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Correlating Periodontal Disease and Cardiovascular Disease: A Comparative Study of Research Methodology

Alexandra Hensen
Western Michigan University, ahensen10@gmail.com

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Alexandra M. Hensen

Western Michigan University

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Thesis Mentor: Dr. Allen Webb

Abstract

This meta-analysis compares and contrasts three types of population-based research methodology currently used to analyze correlations between Periodontal disease and Cardiovascular disease, the most wide spread diseases in the Western industrialized world.

Introduction

Of course, medical practices should be based in science and on knowledge generated from scientific methods and research. As modern scientific knowledge has increased so has the complexity of research. Research is now conducted in diverse ways not only by practitioners but also by communities and institutions of scientists in national and international academic, governmental, and corporate contexts. It is vital for medical practitioners to stay abreast of research developments in their fields, to engage in evidence-based practice. Practitioners need to be able to think scientifically and to understand differing research methods and protocols in order to evaluate their value and relevance to practice. This thesis prepared for the Lee Honors College at Western Michigan University is an examination of research methodology in the exciting and rapidly evolving field of research examining the relationship between periodontal and cardiac health. I consider these research methods a natural and relevant topic for me in my preparation to enter the medical field, particularly my aspiration to enter dental school in the fall and become an outstanding and scientifically sophisticated dentist.

In the broadest historical perspective, the scientific method was proposed by Ibn al-Haytham, an Arab scientist who designed this method in order to demonstrate how and why he figured out that light only travels in straight lines (Pomeroy 2014). Al-Haytham proposed the following protocols: the first step is to ask a question, do background research on the question at hand, construct a hypothesis, test the hypothesis by doing an experiment, analyze the data collected, draw a conclusion based on the analyzed data, and communicate the results with

others. Again, in the broadest possible sense, there are many different ways that research can be described. First it might be broken into two categories: quantitative research and qualitative research. Quantitative research involves numerical and statistical explanations whereas qualitative research involves observational explanations such as from surveys and questionnaires. Other categories of research include correlational analysis which involves determining potency of the relationship between two or more variables. Experimental research involves comparing two groups, one of which is influenced by something and the other which is not. A meta-analysis is a type of research that involves comparing and contrasting different research results and methods that have been done on a particular topic. This honors thesis is a meta-analysis of research methods of three different types of studies attempting to understand the relationship between periodontal disease and cardiovascular disease.

Periodontal disease affects 15-20% of middle-aged people throughout the world (WHO 2012) and approximately 40% of the adult population in the United States (Barros et al. 2013). Periodontal diseases are infections around the teeth; the structures around teeth include the gums, the cementum that covers the root, the alveolar bone, and the periodontal ligament (Colgate 2013). Mild cases of periodontal disease typically only affect the gums and can be treated, however when the disease progresses it can affect all of the structures around the tooth and result in the loss of teeth. Inflammation caused by the infections can result in the breakdown of the connective tissue attaching the alveolar bone and the teeth, this can eventually lead to loss of the bone and cause irreversible damage. The level of attachment is determined by techniques using probing at multiple sites throughout the mouth (Agustsdottir et al. 2011). The inflammatory response caused in periodontal diseases is due to the colonization of bacterial metabolites and biofilms in the subgingival areas. The activation of the host immune system triggers the

synthesis and release of cytokines, proinflammatory mediators, and matrix metalloproteinases that result in the destruction of the tissues in order to fight the infection. The balance between the virulence of the local biofilm and the host immune system ultimately determines the progression and severity of the destruction caused by the infection (Oppermann et al. 2012). Periodontitis, in adults over the age of 40, is the major cause for tooth loss (Groessner-Schreiber et al. 2009). There are many factors that play a role in the cause of periodontal disease and the overall effects it can have on an individual. The cardiovascular system has been linked to periodontal disease, as it can be affected by the progression of periodontal infections.

Cardiovascular disease is disease of the heart and blood vessels. Atherosclerosis is the process that is most commonly correlated with problems associated with heart disease, specifically coronary heart disease. Atherosclerosis develops when plaque builds up in the walls of the arteries, making it more difficult for blood to pass through, often leading to the formation of a clot which can result in a heart attack or stroke. Coronary heart disease is the leading cause of death in both men and women in the United States and accounts for more than 7 million deaths worldwide each year.

