Fusobacterium nucleatum	-0.02 (0.03)	-0.03 (0.03)	0.03 (0.03)	0.6345
Streptococcus intermedius	-0.03 (0.04)	0.01 (0.1)	0.07 (0.1)	**0.0084**
Streptococcus oralis	-0.02 (0.04)	-0.01 (0.1)	-0.01 (0.1)	0.8761
Streptococcus mutans	-0.02 (0.04)	-0.01 (0.04)	0.01 (0.1)	0.6087
Micromonas micros	0.05 (0.03)	0.06 (0.03)	0.004 (0.1)	0.6065
Capnocytophaga ochracea	0.02 (0.03)	-0.02 (0.03)	-0.04 (0.1)	0.1589
Eubacterium nodatum	0.12 (0.04)	0.03 (0.04)	-0.02 (0.1)	**0.0015**
Actinomyces naeslundii	0.12 (0.03)	0.05 (0.03)	-0.02 (0.03)	**0.0024**

Table3.4−Odds Ratios (OR) & 95% CI for Type-2 diabetes and pre-diabetes in different clusters scores

Models OR (95% CI)	**Type 2 DIABETES**				**PRE-DIABETES**			
	Orange-Red	Red-Green	Yellow-Orange	Orange-Blue	Orange-Red	Red-Green	Yellow-Orange	Orange-Blue
Model1	**1.111*** (1.067- 1.157)**	0.979 (0.943 - 1.017)	1.002 (0.955-1.051)	**0.888** (0.831-0.949)**	**1.035* (1.002-1.069)**	1.024 (0.993 - 1.056)	0.971 (0.936-1.008)	**0.927* (0.881-0.949)**
Model2	**1.074* (1.026-1.124)**	0.963 (0.925-1.002)	1.026 (0.978-1.078)	0.962 (0.901-1.028)	1.014 (0.981-1.047)	1.011 (0.979-1.044)	0.988 (0.951-1.027)	0.975 (0.932-1.020)
Model3	**1.080* (1.029-1.133)**	**0.957* (0.919- 0.997)**	1.033 (0.985-1.084)	0.956 (0.897 - 1.019)	1.015 (0.980 - 1.051)	1.014 (0.981- 1.047)	0.983 (0.948 - 1.024)	0.983 (0.939 - 1.029)
Model4	**1.082* (1.032-1.134)**	**0.955* (0.912 -0.999)**	1.049 (0.993-1.109)	0.929 (0.861-1.001)	1.022 (0.988-1.058)	1.005 (0.969 -1.043)	0.993 (0.951-1.037)	0.987 (0. 942 - 1.033)
Model5	**1.079* (1.031-1.130)**	0.956* (0.912 -1.001)	1.048 (0.992-1.108)	0.935 (0.869-1.007)	1.023 (0.988-1.059)	1.005 (0.969 -1.043)	0.993 (0.951-1.038)	0.987 (0. 944 - 1.032)

Model1 with no adjustment for confounders; Model2 adjusted for age (continuous), sex, education, ethnicity, income-to-poverty; Model3 adjusted further for smoking and drinking alcohol; Model4 further for physical activity, BMI (continuous), waist circumference [WC],dentist visit and fibers intake. Model5 adjusted further for missing teeth, daily carbohydrate intake, protein intake, fat intake, and total calories intake.

p-value <0.05, ** p-value <0.001, *** p-value <0.0001

Orange-Red: *P. melaninogenica, P. intermedia, P. nigrescens, P. gingivalis*

Red-Green: T. forsythia, T. denticola, A. actinomycetemcomitans, E. corrodens, S. noxia, V. parvula, C. rectus

Yellow-Orange: S. intermedius, S. oralis, S. mutans, F. nucleatum, M. micros, C. ochracea

Orange-Blue: E. nodatum, A. naeslundii

Table 3.5− Odds Ratios & 95% CI for type-2 diabetes and pre-diabetes in different groups of z-scores titers (classified according to Desvarieux et al., 2005)

Models	Type 2 DIABETES			PRE-DIABETES		
OR (95% CI)	Etiologic	Putative	Health	Etiologic	Putative	Health
Model1	**1.067*** **(1.003 - 1. 135)**	1.003 (0.958- 1.050)	**0.871**** **(0.803-0.945)**	**1.041*** **(1.002 - 1.080)**	1.001 (0.971- 1.031)	**0.927*** **(0.87-0.988)**
Model2	1.047 (0.980-1.118)	0.991 (0.948-1.035)	0.947 (0.87-1.034)	1.037 (0.998-1.077)	0.989 (0.957-1.023)	0.968 (0.90-1.041)
Model3	1.044 (0.976 - 1.115)	0.995 (0.951-1.041)	0.945 (0.867-1.029)	**1.040*** **(1.001 - 1.08)**	0.986 (0.954-1.019)	0.974 (0.906-1.051)
Model4	1.024 (0.957 - 1.096)	1.014 (0.958-1.072)	0.949 (0.868-1.039)	**1.044*** **(1.004 - 1. 086)**	0.986 (0.952-1.021)	0.980 (0.906-1.061)
Model5	1.014 (0.948 - 1.084)	1.019 (0.963-1.079)	0.959 (0.880-1.045)	**1.044*** **(1.004 - 1. 085)**	0.987 (0.954-1.022)	0.980 (0.908-1.058)

Model1 with no adjustment for confounders; Model2 adjusted for age (continuous), sex, education, ethnicity; Model3 adjusted further for smoking and drinking alcohol; Model4 further for physical activity, BMI (continuous), WC, dentist visit and fibers intake. Model5 adjusted further for missing teeth, daily carbohydrate intake, protein intake, fat intake, and total calories intake.

*P-value <0.05, **P-value <0.001,

Etiologic: *P. gingivalis , A. actinomycetemcomitans, T. forsythia, T. denticola*

Putative: *F. nucleatum, M. micros, C. rectus, P. intermedia, E. corrodens*

Health : *V. parvula, A. naeslundii*

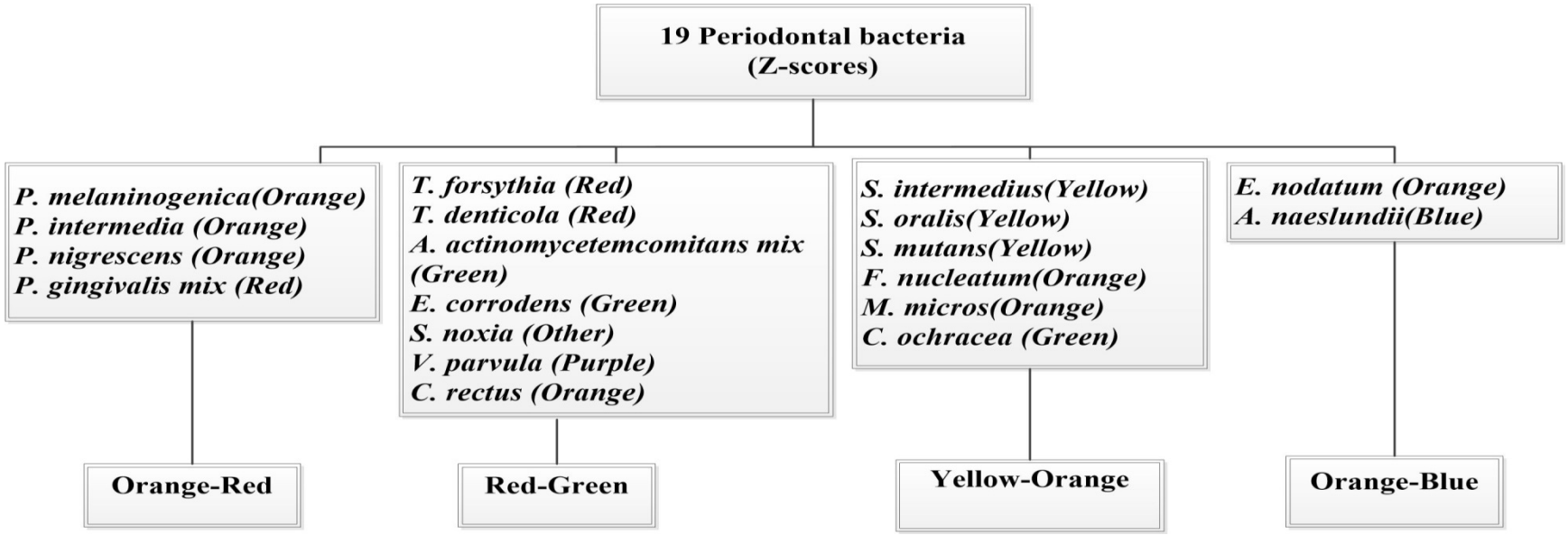

Figure 3.1: Composition of mutually exclusive four clusters formed with cluster analysis of z scores of 19 periodontal serum antibody titers. Naming of clusters was done using predominant Socransky's microbial color complexes as shown in parenthesis in the respective cluster.

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CHAPTER 4

Manuscript 2

Effect modification of the relation between serological periodontal markers and type 2 diabetes by periodontal pocket depth

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Abstract:

Chronic periodontitis is a silent infectious disease that is prevalent worldwide in adults and is associated with chronic diseases. Elevated serum IgG markers against selected periodontal bacteria are associated with periodontal disease or health. The interaction between the serological markers and clinical periodontal measures is understudied in relation to systemic diseases. Our objective was to investigate the joint association of clinical periodontal measures and serological markers in relation to diabetes.

We evaluated the interaction in a cross-sectional study among adult participants who were 40 years or older from nationally representative NHANES III (1988-1994) survey. Serum IgG antibody titers against 19 periodontal bacteria from those participants who had glycated hemoglobin ≥5.7 were grouped into four clusters and were named as Orange-Red, Red-Green, Yellow-Orange and Orange-Blue according to Socransky & Haffagee color complex scheme. Mean clinical attachment loss (CAL) and pocket depth (PD) were divided into tertiles. we further defined the periodontal status as moderate to severe or normal based on CAL and PD and the outcome was defined as having diabetes or normal blood glucose level (n=2908). We performed survey logistic procedures in SAS 9.3 to explore the role of clinical periodontal measures on the relations between serological clusters and diabetes.

The inverse association between the Orange-blue cluster score (that included *E. nodatum* and *A. neslundii* antibody titers) was stronger in the top versus bottom tertile of mean periodontal pocket depth ORTertile $_1$= 1.07, 95% CI (0.89, 1.29), $OR_{Tertile\ 2}$= 0.84, 95% CI (0.72, 0.98) and $OR_{Tertile\ 3}$= 0.74, 95% CI (0.60, 0.91), p-value for

interaction=0.07 after multivariable adjustment.

The relation between IgG antibody groups and diabetes were varied by tertiles of periodontal pocket depth. This suggests that clinical parameters and antibody titers against periodontal antibodies in combination can predict the hyperglycemic condition.

Introduction

Periodontal disease results from low grade chronic inflammation by multiple indigenous organisms (Thomas et al. 2012) that leads to tissue destruction. Traditionally, diagnostic parameters such as clinical attachment loss (CAL) and probing depths are used in assessing the periodontal disease. Despite extensive research in the area of serological makers of periodontal pathogens (Albandar et al. 2002, Dye et al. 2005, Dye et al. 2009, Mustapha et al. 2007, Papapanou et al. 2004, Pussinen et al. 2011), these are not commonly used in periodontal disease assessment. A possible reason may be that the interpretation of elevated antibodies titers against specific periodontal bacteria is not straightforward (Lockhart et al. 2012); high levels of antibodies may either indicate effective host defense, or chronic failure to control the pathogen and be prognostic of major periodontal tissue destruction (Albandar et al. 2002). Hence, both clinical measures (CAL and Probing depth), and host defense measures (antibody titers against periodontal pathogens), may provide important information about different aspects of the periodontal disease process, particularly in relation to systemic disease (Mustapha et al. 2007).

Elevated serum immunoglobulin G (IgG) antibody titers against specific periodontal bacteria e.g. *Porphyromonas gingivalis* , *Prevotella sps* are linked to inflammatory diseases such periodontal pathogenesis, coronary heart disease, type 2

diabetes, myocardial infraction and others (Ebersole et al. 2008, Mustapha et al. 2007, Oppermann, Weidlich, and Musskopf 2012, Pussinen et al. 2007, Pussinen et al. 2004, Pussinen et al. 2005, Albandar et al. 2002); elevated IgG titers against *E. nodatum* are linked to periodontal health (Dye et al. 2009). In general there is a shift in periodontal microbiota associated with systemic health states (Berezow and Darveau 2011). It is well understood that elevated serum IgG antibody titers against specific periodontal bacteria may reflect history of past infection by those bacteria (Lakio et al. 2009, Kinane and Lappin 2001); however, there is very limited knowledge about the combined effect of periodontal destruction status and elevated antibody titers on systemic disease activity.

We previously clustered serum IgG antibody titers against 19 periodontal bacteria into four empirically derived clusters, and investigated their relation to hyperglycemia. We found that measures of hyperglycemia were higher among individuals with high antibody titers against certain oral microbes, and lower among others (unpublished findings). In this present study, we used these four clusters of antibodies against periodontal microorganisms and investigated if the presence of clinical periodontal disease outcome assessed by CAL and PD modified their association with diabetes. This finding will increase understanding of the potential combined effect of measures of periodontal destruction and antibody titers against periodontal microorganisms in relation to diabetes.

Materials and Methods:

Data Source and study population:

The periodontal serum IgG antibody titers analyzed for this cross-sectional study were from adult participants who were 40 years or older who participated in the

nationally representative NHANES III (1988-1994) survey. The NHANES III used a multi-stage probability cluster sampling design to provide nationally representative data on a variety of health risks and behaviors, including both self-reported and objective estimates of diabetes and medical conditions, oral health data from dental examination, and antibody information related to periodontal disease. The selection and sampling methods are described elsewhere (Ezzati et al. 1992).

We included participants with complete information on serum IgG antibodies against 19 oral bacteria (n=8153) and relevant socio-economic, anthropometric, laboratory and nutritional data available. For this purpose, we linked the dataset with information on antibodies against periodontal pathogens (SPSDEPPX) with the following datasets from NHANES III public release data files: NHANES III Household Adult Data File (Catalog Number 77560), NHANES III Examination Data File (Catalog Number 76200); NHANES III Laboratory Data File (Catalog Number 76300); NHANES III Dietary Recall Data Files (Catalog Number 76700) using a unique de-identified respondent identification number (SEQN NO) provided to each participants. All data for this report are available at: http://www.cdc.gov/nchs/nhanes/nh3data.htm

IgG assay for periodontal bacteria

Most sampled persons giving blood samples in the mobile examination center (MEC) provided additional blood sample that were stored at -70^0C for future laboratory tests. 8153 persons had sera analysed for IgG antibodies to 19 oral bacterial species. The following 19 bacterial strains were used to prepare whole cell antigenic extracts by checkerboard immunoassay: Aggregatibacter actinomycetemcomitans (ATCC strains #43718, #29523 and #33384); Porphyromonas gingivalis (ATCC #33277 and #53978);

Tannerella forsythia (ATCC#43037); Treponema denticola (OMGS#3271); Campylobacter rectus (ATCC#33238); Eubacterium nodatum (ATCC#33099); Prevotella intermedia (ATCC#25611); Prevotella nigrescens (ATCC#33563); Prevotella melaninogenica (ATCC#25845); Fusobacterium nucleatum (ATCC#10953); Parvimonas micra (previously named Micromonas micros) (ATCC #33270); Selenomonas noxia (ATCC#43541); Eikenella corrodens (ATCC#23834); Capnocytophaga ochracea (ATCC#33624); Streptococcus intermedius (ATCC#27335); Streptococcus oralis (ATCC#35037); Streptococcus mutans (ATCC#25175); Veillonella parvula (ATCC#10790); and Actinomyces naeslundii (ATCC#49340). The laboratory tests were performed by the Oral and Diagnostic Sciences Laboratory, Columbia University College of Dental Medicine, using the ‘‘checkerboard’’ immunoassay technique measured in gravimetric units and these laboratory reports were made available in 2008. The specific laboratory method and quality control and monitoring for the measurement of serum IgG titers are described in the documentation of NHANES III (National Center for Health Statistics 2008). The detailed procedure of assessing IgG by checkerboard immunoassay is described in NHANES III documentation: ftp://ftp.cdc.gov/pub/Health_Statistics/NCHS/nhanes/nhanes3/30a/spsdeppx.pdf

Cluster Formation and Naming the Clusters

For each bacterial species, serum IgG antibody titer values were natural log transformed and then standardized by dividing these values by the log-transformed population standard deviation (Desvarieux et al. 2013, Desvarieux et al. 2005). We then selected homogenous subpopulation of those individuals who have blood glucose greater than HbA1c $\geq$ 5.7 (n=3049) and by using cluster analysis we derived four mutually

distinct groups of periodontal bacteria. The specific bacteria that were grouped in each cluster were shown in Flowchart1.The standardized z-scores of IgG against those periodontal bacteria in the respective clusters were summed to obtain cluster scores for each group. These four cluster scores were the main exposure variables used in this analysis.

We named each cluster according to the dominant color scheme of microbial complexes given by Socransky and Haffajee (Socransky and Haffajee 2005, 2002). For instance, if a cluster had bacteria mixture of Red, Orange and other complexes, then the cluster was named according to the predominant bacterial complex. We obtained four clusters named as "Orange-Red", "Red-Green", "Yellow- Orange", and "Orange-Blue" as described in Figure 3.1.

Measurement of periodontal health

We assessed periodontal damage by PD and CAL. The detailed method is described elsewhere (Choi et al. 2011). CAL of 1–2 mm is considered to be slight, 3–4 mm moderate, and ≥5 mm severe. Dental examiners trained on the NHANES protocol examined all participants at mobile examination centers. Periodontal health assessment was based on CAL and pocket depth measurements made at two sites (midbuccal and mesiobuccal) on every tooth in each of two randomly chosen quadrants, one in maxilla and the other in mandible as described in the NHANES procedures manuals. In total, 28 sites and 14 teeth per individual were measured if the subject had no history of tooth removal excluding third molars (Choi et al. 2011).

We computed mean CAL and PD and grouped individuals into tertiles; the lowest tertile having least periodontal damage as has been describe elsewhere (Choi et al. 2011).

Definition of Diabetes

Individuals were defined as normal (n=2595) if HbA_{1c} was <5.7% or fasting plasma glucose (FPG) <100 mg/dl (<5.6 mmol/L). Individuals with $HbA_{1c} \geq 6.5\%$ or FPG ≥ 126 mg/dl [7 mmol/L] or those who self-reported diagnosed diabetes were classified as having diabetes (n=738) (Bullard et al. 2013).

Covariate Information

The covariates included demographic, lifestyle and anthropometric measures such as gender, age, race/ethnicity, education, income-poverty ratio, smoking and alcohol drinking habits, physical activity, fiber intake, frequency of regular dentist visits, waist circumference (WC), body mass index (BMI) and diabetes. Race/ethnicity was self-reported and was divided into four groups: non-Hispanic white, non-Hispanic black, Mexican American and others. Income was grouped into three groups of the income-to-poverty ratio (≤1.5, >1.5 to ≤3.0, and >3.0), education level into three groups ≤6 years; 7 to 12 years; and ≥13 year. The number of missing teeth into four groups (0, 1–5, 6–10, and ≥11).

Smokers were defined into three groups (current, past and never smokers). For instance, if they said yes to the question "Do you smoke now" then they were defined as current cigarettes smokers. If not, then based on the question "Have you smoked 100+ cigarettes in life", we defined them as past smoker or not smoker. Alcohol drinkers were divided into three groups (current, past and never alcohol drinkers). If individuals said "No" to the question "Had at least 12 drinks in life" were "Never Drinkers". Those who said "yes" were asked next question "Had at least 12 drinks in last 12 months", if they

said yes then were defined as “Current Drinkers”, else were “Past Drinkers”. The physical activity into three groups based on metabolic equivalents (METs) (active, ≥6; moderate, ≥4 and <6; and less active, <4).

Fiber intake (grams/day) was assessed based on estimates from the 24-hour dietary recall. People who had regular dental checkups were defined as those who had visited a dental clinic at least once in the past year. WC to assess central obesity was measured using a steel measuring tape to the nearest 0.1 cm at the high point of the iliac crest at minimal respiration when the participant was in a standing position . Central adiposity was said to be present if WC was ≥101.6 cm for male subjects and ≥88.9 cm for female subjects.

Statistical Methods

In this analysis, we excluded insulin users (n=299), pre-diabetic individuals (n=3334) and those who had no periodontal examination measures (n=1181); hence the final data for analysis were from 3333 participants.

We used statistical procedures in SAS, version 9.3 (SAS Institute, Cary, NC) to analyze complex survey using cluster, weight and strata statements, provided by Center for Disease Control for all the statistical analysis. We calculated the weighted means and weighted frequencies of serum IgG antibody titers clusters, socio-demographic and other risk factors according to the tertiles of periodontal clinical measures (accessed by tertiles of Pocket depth and tertiles of CAL).

To explore the crude association between periodontal clinical measures and the four z-score clusters of antibody titers, we first ran crude multivariate logistic regression model. The second model was adjusted for age, sex, race-ethnicity, and income-poverty

ratio. The third model was additionally adjusted for smoking and drinking alcohol, and the fourth model additionally included physical activity, WC, BMI, dentist visits, missing teeth, nutrients including intakes of total energy, fibers, carbohydrate, protein, and fat. The adjusted odds ratios (ORs), 95% CIs, and *P* values calculated.

We stratified the analyses described above by tertiles of PD and CAL. To evaluate possible effect modification we created interaction terms for each of serum IgG clusters and tertiles of PD and CAL. For example, to evaluate the interaction between Orange-Red cluster and CAL on diabetes the following variables were included in the model: a multiplicative term between Orange-Red cluster and CAL, scores for all other three clusters, and covariates including CAL. The interaction was considered statistically significant if the p-value for the type III analysis of effect for the interaction term was <0.05.

Results:

Characteristics of the study population across the tertiles of periodontal clinical measures

The CAL and PD showed positive correlation with Orange-Red and inverse correlation with Orange-Blue cluster scores (Spearman correlation coefficients are shown in Table 4.1.

The weighted means (SEs) and weighted frequency percentages of socio-demographic and other potential risk factors are shown in Table 4.2. Orange-Blue cluster scores decreased across the tertiles of clinical attachment loss (p trend =<0.0001) while significant trends were not seen in other cluster scores. The mean of Orange-Red cluster score increased (p trend =0.03) while Orange-Blue cluster scores decreased (p trend=

0.0095) across the tertiles of pocket depth.

Investigation of confounding and effect modification of clinical periodontal destruction measures and serum antibody titers in relation to diabetes

The extent of association of serum antibody titers and diabetes in a full model were not further changed when either of CAL or PD was included (Table 4.3). Hence, no confounding by clinical periodontal destruction was observed.

The association between diabetes and four serum antibody cluster scores stratified by tertiles of mean CAL and PD are shown in Table 4.4 and Table 4.5. The relationship between serum antibody cluster scores and diabetes did not vary when stratified by the tertiles of CAL (Table4. 4), but approached difference within strata of tertiles of PD (Table 4.5). The association between the Orange-Blue cluster score and diabetes was stronger in the highest tertile of PD ($OR_{\text{tertile }1}$=1.07, 95% CI 0.89, 1.29 versus $OR_{\text{tertile }1}$=0.74, 95% CI 0.60, 0.91, p-value for interaction 0.07) (Table 5). The positive association between Orange-Red cluster scores and diabetes likewise seemed stronger in the highest versus lowest tertiles of PD (Table 4.5). We found the association was significantly different in the Orange-Red and Orange-Blue clusters by the periodontal status when periodontal status were defined as moderate or severe if the CAL $\geq$ 4 mm or PD $\geq$ 5mm or else normal. (Appendix A:Table 1).