Together, coronary heart disease and periodontitis are the most widespread diseases in the Western industrialized world (Groessner-Schreiber et al. 2009).

Studying these two detrimental diseases and how they influence one another can be a difficult task, however the strong correlation between the two diseases has inspired increasing research. Diseases are studied in many ways. Acquiring knowledge about how processes in the body take place and what they affect, is crucial in understanding how to treat and fight disease. When considering how a disease or diseases affect a large population researchers are engaged, broadly speaking, in epidemiology, the science that studies the patterns, causes and effects of

health and disease conditions in defined populations. Epidemiology is vital to public health, and informs policy and evidenced-based medical practice. This kind of research draws on surveillance, screening, clinical trials, and biomonitoring. Their studies are typically population-based. Population-based studies offer important ways to examine questions about the relationships between diseases specifically because they do not single out individuals; instead a group of individuals are studied and the results are used to understand a target population. We are faced everyday with impediments in our understanding of the human body, and changes and new diseases arise all the time. As we progress in the ways we understand the relationship between disorders and make more sophisticated our research protocols – the ways in which we study these disorders -- we can progress in learning to treat them.

There are many different types of population-based studies. In this thesis three current and entirely different types of population-based studies were identified and researched based on the type of methodology they used to understand the relationship between periodontal disease and cardiovascular disease. These three studies include: "Gender Differences in the Relationship Between Periodontal Disease, Tooth Loss, and Atherosclerosis," published in the *Journal of Stroke and Cerebrovascular Diseases* (2013); "Periodontal Disease and Mortality in Type 2 Diabetes" published in *Diabetes Care* (2005); and "Identification of a Shared Genetic Susceptibility Locus for Coronary Heart Disease and Periodontitis" published in *PLoS Genetics* (2009). These studies vary dramatically in their specific focus and in the way that they conducted research – their research method--; however, all three of the studies furthered the conclusion that there is a relationship between periodontal disease and cardiovascular disease. The methodologies used and that I studied in these three analyses include a cross-sectional

population-based survey, a longitudinal population-based study, and a population-based

candidate-gene association study.

All three of the analyzed studies were population-based, meaning that the selected

subjects for the study were chosen based on their residence, ethnicity, gender, etc. This term

typically refers to a general population, such as within a city or certain country, rather than

referring to a population of people residing in a specific place, such as a retirement home or

hospital, for example. Whether the population is a country, a state, an ethnic group, or some

other subgroup, the overall goal of a population-based experiment is to use survey sampling

methods to produce a group of subjects that is representative of the target population of interest

for a particular theory (Mutz 2011). The population-based approach to research has evolved

dramatically due to technological advances including the use of telephone interviewing and the

Internet. More specifically, computer-assisted telephone interviewing has allowed researchers to

develop more complex experimental designs and use large and diverse subject pools. The

Internet has increased the potential for telephone-based and face-to-face interviewing methods.

There are advantages to population-based research; when it is done properly it can depict the

target population. For example, an advantage of population-based survey experiments is that

theories can be tested on samples that are representative of the populations to which they are said

to apply (Mutz 2011).

When population-based studies are performed through surveys versus in a laboratory

setting it allows easier studying of sub-populations. In a laboratory participants may not be naive

and results can be thrown off because subject biased is created by prior knowledge of the

purposes of research. Whereas when sub-population research is done via survey subjects will

not be as prone to compare their presence to the others around them. Mutz explains, in her book,

Population-based Survey Experiments, that population-based survey experiments are likely to encourage more widespread use of experiments by social scientists, the use of complex experimental designs, more successive studies comprising a larger research agenda, and research that speaks to real world events and policies.

In a laboratory setting, certainly, it can be difficult to get participants. Many times people will not want to participate in a laboratory setting experiment or research study because it is inconvenient to go to the designated laboratory. However this obstacle can be overcome by the use of incentives, such as a monetary payment, but the downfall of this is that it can result in a research study costing much more than what would seem necessary and often times the research will not be followed through due to funding. Also, it can be difficult to achieve diversity in the population sample when the research is conducted in a laboratory; many times the population of interest is not a group of people that will be easily coaxed into coming into a laboratory.