Discussion:

The association between clusters of IgG antibodies against periodontal microorganisms and diabetes was stronger among individuals with greater pocket depth suggesting effect modification by PD; there was no evidence of effect modification of this association by levels of CAL. The inverse association between the Orange-Blue

cluster score and diabetes was stronger in the highest versus lowest tertile of mean PD. Similarly the positive association between the Orange-Red cluster score and diabetes increased by PD severity. This information is important to consider when information from clinical and serologic measures of periodontal disease is combined to evaluate their relation with systemic outcomes.

Serum IgG antibody titers measure the cumulative life time host response due to persistent infection of periodontitis and/ or the re-occurrence (Papapanou et al. 2004, Rams, Listgarten, and Slots 2006) which tends to remain stable over a longer periods even after treatment (Darby, Mooney, and Kinane 2001). Moreover, elevated serum antibodies titers may not only reflect the protective effect against periodontal disease, but may also indicate active periodontal lesions, in which case antibody titers would be indicators of developing periodontal disease (Dye et al. 2009, Lockhart et al. 2012). Selected serological markers have been used to assess in periodontal disease (Dye et al. 2009). The reasons we used cluster analysis to form 4 groups are, first, periodontal disease is associated with a shift in balance of microorganisms that are ubiquitous in the mouth rather than by any single organism. Second, cluster analysis grouped the antibodies against periodontal microorganisms as they naturally occur is explained in our previous study. In brief, the Hence by clustering these markers into mutually exclusive clusters, we can distinguish whether elevated titers are the markers of systemic health or disease status.

Our findings are consistent with prior reports linking antibodies against periodontal pathogens and diabetes. In our previous studies, we observed that the Orange-Red cluster of antibodies was positively associated with type 2 diabetes and

hyperglycemia, while the Orange-Blue cluster of antibodies showed an inverse relation. . The Orange-Red cluster included serum antibodies against *P. gingivalis* and *Prevotella sps.* The Orange-Blue cluster included *E. nodatum* and *A. naeslundii*. In this current study, no notable relationship was observed in Red-Green and Yellow-Orange Clusters. There are evidences that the elevated antibody titer of some selected bacteria in these clusters is linked to diabetes. The periodontal bacteria of Red-Green cluster score; particularly *A. actinomycetemcomitans* has previously shown inconsistent association with other systemic disease (Hosomi et al. 2012, Pussinen et al. 2005). Studies have reported that periodontal pathogens *P. gingivalis and Prevotella sps* and their serum antibodies titers are higher in individuals with type 2 diabetes (Casarin et al. 2013, Darveau 2010, Darveau, Hajishengallis, and Curtis 2012, Desvarieux et al. 2013, Dye et al. 2009). Although the *E. nodatu*m organism is associated with periodontal destruction, elevated antibody titers against *E. nodatu*m are associated with periodontal health (Dye et al. 2009). Higher concentration of *A. neslundii* has been associated with periodontal health (Williams, Pantalone, and Sherris 1976, Dye et al. 2009, Desvarieux et al. 2005). A recent study reported effect modification of the relation between periodontal damage and diabetes by levels of serum CRP, and antibody titers against periodontal pathogens particularly *P. gingivalis* and *A. actinomycetemcomitans.*

In the present analyses we have built on findings linking antibodies against periodontal pathogens and diabetes by studying their interaction with clinically determined periodontal destruction. Periodontal destruction has being linked to an array of chronic diseases including hyperglycemia through systemic inflammation (Oppermann, Weidlich, and Musskopf 2012, Mustapha et al. 2007, Ebersole et al. 2008,

Brito et al. 2013). Although unclear, there are four biologically meaningful hypotheses (common susceptibility, systemic inflammation, direct infection of blood vessels and the cross-reactivity or molecular mimicry) that explain possible mechanisms kinking periodontal infection with systemic disease in different people (Seymour et al. 2007, Seymour et al. 2009). Understanding the interaction between the clinical periodontal destruction, microbiological, serological periodontal parameters will be useful to minimize bias and spurious relationship while exploring the complex systemic relationship.

This present study has some drawbacks. First, in NHANES III (1988-1994), periodontal examination (CAL and PD) was measured on only 2 sites in each tooth which might underestimate the prevalence and severity of periodontal disease (Papapanou 2012). Although, we measured most of the potential confounders that were examined and measured in this dataset, there could be some unmeasured confounding factors such as oral hygiene that could bias this cross-sectional analysis. Additionally, we studied the commonly identified serum IgG antibody titers of 19 periodontal bacteria but those unidentified and unknown periodontal microbes may change the pattern of clusters hence we recommend this study findings needs to validated in recent population using sophisticated techniques and epidemiological methods. Despite limitations, this study has generated a hypothesis that combination of selected serological markers and clinical assessment of periodontal destruction increase the prediction of the diabetes.

The relation between IgG antibody groups and diabetes was found to vary by tertiles of periodontal pocket depths. This finding is relevant when clinical parameters and periodontal antibodies are studied in combination in relation to systemic disease.

Table 4.1: Correlation of the z-scores of serum antibody clusters with clinical attachment loss and pocket depth

Spearman's correlation (n=3333)	PD	Red-Green	Orange-Red	Yellow-Orange	Orange-Blue
CAL	**0.57****	-0.02	**0.07****	-0.03	-0.13**
PD	-	-0.02	**0.11****	-0.02	-0.07**

** p value <0.0001

Table 4.2: Profile characteristics of study population sample across tertiles of clinical periodontal measures

	Clinical Attachment loss (CAL)				**Pocket Depth (PD)**			
(Total n=3333)	*Tertile1 (n=1188)*	*Tertile II (n=1136)*	*Tertile III (n=1009)*	*P value*	*Tertile1 (n=1111)*	*TertileII (n=1163)*	*Tertile III (n=1059)*	*P Value*
Sex: % Male Female	40.1 59.9	44.3 55.7	58.2 41.8	**<0.0001**	39.1 60.9	45.8 54.2	57.1 42.9	**<0.0001**
Race/ Ethnicity % Non-Hispanic Whiles Non –Hispanic Blacks Mexican American Others	84.5 6.3 3.5 5.7	81.3 6.5 3.6 8.5	79.1 10.4 3.7 6.8	**0.0734**	86.2 4.4 2.6 6.8	82.2 7.2 3.8 6.8	75.2 12.5 4.8 7.5	**<0.0001**
Education % ≤ 6 years 7 – 12 years ≥ 13 years	3.21 38.1 58.7	4.4 52.5 43.1	8.1 61.8 30.1	**<0.0001**	3.5 42.2 54.3	4.1 47.5 48.4	7.9 61.3 30.9	**<0.0001**
Income to poverty ratio % Lower (≤1.5) Middle (≤3.0) Higher (>3.0)	10.6 23.3 63.1	14.2 25.3 60.5	20.0 35.6 44.4	**<0.0001**	11.5 26.6 61.9	12.2 27.0 60.8	21.1 32.2 46.7	**<0.0001**
Smoking % Never Past Current	55.5 33.9 10.6	37.2 47.1 15.7	24.3 50.0 25.7	**<0.0001**	50.4 3805 11.1	40.6 43.9 15.5	29.0 46.4 24.6	**<0.0001**
Alcohol intake %	14.0	14.4	12.2	**0.3813**	14.8	12.7	13.4	**0.1986**

Never Past Current	32.5 53.5	31.4 54.2	37.4 50.4		34.9 50.3	29.8 57.5	35.4 51.2	
Physical activity % Sedentary Moderately active Vigorously active Missing	57.5 12.9 8.3 21.3	54.7 13.1 8.0 24.1	54.0 11.3 5.8 28.9	**0.1336**	55.4 15.0 9.0 20.6	57.2 11.6 7.3 23.9	54.1 10.0 5.8 30.2	**0.0027**
No of missing teeth, % "0" "1-5" "6-10" ">10"	56.1 37.3 5.4 1.1	40.0 49.8 9.2 0.9	35.1 41.2 17.7 5.9	**<0.0001**	51.4 39.6 7.8 1.2	42.3 47.5 8.8 1.3	40.6 40.6 13.8 5.0	**<0.0001**
Waist Circumference Normal Elevated	58.3 41.7	56.7 43.3	49.7 50.3	**0.0270**	60.4 39.6	55.5 44.5	48.2 51.8	**0.0013**
Annual visits to dentist % Yes No	71.2 28.7	65.1 34.9	48.9 51.1	**<0.0001**	72.8 27.2	65.8 34.1	46.0 54.0	**<0.0001**
Diabetes % *Normal*	8.9 91.1	11.3 88.7	25.2 74.8	**<0.0001**	10.1 89.9	12.9 87.1	20.5 79.5	**<0.0001**
Mean (Se)								
Age, years, mean (se)	49.1±0.5	53.9±0.6	59.0 ± 0.6	**<0.0001**	52.5 ±0.5	53.3 ±0.4	53.8 ±0.5	**0.0892**
Fiber intake, g/day, mean (se)	27.04±0.3	27.1±0.3	27.1±0.2	**0.0528**	26.4±0.2	27.1±0.3	28.1±0.3	**0.0834**

Carbohydrate Intake, g/day mean (se)	258.9±5.5	248.8±5.2	243.4±7.6	**0.04**	249.3±5.2	254.3±5.1	252.2±9.0	**0.69**
Protein Intake, g/day mean (se)	79.7±1.8	77.8±1.8	77.5±1.9	**0.35**	76.2±1.7	79.0±1.8	81.7±2.1	**0.07**
Fat Intake, g/day mean (se)	80.4±2.6	77.7±2.3	75.3±2.1	**0.10**	76.5±2.2	80.0±2.1	78.9±2.2	**0.41**
BMI, kg/m^2, mean (se)	17.9±0.5	17.1±0.5	16.4±0.4	**0.8527**	17.7±0.5	17.4±0.4	16.3±0.6	**0.0002**
Red-Green	0.50 ± 0.4	-0.20 ± 0.4	-0.39 ± 0.5	**0.0338**	0.38 ± 0.4	-0.10 ± 0.5	-0.31 ± 0.4	**0.0755**
Orange-Red	-0.30 ± 0.2	-0.14 ± 0.1	-0.12 ± 0.2	0.4487	-0.37 ± 0.2	-0.26 ± 0.2	0.15 ± 0.2	**0.0264**
Yellow-Orange	0.43 ± 0.3	0.07 ± 0.3	-0.35 ± 0.4	**0.0496**	0.26 ± 0.3	0.07 ± 0.3	-0.03 ± 0.2	0.2896
Orange-Blue	0.51 ± 0.1	0.28 ± 0.1	-0.13 ± 0.1	**<0.0001**	0.39 ± 0.1	0.26 ± 0.1	0.13 ± 0.1	**0.0026**

Table 4.3: Exploring confounding effect (Odds Ratio and 95%CI) of clinical periodontal measures in the relation to serum antibody clusters and diabetes

(n=3333)	Red-Green	Orange-Red	Yellow-Orange	Orange-Blue
Adjustment for age, sex, education, ethnicity, smoking , drinking alcohol, physical activity, BMI WC, missing teeth and dentist visit (Reference full model)	0.98 (0.92 ,1.03)	1.04 (0.98, 1.09)	1.05 (0.97, 1.14)	**0.893 (0.81, 0.99)**
Additionally adjusted for CAL	0.97 (0.922, 1.036)	1.03 (0.968, 1.086)	1.06 (0.98, 1.14)	**0.90 (0.81, 0.99)**
Additionally adjusted for PD	0.98 (0.92, 1.04)	1.03 (0.97, 1.09)	1.06 (0.98, 1.14)	**0.89 (0.80, 0.99)**

Table 4.4: Association of the z-scores of serum antibody clusters with diabetes stratified by the tertiles of clinical attachment loss

Serum Antibody z-scores Clusters	Odds Ratio (95% Confidence Interval) Clinical Attachment Loss		
Model*	Tertile I	Tertile II	Tertile III
Red-Green			
Model1	1.01 (0.93, 1.10)	1.01 (0.91, 1.13)	1.01 (0.93, 1.09)
Model2	1.06 (0.94, 1.18)	0.96 (0.88, 1.06)	0.99 (0.92, 1.07)
Model3	1.03 (0.91, 1.15)	0.96 (0.87, 1.06)	0.99 (0.93, 1.08)
Model4	1.04 (0.92, 1.18)	1.01 (0.88, 1.66)	0.95 (0.86, 1.05)
Orange-Red			
Model1	**1.21 (1.08, 1.36)***	1.08 (0.99, 1.18)	1.09 (0.98, 1.20)
Model2	1.08 (0.96, 1.23)	1.04 (0.95, 1.13)	1.05 (0.95, 1.17)
Model3	1.06 (0.91, 1.24)	1.03 (0.94, 1.14)	1.06 (0.96, 1.16)
Model4	1.001 (0.83, 1.15)	0.98 (0.88, 1.10)	1.07 (0.98, 1.16)
Yellow-Orange			
Model1	0.94 (0.82, 1.07)	0.97 (0.87, 1.08)	0.97 (0.88, 1.07)
Model2	0.94 (0.83, 1.07)	1.03 (0.92, 1.13)	1.00 (0.91, 1.10)
Model3	1.02 (0.89, 1.15)	1.03 (0.93, 1.15)	0.99 (0.91, 1.10)
Model4	1.05 (0.90, 1.22)	1.02 (0.88, 1.18)	1.08 (0.96, 1.20)
Orange-Blue			
Model1	**0.82 (0.70, 0.96)***	0.88 (0.76, 1.01)	0.97 (0.86, 1.09)
Model2	0.91 (0.77, 1.07)	0.95 (0.82, 1.09)	0.98 (0.86, 1.12)
Model3	0.88 (0.74, 1.04)	0.94 (0.83, 1.07)	0.99 (0.86, 1.13)
Model4	0.87 (0.72, 1.04)	0.87 (0.73, 1.04)	0.95 (0.78, 1.15)

Model 1: adjusted for the clusters.
Model 2: Model 1 plus additionally adjusted for age, sex, education, income, race
Model 3: Model 2 plus additionally adjusted for smoking, alcohol intake
Model 4: Model 3 plus additionally adjusted further with missing teeth, dental visit, BMI, central adiposity, physical activity, total energy, carbohydrate, fat and protein intake.* p value <0.05

Table 4.5: Association of the z-scores of serum antibody clusters with diabetes stratified by the tertiles of clinical pocket depth measures

Serum Antibody Clusters	Odds Ratio (95% Confidence Interval) Pocket Depth		
Model*	Tertile I	Tertile II	Tertile III
Red-Green			
Model1	0.98 (0.88, 1.08)	1.05 (0.93, 1.18)	1.07 (0.97, 1.19)
Model2	0.97 (0.87, 1.07)	1.02 (0.90, 1.15)	1.03 (0.92, 1.15)
Model3	0.97 (0.87, 1.08)	1.02 (0.90, 1.13)	0.97 (0.86, 1.11)
Model4	0.92 (0.82, 1.03)	1.04 (0.91, 1.17)	0.97 (0.84, 1.12)
Orange-Red			
Model1	**1.13 (1.03, 1.23)***	1.10 (0.99, 1.22)	**1.16** (**1.05**, **1.29**)*
Model2	1.01 (0.91, 1.13)	1.03 (0.94, 1.13)	**1.12** (**1.00**, **1.26**)*
Model3	0.99 (0.88, 1.12)	1.03 (0.94, 1.12)	**1.15** (**1.01**, **1.31**)*
Model4	0.97 (0.86, 1.09)	1.03 (0.94, 1.14)	1.10 (0.95, 1.28)
Yellow-Orange			
Model1	0.99 (0.89, 1.10)	0.96 (0.86, 1.06)	0.92 (0.80, 1.05)
Model2	1.05 (0.91, 1.13)	0.99 (0.89, 1.09)	0.97 (0.83, 1.12)
Model3	1.05 (0.94, 1.16)	0.98 (0.88, 1.08)	1.04 (0.87, 1.24)
Model4	**1.13 (1.01, 1.27)**	0.97 (0.87, 1.09)	1.10 (0.89, 1.36)
Orange-Blue			
Model1	0.94 (0.80, 1.09)	**0.80 (0.70, 0.89)***	**0.86 (0.73, 0.99)***
Model2	1.06 (0.89, 1.26)	0.88 (0.77, 1.00)	0.89 (0.77, 1.04)
Model3	1.06 (0.87, 1.29)	**0.88** (**0.78**, **0.99**)*	**0.84** (**0.70**, **0.99**)*
Model4	1.07 (0.89, 1.29)	**0.84** (**0.72**, **0.98**)*	**0.74** (**0.60**, **0.91**)*

Model 1: adjusted for the clusters. Model 2: Model 1 plus additionally adjusted for age, sex, education, income, race
Model 3: Model 2 plus additionally adjusted for smoking, alcohol intake
Model 4: Model 3 plus additionally adjusted further with missing teeth, dental visit, BMI, central adiposity, physical activity, total energy, carbohydrate, fat and protein intake.* p value <0.05

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CHAPTER 5

MANUSCRIPT 3

Relationship between serological markers of periodontal bacteria and metabolic syndrome and its components

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Abstract:

Metabolic syndrome (MetS), a complex cluster of cardiovascular risk factors, has been linked to periodontal diseases; however the contribution of periodontal bacteria to systemic conditions remains unclear. We studied the magnitude and extent of the relation between serum IgG antibody titers against periodontal bacteria and MetS and its individual components.

The study population comprised of 7848 US adults who participated in an interview, clinical, oral health examination, and had serum IgG titers measured against 19 periodontal bacteria as part of the NHANES III survey. The z-scores antibody titers were clustered into 4 mutually exclusive groups and named after Socransky's classification of periodontal bacteria (Orange-Red, Red-Green, Yellow-Orange, and Orange-Blue). Survey logistic regression was used to investigate the independent associations between the cluster scores, and MetS and each component including hypertension, hypertriglyceridemia, low HDL-cholesterol, central obesity, and elevated fasting glucose.

The Orange-Red cluster score (associated with periodontal disease) was positively associated [OR: 1·067 (1·02-1·12)]) and Orange-Blue cluster score inversely associated [OR= 0·93 (0·88-0·97)] with elevated fasting glucose (≥110 mg/dl) after adjusted for clusters and potential confounders. Neither MetS nor its other remaining MetS components were associated with a particular cluster score.

The associations between different antibody clusters against periodontal bacteria and elevated plasma glucose were in qualitatively opposite directions after multivariable adjustment in a large, adult population. The periodontal bacterial profile was not found

associated with metabolic control other than very moderate association with elevated plasma glucose.

Keywords: Metabolic syndrome, hypertension, hyperlipidemia, central obesity, elevated fasting plasma glucose, serum immunoglobulinG (IgG), periodontal microbiota

Introduction:

Periodontitis is a chronic poly bacterial infection in the tooth-supporting tissues, which may lead to tooth loss. Isolation of selected periodontal pathogens such as *Porphyromonas gingivalis, Prevotella intermedia, Eikenella corrodens, Treponema denticola, Campylobacter rectus* from atherosclerotic lesions in humans and animals, (Kebschull, Demmer, and Papapanou 2010) and the positive association of higher titers of antibodies against *P gingivalis* with coronary heart disease (Yamazaki et al. 2007), stroke (Pussinen, Alfthan, et al. 2007), and myocardial infraction (Holmlund et al. 2011) raised attention to the potential role of periodontal organisms in systemic conditions . Recently, a longitudinal study findings showed that improved clinical and microbial periodontal profile reduced the progression of carotid atherosclerosis over three years (Desvarieux et al. 2013). These microbiological and, serological evidences (Mustapha et al. 2007) have generated interest to better understand related factors underlying the relationship between periodontal disease and cardiovascular disease (CVD), including chronic inflammation, diabetes and the metabolic syndrome (MetS).

Diabetes and periodontal disease are common in middle aged persons, share common risk factors (Taylor, Preshaw, and Lalla 2013), are associated with CVD (Lockhart et al. 2012, Southerland et al. 2012), risk markers of CVD (Oldridge et al. 2001), systemic inflammation (Oppermann, Weidlich, and Musskopf 2012), and

metabolic syndrome (MetS) (Alberti et al. 2009). Diabetes and pre-diabetes prevalence is higher among individuals with more severe periodontal disease (Choi et al. 2011). However, the role that periodontal microorganisms play in these relationships is incompletely understood. A clear insight into this relationship has potential for the development of targeted strategies to prevent and manage these conditions.

In the previous observations, we found that antibody titers against selected periodontal microorganisms group into 4 distinct clusters, and that these are related to diabetes and pre-diabetes in the third National Health and Nutritional Examination survey (NHANES III). In this present study, we aimed to investigate the extent to which clusters of serum IgG antibodies against periodontal bacteria were related to MetS and its individual components including hypertension, hypertriglyceridemia, low high density lipoprotein (HDL)-cholesterol, central adiposity, and elevated plasma glucose. MetS intends to identify those who are at risk stage of cardiovascular disease, pre-diabetes, or diabetes (Alberti et al. 2009). We hypothesized that specific subsets of serum IgG antibodies against periodontal bacteria were linked to periodontal health or disease could associated with metabolic control.

Materials and Methods:

Data Source:

The study population consisted of participants of the NHANES III (1988-1994) survey who were ≥40 years old, and had complete data for serum IgG antibody titers against 19 oral bacteria (n=8153); we excluded those who used insulin (n=299) and/or had gestational diabetes (n=6); leaving 7848 participants for the final analysis. The

NHANES III sample is representative of the non-institutionalized US population; information is collected through interviews, medical and dental examinations, and laboratory tests. The de-identified data are available at: http://www.cdc.gov/nchs/nhanes/nh3data.htm. For this study we merged the following datasets: Periodontal Pathogens (SPSDEPPX), Household Adult Data File (Catalog Number 77560), NHANES III Examination Data File (Catalog Number 76200); NHANES III Laboratory Data File (Catalog Number 76300); NHANES III Dietary Recall Data Files (Catalog Number 76700) using unique de-identified respondent identification numbers.

Assessment of Outcome

The outcomes were each of five components of metabolic syndrome consistent with recent task force guidelines (Alberti et al. 2009): elevated central obesity, hypertriglyceridemia, low HDL cholesterol, high blood pressure, and high elevated fasting glucose. We categorized these variables after excluding implausible values. *Central obesity* was categorized as elevated if waist circumference was ≥ 90 cm for men and ≥ 85 cm for women, else normal. *Hypertension* was defined as present if systolic blood pressure was > 130 mm Hg or Diastolic blood pressure was > 85 mm Hg or if the participant reported taking anti-hypertensive medication, or absent otherwise. *Hypertriglyceridemia* was defined as present if triglycerides were >150 mg/dl or else normal. *High density lipoprotein* was categorized as low if HDL was < 40mg/dl for men and <50 mg/dl for women or optimum otherwise. *Elevated plasma glucose* was defined as present if fasting plasma glucose was >100 g/dl or else normal. Additionally, participants were assessed as having MetS if they had at least three of these components

(Alberti et al. 2009).

IgG assay for periodontal bacteria

Stored sera of NHANES III participants, aged 40 years or older were analyzed for IgG antibodies against 19 oral bacterial species, and the data released in 2008 (N=8153). The following 19 bacterial strains were used to prepare whole cell antigenic extracts by checkerboard immunoassay: *Aggregatibacter actinomycetemcomitans (*ATCC strains #43718, #29523 and #33384); *Porphyromonas gingivalis (*ATCC #33277 and #53978); *Tannerella forsythia (*ATCC#43037); *Treponema denticola (*OMGS#3271); *Campylobacter rectus (*ATCC#33238); *Eubacterium nodatum (*ATCC#33099); *Prevotella intermedia (*ATCC#25611); *Prevotella nigrescens (*ATCC#33563); *Prevotella melaninogenica (*ATCC#25845); *Fusobacterium nucleatum (*ATCC#10953); *Micromonas micros (*ATCC #33270); *Selenomonas noxia (*ATCC#43541); *Eikenella corrodens (*ATCC#23834); *Capnocytophaga ochracea (*ATCC#33624); *Streptococcus intermedius (*ATCC#27335); *Streptococcus oralis (*ATCC#35037); *Streptococcus mutans (*ATCC#25175); *Veillonella parvula (*ATCC#10790); and *Actinomyces naeslundii (*ATCC#49340). The Oral and Diagnostic Sciences Laboratory, Columbia University College of Dental Medicine analyzed the sera The using the ‘‘checkerboard’’ immunoassay technique measured in gravimetric units. The detailed procedure is described in NHANES III documentation: ftp://ftp.cdc.gov/pub/Health_Statistics/NCHS/nhanes/nhanes3/30a/spsdeppx.pdf

Definition of the periodontal exposures

The IgG antibody titers were log transformed and then converted to standardized z scores (Desvarieux et al. 2010). Participants with hyperglycemia (HbA1c ≥ 5.7) were included in cluster analysis to derive four mutually distinct groups of periodontal bacteria. The z-scores against periodontal bacteria in the respective clusters were summed to obtain cluster scores for each group. These four cluster scores [Orange-Red, Red-Green, Yellow-Orange, and Orange-Blue] were named according to Socransky's color classification scheme (Figure 3.1) (Socransky and Haffajee 2002).