Another challenge is funding for such research; it is very important to have a large sample size for this type of study and that involves spending money. In population-based, as with any type of study, there are important challenges when attempting to determine causation. In order for one variable to be said to "cause" another, three conditions must be met. First of all, the two must co-vary, whether over time or across units of analysis, this criterion seems to be the most difficult one to obtain; next, the cause must precede the effect in time, this criterion can be tricky for cross-sectional studies; and finally, the relationship between the cause and effect must not be explainable through some other third variable, this is part of the reason that experiments, versus surveys, are referred to as the "gold standard" for inferring causality, experiments are good methods to address the issue of third variables (Mutz 2011).

Population-based laboratory setting research and population-based survey setting research both cannot fully protect against people being biased or untruthful. In a survey bias and untruth might seem easy for subjects and perhaps be unnoticed by researchers. In a laboratory it may seem bias and untruth might seem necessary to a subject in order to avoid being seen negatively by the in person researcher. Certainly, in order to properly perform any type of research that involves participants there needs to be guidelines and careful procedures.

The three studies that were analyzed in this thesis are summarized below. These "summaries" are not simplistic cribbing from the articles, but include additional information and analysis. For example, frequently I had to research referenced terms and procedures. I attempt to define and explain the studies so as to make them clearly understandable to the reader. Much of the analysis represents my own best thinking about the strengths and weaknesses of the studies and their methodology.

Three Population-Based Studies

"Gender Differences in the Relationship Between Periodontal Disease, Tooth Loss, and Atherosclerosis" published in *Journal of Stroke and Cerebrovascular Diseases* (2013)

There have been studies on the difference in genders correlating periodontal disease and cardiovascular disease. A particular study was done and written about in the Stroke Journal, published by the American Heart Association, titled "Gender Differences in the Relationship Between Periodontal Disease, Tooth Loss, and Atherosclerosis." This study, a study of health in

Pomerania, was a cross-sectional population-based survey in Germany, the cities involved in the survey included Greifswald, Stralsund, Anklam, and 29 surrounding villages. Participants were selected with no history of myocardial infarction or stroke, however an assessment of the subjects resulted in elimination due to missing tooth count, missing periodontal measurements resulting from crowns, absence of teeth in the quadrants measured, incomplete lipids, risk factors, and those who refused participation. There were 1740 participants, ages 45 to 75 years old, remaining in the study, which took place from October 1997 until May 2001.

A cross-sectional study approach is great for this type of research because different populations are being compared at a single point in time and also over the course of years. Also, since a cross-sectional study is an observational study, it is good that the participants' lives are not influenced by the researchers. This gives the results a more accurate outcome. I was under the impression that in a cross-sectional study previous occurrences were not considered, however in this study they selected participants with no history of myocardial infarction or stroke. However, I do not think this study could be done without knowing about previous health conditions, so this strategy should be effective and appropriate for this study.

From this information I think it would be interesting to see what the outcomes would have been if they also included a younger population, this way perhaps puberty could be taken into consideration between males and females. A follow up with the younger population when they reach their fifties to see what has happened to their oral health and within their cardiovascular system as they aged and depending on the lifestyles they lived.

Dental history and oral examinations were done by calibrated licensed dentists to assess tooth count and location, also participants reported their flossing and brushing habits. All participants had measurements taken at four sites per tooth (mesiobuccal, midbuccal,

distobuccal, and milingual). These measurements were taken with the periodontal probe PCP 11, this assessed probing depth, gingival recession, and clinical attachment loss. Current periodontal disease was measured by probing depth and long-term disease was measured by attachment loss. Both long-term and current disease accounted for the severity of a participant's disease, which was defined by the percentage of periodontal sites meeting the severity criteria, ≥5mm for probing depth and ≥4mm for attachment loss. The percentage of sites was calculated within each mouth by dividing the number of sites with probing depth ≥5mm by the total number of sites measured and similarly for attachment loss. Every 6-12 months the calibration exercises were performed on subjects not connected to the study, this yielded an intraclass correlation of 0.82 to 0.91 per examiner and an inter-rater correlation of 0.84 relative to attachment loss. Including people not connected to the study seems to be beneficial because they will not be biased in their results and the information obtained from the calibration could lead to further information when determining the results of the study at hand.

How the non-connected subjects were found and placed into the calibration would be good to know. Were they just random people, patients of the calibrated licensed dentists, were they aware of the study? Not knowing this information seems to be a potential drawback.