Covariate Information

Demographic, lifestyle and anthropometric measures included gender, age, race/ethnicity, education, income-poverty ratio, smoking and alcohol drinking habits, physical activity, fiber intake, frequency of regular dentist visits, waist circumference (WC), body mass index (BMI), and diabetes. Race/ethnicity was self-reported and was divided into four groups: non-Hispanic white, non-Hispanic black, Mexican American and others. Income was grouped into three groups of the income-to-poverty ratio (≤1.5, >1.5 to ≤3.0, and >3.0), education level into three groups ≤6 years; 7 to 12 years; and ≥13 years).

Smoking status was classified into three groups (current, past and never smokers). Alcohol consumption was divided into three groups (current, past and never alcohol drinkers). Physical activity was divided into three groups based on metabolic equivalents (METs) (active, ≥6; moderate, ≥4 and <6; and less active, <4) (Choi et al. 2011).

Fiber intake was assessed based on estimates from 24-hour dietary recall data.

People who had regular dental checkups were defined as those who had visited a dental clinic at least once in the past year. WC to assess central obesity was measured using a steel measuring tape to the nearest 0.1 cm at the high point of the iliac crest at minimal respiration when the participant was in a standing position (Choi et al. 2011). Central adiposity was said to be present if WC was ≥101.6 cm for male subjects and ≥88.9 cm for female subjects. WC was not included in final adjusted model where WC was the outcome variable and similarly, diabetes was not used as confounder when elevated plasma glucose was the outcome variable. We defined diabetes status as diabetes, pre-diabetes and normal blood glucose level. Pre-diabetes was defined as having HbA_{1c} 5.7 to <6.5% or fasting plasma glucose (FPG) 100 to <126 mg/dl (5.6-6.9 mmol/L). Individuals with HbA_{1c} ≥ 6.5% or FPG ≥ 126 mg/dl [7 mmol/L] or those who self-reported diagnosed diabetes were classified as having diabetes or else classified as normal blood glucose level(Bullard et al. 2013).

Statistical Methods

SAS, version 9.3 (SAS Institute, Cary, NC) was used for data management and statistical analyses. SAS survey procedures were used to take into account the complex weighted sampling design and yielded unbiased parameter estimates and standard errors. Sample weights, cluster, and strata variables provided by CDC were used. A statistically significant α level was considered at 0.05.

Descriptive statistics were estimated using procedures for complex surveys in SAS (proc surveymeans and proc surveyfreq). The distribution of socio-demographic, lifestyle, anthropometric and outcome measures across tertiles of cluster scores were estimated.

Using the survey regression multivariable model, we then regressed each of these outcomes with the four z-score clusters. Secondly, the model was adjusted for age (continuous) and sex. Thirdly, we adjusted further for race and ethnicity, income-poverty ratio, smoking, alcohol, physical activity, fiber intake (continuous), dentist visits, WC and BMI (continuous). In the fourth model, diabetes status was further added in the analysis of hypertension, hyper triglycerides, low HDL-cholesterol, and central adiposity.

Results:

In this population, 37·8% had low HDL-cholesterol, 37·4% hypertriglyceridemia, 60.02% hypertension, 52.01 % elevated central adiposity, 38.2 % elevated plasma glucose and 41.8% MetS. The adverse health conditions were generally more common among Non-Hispanic Whites, females, high school graduates (7-12 years education level), never or past smokers, current or former alcohol drinkers, individuals who were hypertensive, overweight, sedentary, and who did not have annual dental visits. The characteristics of the study population across extreme tertiles of four cluster scores are shown in Table 5.1. There were some notable differences in participant characteristics across Orange-Red and Orange-Blue clusters. The top tertile of Orange Red cluster (which included *P gingivalis and Prevotella species)* had larger proportions of individuals who were male, less educated, low income, Non-Hispanic Black, never smokers, normal triglycerides, hypertensive and insulin resistant compared to the lowest tertile; the highest tertile of the Orange-Blue cluster (which included *A naeslundii and E nodatum*) had individuals who were highly educated, high income, Non-Hispanic White, never smokers, current drinkers, visited the dentist regularly at least once a year, and had optimum high density lipoproteins, tri-glyceride and blood glucose levels. There were

higher proportions of individuals with diabetes and pre-diabetes in the top versus bottom tertiles (11·2 vs 8·1) in the Orange-Red cluster, however the opposite trend for diabetes (8·6 vs 11·1) was observed in the Orange-Blue cluster; no such differences were seen in other clusters (Table 5.1).

Correlations between clusters scores are shown in Table 5.2. The highest correlation is in between Yellow-Orange (predominantly *Streptococcus sps*) and Red-Green *(T denticola, T forsythia, A actinomycetemcomitans and others)* scores (0·91, p value=<0·0001) whereas lowest is in between Orange-Red and Orange-Blue clusters (0·34, p value=<0·0001). Mean values of serum IgG z-scores of four clusters by different outcome measures are shown in Table 3. Only Orange-Red scores were higher in individuals having MetS and those with elevated plasma glucose levels; increased Orange-Blue scores were observed in individuals who had no hypertension and normal plasma blood glucose.

After full adjustment of potential confounders, no association was seen in four cluster scores with MetS and any of the individual components except for elevated plasma glucose. For each unit increase in Orange-Red cluster scores the odds of elevated plasma glucose 6% [OR: 1·06 (1·02-1·10)] with; with the National Cholesterol Education Program (NCEP)-Adult treatment panel III criteria (elevated plasma blood glucose level, ≥110 mg/dl) (Lorenzo et al. 2007)(17)(Lorenzo et al. 2007)(Lorenzo et al. 2007) there were 7% higher odds [OR: 1·06 (1·02-1·12)] and 1 unit higher Orange-Blue cluster score was associated with 7% lower odds of hyperglycemia [OR:0·93 (0·88-0·98)]. No association was seen with Orange-Red and Yellow-Orange cluster scores (Table 5.4).

Similar analyses conducted among people with no missing teeth yielded qualitatively similar results (Table 5.5).

Discussion:

Elevated plasma glucose was more common among individuals with higher serum IgG antibody titer scores against organisms belonging to the Orange-Red cluster (indicating periodontal disease), and lower in titer scores against organisms from the Orange-Blue cluster after adjustment for several socio-demographic, anthropometric and behavioral factors. There was no association between antibody score clusters and MetS or other individual components of MetS (hypertension, dyslipidemia, central obesity).

Periodontitis is not due to a single pathogen, but results from a complex biological alteration of the periodontal microenvironment, and a distributional shift of key periodontal pathogens(Berezow and Darveau 2011). Ebersole et al (Ebersole et al. 2008) postulated that increased severity may be due to alterations in the pathogenicity of the periodontal bacteria in the plaque biofilm, which in turn may imbalance the host immune system and impact systemic inflammation. Although there is biological plausibility for the hypothesis, some study findings refute a causal role of direct periodontal pathogen invasion in the systemic disease (Lockhart et al. 2012). Moreover, periodontal pathogen coexists with hundreds of others, making it difficult to isolate the potential effect of a particular pathogen, and predisposing such studies to type 1 error (Wang et al. 2007). To overcome this limitation investigators have grouped the periodontal organisms together either using empirical (Socransky and Haffajee 2002) or theoretical (Desvarieux et al. 2013) approaches. Socransky et al grouped pathogens

related to periodontal disease status, while Desverieux et al grouped the periodontal organisms that were hypothesized to be related to cardiovascular disease. Similar analyses for type 2- diabetes were not found in the literature.

In the current study, we used an empirical approach to cluster oral pathogens among individuals with hyperglycemia and measured the extent of association of these clusters with metabolic control. This was an appropriate method to classify antibodies against the 19 oral microorganisms because the microorganisms are correlated with each other and their roles are not yet clearly defined. We obtained cluster scores by summing z-scores of the titers because we were interested in evaluating the relative (rather than absolute) contributions of the titers to the overall score.

Both the total study participants (Table 5.4) as well as only dentate (Table 5.5), the Orange-Red cluster (associated with periodontal disease) include antibody titers against *P.gingivalis and Prevotella sps* and increased the odds while the elevated serum IgG antibody against Orange-Blue cluster score that included *E. nodadum* and *A. naeslundii* antibody lowered the odds of having elevated plasma glucose. This relationship was more distinct when elevated plasma level cut point was >110 mg/dl which will include only diabetes population. *P.gingivalis and Prevotella sps* have been consistently associated not only with periodontal disease status but also different systemic diseases such as CVD, diabetes or arthritis. *A. naeslundii* has been consistently related to healthy periodontal and systemic states (Socransky and Haffajee 2002, Desvarieux et al. 2013). One study has reported higher microbial count of *E. nodatum* associated with periodontitis lesions (Haffajee, Teles, and Socransky 2006) but the antibody titers against *E. nodatum* were associated with periodontal health (Dye et al.

2009). No prior studies used cluster methodology to study the relationship in past literatures; hence we could not compare our study findings with other studies. Clinically, periodontitis is diagnosed only for the dentate individuals. However, serum antibody titers against the periodontal bacteria may reflect current or past exposures to periodontal bacteria, hence measures the cumulative life time exposures. Excluding edentulous individuals did not alter the results (Table 5.5). There was no significant finding when the association was observed using three groups (Etiologic, putative and Health) (Table 5.6).

Prior studies reported an association of periodontal disease independently with MetS (Nibali et al. 2013) and their individual components including hypertension (Desvarieux et al. 2010), hyperlipidemia (Moeintaghavi et al. 2005)and central obesity(Kim, Jin, and Bae 2011). Periodontal disease in these studies was assessed by clinical measures index (such as clinical attachment loss or pocket depth). In the current study, upon full adjustment for potential confounders, no association was seen with serological markers of periodontal bacteria with MetS and any of other MetS (Table 5.4 and 5.5). However, Pussinen et al., (2003) (Pussinen et al. 2003) reported the combination of serum IgG antibodies against *P. gingivalis* and *A. actinomycetemcomitans* had inverse association with serum HDL concentration.

This present study has some drawbacks. First, the cross-sectional design does not explain when the serum IgG antibody titer response evolved. Second is the possibility of unmeasured confounding factors because oral health behaviors were not evaluated. Third, this study evaluates 19 antibodies against periodontal microbiota; hence additional pathogens could modify the overall relationship. Research finding has shown that presence of high virulence strain of *P. gingivalis* may affect CHD (Yamazaki et al. 2007),

so use of serum antibody titers against *P. gingivalis* mixture and *A. actinomycetemcomitans mixture* could underestimate our analysis. Fourth, although highly sensitive C-reactive protein (Hs-CRP) is an indicator of systemic inflammation, we did not adjust for it or clinically defined periodontitis because they may be on the causal pathway and would create bias (Cole and Hernan 2002).

The strengths of this study were first, this was a large, representative, population based sample, with careful measurement of potential confounders, reducing the likelihood of false negative or spurious results. To our knowledge, this is the largest study to evaluate the relation between titers against oral microorganisms and MetS and its individual components. Second, we adjusted for a number of potential confounders. Third, serum IgG antibody titers measure persistent periodontal infection and/ or recurrence, (Papapanou et al. 2004, Rams, Listgarten, and Slots 2006); these serum IgG measures are a therefore a measure of cumulative life exposure to periodontal diseases. Fourth, cluster analysis resulted in reduced data and mutually exclusive groups, minimizing the chance of spurious results from multiple testing. Fifth, in the multivariable models we evaluated the clusters together. This approach mimics the biological processes that take place in the mouth because oral microorganisms are ubiquitous, and changes in microbial composition occur relative to other organisms in the mouth. Thus, the relative composition oral microorganisms may tilt the balance between health and disease (Socransky and Haffajee 2002, Oppermann, Weidlich, and Musskopf 2012). Sixth, empirical clustering of serum IgG reduces the influence of individual antibody titers, making it a more stable measure of cumulative exposure to periodontal

pathogens, and possibly advantageous in the study the periodontal-systemic disease relations.

In summary, we found that antibodies against periodontal microorganisms were moderately associated with elevated plasma glucose but not with MetS or its other components. These findings together with emerging evidence that gut microbiota are correlated with MetS traits (Zupancic et al. 2012), are motivation for the conduct of sophisticated longitudinal studies with clinical, microbiological and immunological data to increase our understanding the relation between microorganisms in the mouth and gut and the metabolic state. This may lead to new approaches to address these conditions.

Table 5.1: Profile characteristics of metabolic syndrome components in lower vs upper tertiles of four cluster scores

Variables	[a]n	Orange-Red		Red-Green		Yellow-Orange		Orange-Blue	
		Tertile1	Tertile3	Tertile1	Tertile3	Tertile1	Tertile3	Tertile1	Tertile3
Sex (%)									
Male	3677	40.1	50.3	41.3	49.8	42.9	47.0	45.9	46.7
Female	4171	59.9	49.7	58.7	50.2	57.1	52.4	54.1	53.3
Education (%)									
≤ 6 years	1403	5.5	9.2	5.7	7.3	7.0	7.1	7.9	5.1
7 – 12 years	4344	57.2	52.1	58.5	50.5	58.6	52.0	58.7	49.3
≥ 13 years	2046	37.3	38.7	35.8	42.3	34.4	40.9	33.4	45.6
[b]Income to poverty ratio									
Lower (≤1.5)	2387	18.5	20.6	19.3	17.7	22.4	18.4	22.8	15.0
Middle (≤3.0)	2214	28.8	28.8	30.6	30.4	28.8	30.8	33.0	27.2
High (>3.0)	2496	52.7	50.6	50.1	51.9	48.8	50.8	44.1	57.8
Race/ethnicity (%)									
Non-Hispanic Whites	4012	87.6	71.9	85.8	76.2	84.0	76.7	80.4	82.6
Non-Hispanic Blacks	1817	5.6	13.4	6.8	10.8	7.4	11.2	9.8	8.3
Mexican Americans	1699	2.2	4.9	2.8	4.1	2.9	3.8	3.1	3.6
Other	320	4.6	9.8	4.6	8.9	5.7	8.3	6.7	5.5
Cigarette Smoker (%)									
Never smoker	3562	39.5	46.3	37.5	45.9	38.7	45.7	38.6	46.5
Past smoker	2521	33.2	35.5	34.2	36.7	33.3	35.3	32.7	35.4
Current Smoker	1765	27.3	18.2	28.3	17.4	28.0	19.0	28.7	18.1
Alcohol (%)									
Never drinker	1480	15.6	15.7	14.7	16.1	15.2	16.2	17.2	13.6
Past drinker	3185	38.3	37.4	38.5	38.9	38.7	37.6	39.6	38.3
Current drinker	3031	46.1	46.9	46.7	45.0	46.1	46.2	43.2	48.1
Dentist visit once in year (%)	3010	51.7	52.0	49.4	52.2	48.1	54.6	47.9	55.3

Yes									
No	4588	48.2	8.1	50.6	47.8	51.9	45.4	52.0	44.7
Physical Activity (%)									
Sedentary	3550	53.6	51.7	54.4	50.3	51.6	50.5	51.3	54.5
Active	927	12.3	11.4	11.1	12.3	12.0	11.8	12.1	12.4
Vigorously active	500	6.8	7.8	7.2	7.8	7.6	8.2	6.8	8.1
[c]Central Obesity (%)									
Normal	3602	50.1	49.4	50.5	49.4	51.9	51.2	50.9	52.0
Elevated	3904	49.9	50.6	49.5	50.6	48.2	48.8	49.1	48.0
High density lipoproteins (%)									
Normal	4820	61.1	61.0	61.9	60.3	62.4	60.9	59.6	61.9
Lower	3028	38.9	39.0	38.0	39.7	37.6	39.1	40.4	38.1
Triglycerides (%)									
Normal	4911	61.9	65.2	62.6	62.5	62.7	63.8	61.3	64.0
High	2937	38.1	34.8	37.1	37.5	37.3	36.2	38.7	36.0
Hypertension (%)									
Yes	4552	50.1	54.1	50.2	53.6	51.3	51.7	53.7	53.0
No	3035	49.9	45.9	49.8	46.4	48.7	48.3	46.3	47.0
Insulin Resistance (%)									
Yes	1466	12.6	15.5	13.8	13.5	15.2	14.1	16.3	12.6
No	6382	87.4	84.5	86.2	86.4	84.8	85.9	83.7	87.4
Metabolic Syndrome (%)									
Yes	2800	32.1	33.3	31.3	34.6	31.2	33.7	33.1	31.3
No	5048	67.9	66.7	68.7	65.4	68.8	66.3	66.9	68.7
Diabetes Status (%)									
Diabetes	1090	8.1	11.2	9.2	9.8	10.4	10.5	11.1	8.6
Pre-diabetes	3334	35.9	38.5	37.1	37.6	38.1	36.2	39.9	35.1
Normal	3424	56.1	50.3	53.7	52.6	51.5	53.3	49.0	56.4
					Mean ± SD				

AGE (years)	56.9±0.5	56.6±0.6	56.8±0.5	56.5±0.6	57.8±0.6	56.2±0.6	58.4±0.6	57.1±0.1
BMI (kg/m2)	26.9±0.2	27.8±0.2	27.9±0.2	27.7±0.2	26.9±0.2	27.5±0.2	26.9±0.2	27.3±0.2
Waist Circumference (cm)	94.7±0.3	96.6±0.5	95.1±0.03	96.4±0.4	94.9±0.4	95.7±0.4	95.4±0.3	95.8±0.4
High density lipoprotein (mg/dl)	51.1±0.6	51.2±0.6	51.5±0.6	50.7±0.6	51.7±0.6	50.8±0.6	50.5±0.5	50.9±0.5
Systolic Blood Pressure (mmHg)	128.9±0.5	129.9±0.6	128.7±0.6	129.6±0.6	129.5±0.6	129.1±0.7	130.3±0.7	127.6±0.4
Diastolic Blood Pressure (mmHg)	75.5±0.3	77.3±0.3	75.7±0.3	76.8±0.4	75.3±0.3	76.6±0.3	75.9±1.1	76.4±0.3
Triglycerides (mg/dl)	155.6±2.9	145.6±3.4	152.6±2.3	151.9±3.1	152.2±2.2	149.0±2.9	155.3±2.6	150.2±0.5
HbA1c(%)	5.5±0.0	5.6±0.0	5.5±0.03	5.5±0.0	5.6±0.0	5.6±0.0	5.6±0.0	5.6±0.0
Fasting plasma Glucose(mg/dl)	99.8±0.6	103.5±1.1	101.7±0.8	101.8±0.9	102.7±0.9	102.1±1.1	103.9±1.1	101.3±0.9
Fiber Intake (g/day)	16.1±0.3	17.0±0.3	15.8±0.3	17.3±0.3	16.0±0.3	16.9±0.2	16.3±0.3	16.5±0.3

[a] Total numbers may be different due to missing data

[b]Income-to-poverty ratio: (midpoint family income)/(poverty threshold values based on calendar years and inflation)

[c]Central adiposity present if waist for male subjects ≥101.6 cm and for female subjects ≥88.9 cm

Table 5.2: Correlation between the four Z cluster scores

	Orange-Red	Red-Green	Yellow-Orange	Orange-Blue
Orange-Red	-	0.64**	0.68**	0.34**
Red-Green	0.64**	-	0.91**	0.49**
Yellow-Orange	0.68**	0.91**	-	0.50**
Orange-Blue	0.34**	0.49**	0.50**	-

****P Value = <.0001**

Orange-Red: *P melaninogenica, P intermedia, P nigrescens, P gingivalis*

Red-Green: T forsythia, T denticola, A actinomycetemcomitans, E corrodens, S noxia, V parvula, C rectus

Yellow-Orange: S intermedius, S oralis, S mutans, F nucleatum, M micros, C ochracea

Orange-Blue: E nodatum, A naeslundii

Table 5.3: Mean (Standard Error of Mean) values of serum IgG z-scores of four cluster scores

	Orange-Red	Red-Green	Yellow-Orange	Orange-Blue
Hypertension				
Yes (4696)	-0.25 (0.1)	0.03 (0.4)	0.03 (0.2)	0.05 (0.1)
No (3128)	-0.40 (0.1)	-0.16 (0.4)	-0.03 (0.2)	0.25 (0.1)
P value	*0.09*	*0.32*	*0.70*	***0.001***
Hypertriglyceridemia				
Yes (2973)	-0.40 (0.1)	-0.17 (0.4)	-0.12 (0.3)	*0.09 (0.1)*
No (4911)	-0.28 (0.1)	-0.004 (0.3)	0.07 (0.2)	*0.18 (0.1)*
P value	*0.32*	*0.43*	*0.28*	*0.14*
Low HDL- cholesterol				
Yes (2934)	-0.34 (0.1)	-0.09 (0.4)	0.01 (0.3)	0.12 (0.1)
No (4820)	-0.30 (0.1)	-0.06 (0.4)	-0.02 (0.2)	0.17 (0.1)
P value	*0.74*	*0.90*	*0.90*	*0.42*
Central Adiposity				
Yes (3904)	*-0.31 (0.1)*	-0.06 (0.4)	0.01 (0.2)	0.13 (0.1)
No (3602)	*-0.31 (0.1)*	-0.02 (0.3)	0.04 (0.2)	0.17 (0.1)
P value	*0.99*	*0.83*	*0.86*	*0.46*
Elevated plasma glucose				
Yes (2988)	-0.08 (0.1)	0.02(0.4)	0.04 (0.2)	0.07 (0.1)
No (4845)	-0.40 (0.1)	-0.1(0.3)	-0.01 (0.2)	0.19 (0.1)
P value	***0.0007***	*0.41*	*0.64*	***0.02***
Metabolic Syndrome				
Yes(2867)	-0.21 (0.1)	0.07 (0.4)	0.09 (0.2)	0.10 (0.1)
No (3829)	-0.40 (0.1)	-0.14 (0.3)	-0.05 (0.2)	0.18 (0.1)
P value	***0.04***	*0.25*	*0.34*	*0.17*
#Adult Treatment Panel (ATP) III-Criteria				
#Elevated Fasting plasma glucose (>110 mg/dl)				

Yes (1466)	-0.17 (0.1)	-0.3 (0.4)	-0.17 (0.2)	-0.09 (0.1)
No (6367)	-0.35 (0.1)	-0.03(0.3)	0.03 (0.2)	0.19 (0.1)
P value	*0.2146*	*0.2504*	*0.2727*	***0.0002***
#Metabolic syndrome				
Yes (3277)	-0.25 (0.1)	0.1 (0.4)	0.13 (0.3)	0.10 (0.1)
No (4571)	-0.36 (0.1)	-0.15 (0.3)	-0.06 (0.2)	0.17 (0.1)
P value	*0.26*	*0.25*	*0.31*	*0.22*

Orange-Red: *P melaninogenica, P intermedia, P nigrescens, P gingivalis*
Red-Green: T forsythia, T denticola, A actinomycetemcomitans, E corrodens, S noxia, V parvula, C rectus
Yellow-Orange: S intermedius, S oralis, S mutans, F nucleatum, M micros, C ochracea
Orange-Blue: E nodatum, A naeslundii
 # Defined as per ATP III criteria

Table 5.4: Odds Ratio (95% CI) between four cluster scores in relation to metabolic syndrome and its individual components