An ultrasound examination was performed on the participants by certified examiners. Scans were performed on the participants in the horizontal position and were done on the extracranial carotid arteries bilaterally with B-mode ultrasound using a 5-MHz linear probe array transducer and a high-resolution instrument. Plaques were present if there was a focal widening of the vessel wall relative to adjacent segments; this was diagnosed during the examination in the common carotid arteries, the bifurcation, and the internal/external carotid artery. Presence of plaque was defined as ≥1 plaque in any of the carotid arteries.

All participants received a risk factor assessment which included an extensive medical examination, a computer-guided interview, and a self-administered questionnaire. Patients medical histories were assessed regarding hypertension (defined by self-report of diagnosed hypertension or use of antihypertensive medications, mean systolic blood pressure $\geq$140mm Hg, or mean diastolic blood pressure $\geq$90mm Hg), diabetes (which was defined as self-reported physician diagnosis of diabetes and pharmacological treatment for diabetes or hemoglobin A1c values $\geq$7%), angina pectoris, history of myocardial infarction, stroke, rehabilitation history, heart surgery, congestive heart failure, peripheral vascular disease, alcohol use, and current smoking (categorized as never, former, occasional, and current smoker). Height and weight were determined using calibrated scales. Nonfasting blood samples were taken and sent for complete blood count to two laboratories, duplicate samples were collected and analyzed for internal quality assurance protocol in each examination center once per week. The total cholesterol of participants was measured enzymatically, high-density lipoprotein-cholesterol enzymatically after magnesium chloride precipitation, and low-density lipoprotein-cholesterol after dextrane-sulfate precipitation. It is clear that nonfasting blood samples were used as to simplify this study, however this could lead to inaccurate results for determining cholesterol.

Having the participants self-report on medical conditions such as hypertension and diabetes seems to be a potentially complicating factor to this study because there could be a possibility that the participants may have not gone to ever be checked for these conditions in their life and the self-report may not be 100% accurate.

Participants were assessed on cultural, life style, and social network variable through a questionnaire. Education was categorized as <9, 9-10, and 11 years of schooling. Assessed social variables covered marital status, number of and contact with friends and children, and the

expressed desire to have more friends or family and the availability of support for personal

problems or transportation. Leisure physical activity was converted to an established measure of

metabolic output (METS) and was assessed as reporting >2 hours per week, 1 to 2 hours per

week, and <1 hour per week, and no activity. Frequency and type of alcohol consumption was

determined by the intake during the previous week and weekend, and the mean daily alcohol

consumption for the last week was calculated by the beverage-specific quantity/frequency

method.

Another potentially complicating factor would be that the frequency of alcohol

consumption may not be accurate due to what the participants had done the previous week. One

week's worth of information is not adequate for an overall judgment of alcohol consumption.

All statistical analyses were performed in PC-SAS for Windows 8.0 and linear and

logistic regression models were used.

The results of this experiment demonstrate that atherosclerosis was related to attachment

loss and tooth loss in males but not females. This could be better understood based on the

knowledge of the participants in this study, the males were older than the females (60±8 versus

59±8 years) which could result in higher chances of atherosclerosis as well as the fact that the

males smoked more than the females. It is important to realize that smoking can sufficiently

increase the risk of heart problems, this could have been a huge part in the results of this study

leading more toward males. They do note that the findings were qualitatively similar in

nonsmokers, however with males smoking more and slightly older it does seem to play a

potential effect in the results. Also, plaque was 10% more prevalent among males in the highest

tertile of attachment loss compared to those in the lowest tertile. However, women had an equal

amount of tooth loss but a substantially lower rate of periodontal disease, the loss of their teeth

could have resulted from independent reasons. There have been studies that demonstrated a relationship between osteoporosis, periodontal disease, and tooth loss, which could also account for the differences in female versus male reasoning for tooth loss.

This study was very interesting in that it compared gender influence on periodontal disease and atherosclerosis, it is common that males and females suffer from diseases differently. From here I think a study focused on only males and only females to compare the effects within one gender would be important.