	Orange-Red	Red-Green	Yellow- Orange	Orange-Blue
Hypertension				
Model1	**1.02 (1.001-1.05)***	1.03 (0.99-1.06)	0.98 (0.95-1.01)	**0.91 (0.87-0.95)***
Model2	1.02 (0.99-1.04)	1.01 (0.98-1.04)	0.99 (0.96-1.04)	0.95 (0.92-1.00)
Model3	1.01 (0.97-1.05)	1.02 (0.98-1.05)	0.99 (0.95-1.03)	0.96 (0.91-1.01)
Model4	1.01 (0.97-1.05)	1.02 (0.98-1.06)	0.99 (0.95-1.03)	0.96 (0.92-1.02)
Hypertriglyceridemia				
Model1	0.99 (0.97-1.02)	1.01 (0.98-1.04)	0.99 (0.96-1.02)	0.97 (0.93-1.03)
Model2	0.98 (0.95-1.01)	1.0 (0.97-1.03)	1.003 (0.97-1.03)	0.99 (0.94-1.04)
Model3	0.98 (0.94-1.02)	1.002 (0.96-1.04)	1.01 (0.96-1.05)	0.99 (0.92-1.05)
Model4	0.97 (0.94-1.01)	1.01 (0.97-1.05)	1.004 (0.96-1.05)	0.99 (0.92-1.06)
Low HDL-Cholesterol				
Model1	0.99 (0.97-1.01)	0.99 (0.96-1.03)	1.02 (0.98-1.06)	0.98 (0.94-1.03)
Model2	0.99 (0.97-1.01)	0.99 (0.96-1.03)	1.02 (0.98-1.06)	0.98 (0.93-1.02)
Model3	0.98 (0.95-1.01)	0.99 (0.96-1.04)	1.01 (0.97-1.06)	0.99 (0.94-1.06)
Model4	0.98 (0.95-1.01)	1.002 (0.96-1.04)	1.01 (0.97-1.06)	0.99 (0.94-1.06)
Central Obesity				
Model1	1.003 (0.97-1.04)	1.00 (0.98-1.03)	1.00 (0.97-1.04)	0.98 (0.94-1.03)
Model2	1.02 (0.99-1.05)	0.99 (0.97-1.02)	0.99 (0.96-1.04)	0.99 (0.96-1.04)
Model3	1.01 (0.96-1.07)	1.0 (0.95-1.05)	1.02 (0.95-1.08)	0.95 (0.88-1.03)
Model4	1.01 (0.96-1.06)	0.99 (0.94-1.05)	1.02 (0.95-1.09)	0.95 (0.88-1.03)
Elevated Plasma Glucose				
Model1	**1.07 (1.03-1.1)***	1.01 (0.98-1.04)	0.97 (0.94-1.001)	**0.94 (0.89-0.98)***
Model2	**1.06 (1.02-1.1)***	0.99 (0.97-1.03)	0.99 (0.96-1.02)	0.97 (0.927-1.02)
Model3	**1.06 (1.02-1.1)***	0.99 (0.96-1.03)	0.99 (0.96-1.03)	0.98 (0.93-1.03)
Metabolic Syndrome				
Model1	**1.02 (1.002-1.04)***	1.01 (0.99-1.04)	0.99 (0.96-1.02)	**0.96 (0.92-0.99)***

Model2	1.02 (0.99-1.04)	1.0 (0.97-1.03)	1.00 (0.97-1.04)	0.99 (0.95-1.03)
Model3	1.02 (0.99-1.04)	1.003 (0.97-1.04)	0.99 (0.96-1.04)	0.99 (0.94-1.05)
#Adult Treatment Panel (ATP) III-Criteria				
Elevated Plasma Glucose				
Model1	**1.05 (1.01-1.10)***	0.99 (0.96-1.04)	0.99 (0.94-1.04)	**0.91 (0.87-0.95)***
Model2	1.04 (0.99-1.10)	0.98 (0.95-1.02)	1.01 (0.96-1.06)	**0.94 (0.89-0.98)***
Model3	**1.07 (1.02-1.12)***	0.98 (0.94-1.02)	1.02 (0.97-1.07)	**0.93 (0.88-0.98)***
Metabolic Syndrome				
Model1	1.004 (0.99-1.02)	1.01 (0.99-1.04)	1.001 (0.97-1.04)	**0.95 (0.91-0.99)***
Model2	1.001 (0.98-1.02)	1.001 (0.97-1.03)	1.01 (0.97-1.05)	0.98 (0.94-1.03)
Model3	0.99 (0.96-1.02)	1.002 (0.97-1.03)	1.02 (0.97-1.07)	0.98 (0.92-1.04)

Model1 with no adjustment for confounders;
Model2 adjusted for age (continuous) and sex;
Model3 adjusted further for education, ethnicity, income-to-poverty, smoking, drinking alcohol, physical activity, BMI (continuous), waist circumference [WC], and dentist visit;
Model4 further for diabetes
* P Value <0.05
Orange-Red: *P melaninogenica, P intermedia, P nigrescens, P gingivalis*
Red-Green: T forsythia, T denticola, A actinomycetemcomitans, E corrodens, S noxia, V parvula, C rectus
Yellow-Orange: S intermedius, S oralis, S mutans, F nucleatum, M micros, C ochracea
Orange-Blue: E nodatum, A naeslundii
 # Defined as per ATP III criteria

Table 5.5: Odds Ratio (95% CI) between four cluster scores in relation to metabolic syndrome and its individual components among the dentate participants (N=3716)

	Orange-Red	Red-Green	Yellow- Orange	Orange-Blue
Hypertension				
Model1	**1.01 (0.97-1.05)**	1.02 (0.98-1.06)	0.98 (0.93-1.02)	**0.92 (0.87-0.98)***
Model2	0.99 (0.96-1.04)	1.01 (0.97-1.05)	0.99 (0.95-1.04)	0.98 (0.92-1.04)
Model3	0.99 (0.95-1.04)	1.01 (0.97-1.05)	0.99 (0.94-1.06)	0.98 (0.91-1.05)
Model4	0.99 (0.94-1.04)	1.01 (0.97-1.05)	0.99 (0.94-1.06)	0.98 (0.91-1.05)
Hypertriglyceridemia				
Model1	1.003 (0.97-1.04)	1.02 (0.98-1.06)	0.97 (0.93-1.01)	0.96 (0.90-1.04)
Model2	0.99 (0.96-1.03)	1.01 (0.97-1.05)	0.98 (0.95-1.02)	0.98 (0.91-1.06)
Model3	1.002 (0.96-1.05)	1.01 (0.97-1.06)	0.99 (0.94-1.03)	0.96 (0.89-1.05)
Model4	0.99 (0.95-1.04)	1.02 (0.97-1.06)	0.98 (0.94-1.03)	0.97 (0.89-1.05)
Low HDL-Cholesterol				
Model1	0.99 (0.96-1.02)	1.001 (0.96-1.05)	1.01 (0.96-1.06)	0.97 (0.91-1.04)
Model2	0.99 (0.96-1.02)	1.001 (0.96-1.05)	1.01 (0.96-1.06)	0.97 (0.91-1.04)
Model3	0.98 (0.95-1.01)	0.99 (0.95-1.04)	1.03 (0.97-1.09)	0.98 (0.91-1.06)
Model4	0.97 (0.94-1.01)	0.99 (0.95-1.04)	1.03 (0.97-1.09)	0.98 (0.91-1.07)
Central Obesity				
Model1	1.01 (0.97-1.05)	1.01(0.97-1.04)	0.98(0.94-1.03)	1.01 (0.95-1.07)
Model2	1.02 (0.98-1.06)	1.01 (0.97-1.05)	0.98 (0.93-1.02)	1.04 (0.98-1.10)
Model3	1.03 (0.95-1.11)	1.02 (0.95-1.08)	0.98 (0.90-1.07)	0.96 (0.87-1.06)
Model4	1.03 (0.95-1.11)	1.01 (0.95-1.08)	0.98 (0.90-1.07)	0.96 (0.87-1.06)
Elevated Plasma Glucose				
Model1	**1.07 (1.03-1.11)***	1.03 (0.97-1.09)	0.95 (0.89-1.01)	0.97 (0.91-1.02)
Model2	1.06 (1.02-1.11)	1.02 (0.96-1.08)	0.96 (0.90-1.03)	1.01 (0.95-1.07)
Model3	**1.06 (1.02-1.11)***	1.002 (0.95-1.06)	0.98 (0.92-1.05)	1.01 (0.95-1.08)

Metabolic Syndrome				
Model1	1.02 (0.99-1.06)	1.03 (0.99-1.07)	0.96 (0.91-1.01)	0.99 (0.93-1.05)
Model2	1.02 (0.99-1.05)	1.02 (0.98-1.06)	0.97 (0.92-1.02)	1.03 (0.97-1.10)
Model3	1.04 (1.001-1.09)	1.02 (0.98-1.06)	0.96 (0.91-1.01)	1.03 (0.96-1.11)
#Adult Treatment Panel (ATP) III-Criteria				
#Elevated Plasma Glucose				
Model1	**1.06 (1.01-1.11)***	1.03 (0.96-1.10)	0.95 (0.89-1.01)	**0.92 (0.88-0.97)***
Model2	1.06 (0.99-1.12)	1.01 (0.95-1.08)	0.96 (0.91-1.03)	0.96 (0.92-1.02)
Model3	**1.07 (1.02-1.13)***	0.99 (0.94-1.05)	0.98 (0.93-1.04)	0.96 (0.89-1.03)
#Metabolic Syndrome				
Model1	**1.002 (0.97-1.03)**	1.02 (0.98-1.06)	0.98 (0.92-1.03)	**0.98 (0.92-1.05)***
Model2	1.0 (0.97-1.03)	1.01 (0.97-1.06)	0.98 (0.93-1.04)	1.02 (0.96-1.09)
Model3	1.01 (0.97-1.05)	1.002 (0.96-1.05)	0.99 (0.94-1.05)	1.01 (0.93-1.09)

Model1 with no adjustment for confounders;
Model2 adjusted for age (continuous) and sex;
Model3 adjusted further for education, ethnicity, income-to-poverty, smoking, drinking alcohol, physical activity, BMI (continuous), waist circumference [WC], and dentist visit;
Model4 further for diabetes
*** P Value <0.05**
Orange-Red: *P melaninogenica, P intermedia, P nigrescens, P gingivalis*
Red-Green: T forsythia, T denticola, A actinomycetemcomitans, E corrodens, S noxia, V parvula, C rectus
Yellow-Orange: S intermedius, S oralis, S mutans, F nucleatum, M micros, C ochracea
Orange-Blue: E nodatum, A naeslundii
 # Defined as per ATP III criteria

Table 5.6: Odds Ratio (95% CI) between three groups of z-scores in relation to metabolic syndrome and its individual components

	Etiology	Putative	Health
Hypertension			
Model1	1.03 (0.99-1.07)	1.00 (0.96-1.04)	0.95 (0.88-1.01)
Model2	1.04 (0.99-1.04)	0.99 (0.95-1.04)	0.97 (0.90-1.04)
Model3	1.02 (0.96-1.08)	1.00 (0.95-1.05)	0.98 (0.91-1.07)
Model4	1.02 (0.96-1.08)	1.00 (0.95-1.05)	0.99 (0.91-1.07)
Hypertriglyceridemia			
Model1	1.00 (0.96-1.05)	0.99 (0.97-1.02)	0.97 (0.91-1.05)
Model2	1.01 (0.96-1.05)	0.99 (0.96-1.02)	0.98 (0.91-1.06)
Model3	1.01 (0.96-1.05)	0.99 (0.96-1.03)	0.99 (0.90-1.08)
Model4	1.00 (0.96-1.05)	0.99 (0.96-1.03)	0.99 (0.90-1.09)
Low HDL-Cholesterol			
Model1	0.99 (0.94-1.03)	1.03 (1.00-1.07)	0.94 (0.88-1.00)
Model2	0.99 (0.94-1.03)	1.03 (1.00-1.07)	0.94 (0.88-1.00)
Model3	0.97 (0.92-1.02)	1.04 (1.00-1.08)	0.97 (0.90-1.04)
Model4	0.97 (0.92-1.02)	1.04 (1.00-1.08)	0.97 (0.90-1.05)
Central Obesity			
Model1	1.00 (0.96-1.04)	1.01 (0.98-1.04)	0.97 (0.91-1.04)
Model2	1.00 (0.96-1.04)	1.01 (0.98-1.05)	0.97 (0.92-1.03)
Model3	**0.93 (0.86-0.99)**	**1.08 (1.02-1.15)**	0.94 (0.86-1.03)
Model4	**0.92 (0.86-0.99)**	**1.08 (1.02-1.15)**	0.95 (0.86-1.04)
Elevated Plasma Glucose			
Model1	1.03 (0.99-1.07)	1.00 (0.97-1.03)	0.94 (0.88-1.01)
Model2	1.03 (0.99-1.07)	0.99 (0.97-1.02)	0.96 (0.89-1.03)
Model3	1.03 (0.99-1.08)*	0.99 (0.96-1.03)	0.98 (0.91-1.07)
Metabolic Syndrome			

Model1	1.02 (0.98-1.06)	1.01 (0.98-1.03)	0.96 (0.90-1.01)
Model2	1.02 (0.99-1.06)	1.00 (0.97-1.03)	0.97 (0.91-1.03)
Model3	1.01(0.96-1.06)	1.01 (0.97-1.04)	0.99 (0.92-1.06)
ATP III Criteria			
Elevated Plasma Glucose			
Model1	1.02 (0.96-1.08)	1.02 (0.97-1.06)	**0.89 (0.84-0.95)**
Model2	1.02 (0.96-1.09)	1.01 (0.97-1.05)	**0.90 (0.84-0.97)**
Model3	1.00 (0.94-1.07)	1.02 (0.97-1.07)	0.94 (0.87-1.02)
Metabolic Syndrome			
Model1	1.01(0.97-1.05)	1.01 (0.98-1.05)	0.96 (0.91-1.02)
Model2	1.01(0.97-1.05)	1.01 (0.98-1.05)	0.97 (0.92-1.03)
Model3	0.98(0.94-1.03)	1.03 (0.98-1.07)	1.00 (0.92-1.08)

Model1 with no adjustment for confounders;
Model2 adjusted for age (continuous) and sex;
Model3 adjusted further for education, ethnicity, income-to-poverty, smoking, drinking alcohol, physical activity, BMI (continuous), waist circumference [WC], and dentist visit;
Model4 further for diabetes
*** P Value <0.05**
Orange-Red: *P melaninogenica, P intermedia, P nigrescens, P gingivalis*
Red-Green: T forsythia, T denticola, A actinomycetemcomitans, E corrodens, S noxia, V parvula, C rectus
Yellow-Orange: S intermedius, S oralis, S mutans, F nucleatum, M micros, C ochracea
Orange-Blue: E nodatum, A naeslundii
 # Defined as per ATP III criteria

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CHAPTER 6

DISSERTATION SUMMARY

Periodontal disease, a polybacterial infection, is associated with different systemic diseases including hyperglycemia and cardiovascular risk factors. From the literature, there is emerging evidence that effect of periodontal infection on cardiovascular outcomes depends upon the type of bacteria predominating the infection(Desvarieux et al. 2013, Desvarieux et al. 2010, Desvarieux et al. 2005, Ebersole et al. 2008, Kebschull, Demmer, and Papapanou 2010, Lockhart et al. 2012), but this has not been evaluated for type 2 diabetes thoroughly. However, the microbiological evidence supports that periodontal bacteria or their antibodies are associated with diabetes(Casarin et al. 2013, Field et al. 2012, Ohlrich, Cullinan, and Leichter 2010, Vlachojannis et al. 2010, Yuan et al. 2001, Zhou et al. 2013).

There are several questions but a dearth of knowledge in the area of periodontal research in relation to the systemic outcomes. Through our dissertation research questions, we have tried to find some answer for some of gaps that existed in the periodontal research with a systemic association such

1. How will we characterize the polybacterial infection or the antibody response of the poly bacterial challenges through the biofilm?
2. What is the extent of association of the elevated antibody titers that formed due to specific periodontal bacteria with respect to T2DM, pre-diabetes and Mets and each of its individual components?

3. Does periodontal destruction modify the association of host response against the periodontal bacteria and diabetes?

We characterized the antibody response of the polybacterial challenges by using both the data derived clustering approach and the theoretical grouping approach. From the empirical approach, we found that the predominance of the elevated serum IgG Orange-Red cluster that included *P gingivalis* and *Prevotella sps* was associated with higher diabetes prevalence whereas elevated Orange-Blue clusters that included the *E. nodatum* and *A neslundii* antibodies lowered the odds of having type 2 diabetes , while predominance of the elevated Red-Green cluster that included *A. actinomycetemcomitans mix, S. noxia, E. corrodens, V. parvula, T. forsythia, T. denticola, C. rectus* antibodies was slightly associated with lower odds of having diabetes. Yellow-orange cluster which consist majority of *Streptococcus sps* antibodies were not associated with diabetes or pre-diabetes. From the theoretical grouping approach, we found the elevated etiologic group that consists *P gingivalis, A. actinomycetemcomitans mix, T. forsythia, and T. denticola,* to be mainly associated with pre-diabetes condition while the putative or Health related groups were not associated with diabetes, pre-diabetes , MetS or any of its individual components.

Mainly our study finding shows that serological markers of periodontal bacteria are related to diabetes prevalence and monitoring the type of periodontal bacteria antibodies in the serum may be important in finding the individuals with high risk of diabetes and pre-diabetes. Combination of clinical periodontal destruction measures and serological markers can provide more information on the hyperglycemia condition.

Strengths and Limitations

There were several strengths in this study. First, plasma IgG antibodies against the periodontal pathogen of *A Actinomycetecomitans* and *P. gingivalis* have been found to be stable for 15 years (Lakio et al. 2009). IgG antibodies against periodontal pathogen therefore represent the cumulative lifetime infection of periodontal disease and host response towards it. Second, we had enough power, sample size and data on serum antibodies of 19 different periodontal bacteria to analyze on the immune -inflammatory response to these periodontal bacteria in relation to diabetes, pre-diabetes as well as other key markers of cardiovascular risk factors adjusting for known confounders. Third, large sample size and clustering of serum IgG markers reduced the chances of type 1 error and gave confidence in the interpretation of the findings. We believe that this study will provide a perspective to understand the magnitude of host immune response to different periodontal bacteria in different clinical biomarkers of inflammatory diseases.

However, our study also has some drawbacks. First, it should be noted that our study being a cross sectional study cannot identify temporality or causality. Studies evaluating the accuracy of the dental examination in NHANES III and NHANES (2001-2004) showed that periodontitis prevalence was underestimated in of in those surveys because of the examination protocol and periodontal disease definitions that were used which may impact validity in surveillance and researches (Eke et al. 2010). Third, our study dataset includes information from 1988 -1994, the smoking habits, nutritional behaviors, diabetes awareness might have changed amply since then, hence the external validity of the findings should be assessed with more current information (Dye, Morin, and Robison 2006, Dye et al. 2007, Morin, Dye, and Hooper 2005). Fourth, there were

many missing data (about 25%) for the CAL and pocket depth which might have underestimated the estimation of interaction between the clusters and clinical periodontal measures with diabetes. Fifth, the periodontal examination was done not done on the full mouth in NHANES III survey which might have also under estimated the prevalence of periodontitis in the US population(Papapanou 2012).

Clinical Implication of our study and Recommendation to the clinicians

Our findings have a strong implication for clinical management of high risk population for diabetes and periodontal disease. Elevated serum antibody titers of selective periodontal bacteria (such as *P. gingivalis* and *Prevotella sps.)* could increase the odds of having diabetes. The presence of elevated titers of Orange-Blue cluster could significantly reduce the odds of having diabetes even in the presence of higher pocket depth destruction. This suggests that combination of serological markers and clinical periodontal measures may be useful to predict the hyperglycemia condition. Dental visits combined with the test of selected serological markers such as *P gingivalis, Prevotella species, E nodatum* and *A neslundii* may be used to screen the high risk individuals for the diabetes or pre-diabetes.

Recommendation to the public health professions

In recent years, the prevalence of chronic diseases such as type 2 diabetes, obesity, hypertension, lipidemia, MetS are in increasing. Oral health can be important lifestyle modulator that is associated with diabetes risk and can also be used to predict the risk of systemic health of an individual. More research is necessary to understand the biological pathways for the association of periodontal disease with different chronic systemic outcomes. Several evidences support that oral infection that is often hidden and

neglected and could be one of seeds of infection that can ultimately lead to systemic inflammation which is the key of several chronic systemic diseases including hyperglycemia. Hence, we recommend that Oral health is a priority in the research and policy of overall public health.

Current work and suggestions for future research

All of the aims encompassed by my dissertation relate to US participants. This is the first attempt to cluster the antibody titers against periodontal bacteria and explore these clusters in relation to systemic outcomes. We found the specific serological markers are associated with a protective role or potential risk of systemic diseases while some are unrelated. We would like emphasize that further research with new sophisticated molecular technology, epidemiological methods and clinical periodontal examination in the recent population are needed to validate our findings in different population.

Recent reports of gut microbiota being associated with diabetes, MetS and other systemic conditions such as obesity strengthens the possibility of linkage of periodontal bacteria (Zupancic et al. 2012) and underscores the need of further research to identify the systemic pathways. Future research could explore the correlation of the gut microbiota with periodontal microbiota. Further exploration of the inter-relationship with the systemic outcomes could characterize the microbiota role in systemic health.

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APPENDIX A – PERIODONTAL CLINICAL MEASURES AND FOUR CLUSTERS

Table A.1: Association of the z-scores of serum antibody clusters with diabetes stratified by the periodontal status

Serum Antibody z-scores Clusters	Odds Ratio (95% Confidence Interval) Periodontal status	
Model*	Yes	No
Red-Green		
Model1	1.03 (0.95, 1.11)	1.02 (0.93, 1.11)
Model2	1.00 (0.92, 1.09)	1.03 (0.93, 1.13)
Model3	1.01 (0.92, 1.10)	1.00 (0.92, 1.09)
Model4	0.99 (0.87, 1.12)	1.00 (0.92, 1.10)
Orange-Red		
Model1	**1.14 (1.05, 1.25)**	1.12 (1.03, 1.22)
Model2	**1.09 (1.00, 1.20)**	1.01 (0.92, 1.12)
Model3	**1.10 (1.00, 1.21)**	1.01 (0.90, 1.14)
Model4	**1.14 (1.02, 1.27)**	0.91 (0.81, 1.12)
Yellow-Orange		
Model1	0.96 (0.86, 1.06)	0.95 (0.86, 1.06)
Model2	1.00 (0.90, 1.11)	0.98 (0.88, 1.08)
Model3	0.98 (0.88, 1.10)	1.01 (0.93, 1.11)
Model4	1.04 (0.89, 1.21)	1.06 (0.94, 1.18)
Orange-Blue		
Model1	**0.83 (0.74, 0.92)**	**0.86 (0.75, 0.98)**
Model2	**0.85 (0.76, 0.95)**	0.98 (0.85, 1.13)
Model3	**0.85 (0.76, 0.95)**	0.95 (0.82, 1.12)
Model4	**0.75 (0.64, 0.88)**	0.93 (0.77, 1.13)

*Bold p value <0.05

Model1 with no adjustment for confounders; Model2 adjusted for age (continuous), sex, education, ethnicity, income-to-poverty; Model3 adjusted further for smoking and drinking alcohol; Model4 further for physical activity, BMI (continuous), waist circumference [WC],dentist visit and fibers intake.

Orange-Red: *P. melaninogenica, P. intermedia, P. nigrescens, P. gingivalis*

Red-Green: T. forsythia, T. denticola, A. actinomycetemcomitans, E. corrodens, S. noxia, V. parvula, C. rectus

Yellow-Orange: S. intermedius, S. oralis, S. mutans, F. nucleatum, M. micros, C. ochracea

Orange-Blue: E. nodatum, A. naeslundii