"Periodontal Disease and Mortality in Type 2 Diabetes" published in *Diabetes Care* (2005)

"Periodontal Disease and Mortality in Type 2 Diabetes" is a study examining the effect of periodontal disease on overall and cardiovascular disease mortality in Pima Indians with type 2 diabetes. Individuals with diabetes and severe periodontal disease may be particularly susceptible to microvascular and macrovascular complications (Bennett et al. 2005). There is evidence suggesting that periodontal disease in diabetes may result from prolonged exposure to hyperglycemia, which is when blood-glucose levels get too high normally because there is not enough insulin or because the body is not using the insulin properly.

Of a total of 772 diabetic individuals there were 628 residents of the Gila River Indian Community of Arizona, mostly Pima or Tohono O'odham Indians, participating in this longitudinal population-based study. A longitudinal study is one in which the same variables are observed over long periods of time, this can be a beneficial in medicine because it can allow

researchers to uncover certain predictors of a disease. This study was conducted by the National

Institute of Diabetes and Digestive and Kidney Diseases.

In the beginning of the study, before the participants were chosen, every two years, all

members of the community five years or older was invited to participate in a standardized

examination that included a medical history, physical examination, and measurement of BMI,

blood pressure, plasma glucose level, HbA^sub 1c^ level, serum cholesterol concentration, and

albuminuria. Subjects were considered hypertensive if their systolic blood pressure was ≥140

mmHg, their diastolic blood pressure was ≥90 mmHg, or they were taking any antihypertensive

drugs.

Smoking was assessed through a questionnaire, current smoking was considered any

amount of smoking in the past year and nonsmokers included those who previously smoked but

not within the last year. This part of the study could have been problematic because some

subjects might not be honest about their smoking or nonsmoking habits and this could lead to

inaccurate data. Also, subjects who previously smoked in their lives but not within the past year

were put into the nonsmoking category, this is a difficult area to define because those subjects

were nonsmokers at the time however their previous smoking habits could influence the outcome

of results. Twelve-lead electrocardiograms (ECGs) were recorded in all of the subjects during

the first biennial examinations and during each examination subsequent to the fifteenth birthday,

they were all interpreted and classified according to the Minnesota Code by the same cardiologist

who had no knowledge of the clinical data. Total serum cholesterol was measured by a

colorimetric method, a urinary albumin-to-creatinine ratio was used as an estimate of albumin

excretion rate. The 1985 World Health Organization criteria was used to define diabetes if

plasma glucose concentration was ≥200 mg/dl 2 hours after a 75-g oral glucose load or if a clinical diagnosis was documented in the medical record.

Over the duration of seven years, January 1983 until September 1990, periodic dental examinations were done by dentists without any knowledge of the patient's medical status. These examinations evaluated the oral mucous membranes and alveolar bone loss, also probing attachment levels were measured at the following sites: midbuccal, midlingual, mesial interproximal point, and distal interproximal point. This evaluation determined the presence and severity of periodontal disease.

The subjects vital statuses were ascertained as of the end of 1998 and the causes of death were defined using statistical codes. Deaths due to disease were coded as "natural", those due to injury or poisoning were coded as "external", and those due to IHD and diabetes were considered as cardiorenal disease. Periodontal disease effects on mortality were examined using a time-dependent Cox proportional hazards model. The Cox model is a method of analyzing time to event data. It assumes that the hazards are proportional and uses partial likelihood to estimate the model. The possibility of effect modification was assessed by evaluating pairwise interaction terms between periodontal disease and sex, age, duration of diabetes, and smoking. A pairwise interaction is between three or more variables that may influence the situation at hand; typically one of the variables is not influenced by the others.

About 60% of the population had severe periodontal disease and 70% of those participants were edentulous, meaning that they did not have teeth. The follow up for this study averaged eleven years and 204 of the 628 subjects had died. Most of the cardiovascular disease deaths were attributed to IHD and most of the diabetes related deaths were attributed to diabetic nephropathy, other CVD and diabetes related deaths were not associated with periodontal disease

all that significantly. However, in subjects with severe periodontal disease the death rate from IHD was 2.3 times as high and the death rate from diabetic nephropathy was 8.5 times as high as in those with less severe periodontal disease. This suggests that periodontal disease may lead to other systemic disease but other systemic disease does not typically lead to periodontal disease.

Although this study was only focused on a very specific population it demonstrates well that periodontal disease can be a huge factor in the onset of other diseases. The study contributes to our understanding of the importance of oral health to overall health.

"Identification of a Shared Genetic Susceptibility Locus for Coronary Heart Disease and Periodontitis" published in *PLoS Genetics* (2009)

The study titled, "Identification of a Shared Genetic Susceptibility Locus for Coronary Heart Disease and Periodontitis," is a population-based candidate-gene association study with participants from Schleswig-Holstein, Germany. A candidate-gene association study is one that is done in order to better understand a hypothesis about a particular gene and what it might be responsible for. This type of study is focused on genes that are selected due to previous hypotheses about their role and sometimes to better understand their biological pathway. This type of study can be beneficial if the researchers' hypothesis is well educated, for a candidate-gene study could lead to finding an association between the gene of interest and the potential role it may play in the biological pathway of the trait of study. If an association is found it could provide information about the genes functionality in the pathway of interest. If knowledge of the

biological pathway and trait of interest is not well known, a candidate-gene association study may not be beneficial to the researcher (*Genetics and Social Science*).

The participants in this study were unrelated and of German ethnicity, determined by both parental birthplaces. Participants were recruited through the population-based PopGen biobank, established in 2003 for research into the genetic risk factors for complex diseases. This study has recently developed into a prospective cohort study to investigate longitudinal changes of inflammatory biomarkers (Krawczak et al. 2006). The participants in this study were required to have coronary catheterization demonstrating significant coronary heart disease, meaning they had to show at least seventy percent stenosis in one major epicardial coronary vessel. Stenosis is also known as the abnormal narrowing of a passage in the body. Of the participants found through the PopGen biobank there were 1,104 that had a diagnosed disease onset less than fifty-five years. From that group of people there were 596 that had suffered a myocardial infarction, also known as a heart attack. Having a large population size to study is important for a candidate-gene association study because if the population were small it could become difficult to identify the independent contribution of a single genetic variant in the pathway. This is because most traits are influenced by many different genes, any one of which could contribute a small amount to the overall risk at hand. About ninety percent of the participants had a history of severe coronary heart disease and had a coronary re-vascularization procedure, either percutaneous coronary intervention, a non-surgical procedure performed with a deflated balloon that is inflated at the site of blockage that is done to treat the stenotic coronary arteries, or coronary artery bypass grafting, this is a surgical procedure done with arteries or veins from elsewhere in the body that are grafted to the coronary arteries to improve the blood supply to the heart. There were 736 healthy ethnically matched control subjects, which were found from the

Blood Service of the University Hospital Schleswig-Holstein. Information about the gender and

age was available and all of the participants gave written informed consent. This study was

approved by the institutional ethics review board and data protection authorities.

The inclusion criterion of the participants included age at diagnosis of 35 years or less,

two or more teeth with 50% or greater periodontal bone loss. A set of full-mouth dental

radiographs were available for confirmative periodontal bone scoring. The sub-phenotype of

localized aggressive periodontitis was characterized by 50% or greater bone loss at two to six

teeth and for the sub-phenotype of generalized aggressive periodontitis it was characterized by

50% or greater bone loss at seven or more teeth. The ethnically matched controls used for the

generalized aggressive periodontitis study were randomly chosen on the basis of the local

population registry and for the localized aggressive periodontitis the controls were obtained from

the Blood Service of the University Hospital Schleswig-Holstein.

All of the control participants reported to be free of periodontitis, this was based on self-

report which could be problematic if the participants had not received specific periodontal dental

examinations recent to the study. The control participants did undergo additional physical

examination at the PopGen facilities in order to obtain information on their general health status.

The oral health status and physical risk factors of the control participants were obtained from

questionnaires that were completed during medical consultations; this was followed by a clinical

checkup.

The genotyping part of this study was performed using the SNPlex and TaqMan

GenotypingSystem on an automated platform, employing TECAN Freedom EVO and 96-well

and 384-well TEMO liquid handling robots (TECAN, Mannedorf, Switzerland). These liquid

handling robots allow for the extraction of DNA as well as amplification set-up, sample dilution,

normalization, and assay development. Using machines that can perform these tasks is necessary

for this type of research for everything must be amplified in order to observe and understand it.

SNPlex is a platform for SNP genotyping; it is based on capillary electrophoresis. SNP

genotyping is the measurement of genetic variations of single nucleotide polymorphisms (SNPs)

between members of a species. SNPs occur throughout our DNA and are commonly used as

biological markers to locate genes that are associated with certain diseases, in this case

periodontal and cardiovascular disease. The TaqMan Genotyping System is a system that

provides PCR (polymerase chain reaction)-based SNP analysis of samples and candidate SNPs.

Genomic DNA was extracted from blood samples, using the Invisorb Blood Universal Kit,

Invitek, Berlin, Germany, and amplified by whole genome amplification. This Invisorb Blood

Universal Kit enables isolation of genomic DNA from variable amounts of whole blood or from

corresponding amounts of buffy coat. The genotypes were generated by automatic calling using

the Genemapper 4.0 software (Applied Biosystems) with the settings as followed: sigma

separation >6, angle separation for two cluster SNPs <1.2 radians, median cluster intensity >2.2

logs. The genotypes were additionally reviewed manually and call rates >95% in each sample

set were required.

Previous genome-wide association studies (GWAS) found a strong association of a

region of elevated linkage disequilibrium (LD) on human chromosome *9p21.3* which is located

upstream of the *CDKN2A* and *CDKN2B* genes. Linkage disequilibrium is the non-random

association of alleles at two or more loci that descend from single, ancestral chromosomes.

Some factors that may influence the level of linkage disequilibrium include genetic linkage,

selection, the rate of recombination, the rate of mutation, and population structure. Different

SNPs were identified that were in high linkage disequilibrium, the association of rs1333049 was

confirmed, through a meta-analysis in seven different populations, the association of this region

with coronary heart disease, therefore making it the best replicated genetic risk locus of CHD to

date. This study investigated a possible association of this linkage disequilibrium region with

aggressive periodontitis to further investigate whether this locus may also relate to the risk for

periodontitis.

To begin the first stage of this experiment three SNPs were selected from the LD region

of interest, the SNPs were rs2891168, rs1333042, and rs1333048. After thorough testing and

replication it was confirmed that all three of the SNPs gave evidence for association with

coronary heart disease, this allowed for the study to continue. The SNPs were then genotyped in

159 German periodontitis patients with the most extreme phenotype of aggressive periodontitis

and also 736 independent ethnically matched healthy control participants. This demonstrated

that the SNPs were associated with periodontitis.

The procedure was replicated in an independent population of 146 German periodontitis

patients with the less severe aggressive periodontitis phenotype localized aggressive periodontitis

and 368 ethnically matched healthy control participants. The SNPs were, again, significantly

associated with periodontitis, at both the multiplicative and genotypic level.

Adjustments were made in both experiments to account for common coronary heart

disease risk factors, such as smoking, type 2 diabetes, and gender, and results demonstrated that

all of the SNPs remained significantly associated with periodontitis.

Statistical analysis was completed with the following software: PS Power and Sample

Size Calculations for power calculations. This is an interactive program used for performing

power and sample size calculations that may be used in studies with dichotomous, continuous, or

survival response measures. For single-marker case-control analysis the following programs

were used: Haploview v4.0, which is designed to simplify and expedite the process of haplotype analysis by providing a common interface to several tasks relating to such analyses; PLINK v2.049, a whole genome association analysis toolset designed to perform a range of basic, large-scale analyses in a computationally efficient manner; and FamHap, a software for single-marker analysis and joint analysis of unphased genotype data from tightly linked markers. The GOLD program, a software package that provides a graphical summary of linkage disequilibrium in human genetic data, was used for LD measure plots. As mentioned previously, LD is the non-random association of alleles at adjacent loci. Also, the program R v2.7.2 was used for logistic regression analysis. Logistic regression is a statistical method used to analyze a dataset in which there are one or more independent variables that determine an outcome. The goal of this analysis is to find the best fitting model to describe the relationship between the dichotomous characteristic of interest (in which there are only two possible outcomes) and a set of independent variables. The Hardy-Weinberg equilibrium, which states that allele and genotype frequencies in a population will remain constant from generation to generation in the absence of other evolutionary influences, was used to test inclusion markers for deviations and significance was assessed by a Wald test as well as by a likelihood-ratio test. The Wald test can be used to test the true value of the parameter based on the sample estimate, this is used whenever a relationship within or between data items can be expressed as a statistical model with parameters to be estimated from a sample.

This study was performed because there had been recent studies indicative of a mutual epidemiological relationship between coronary heart disease and periodontitis, both of which share similar risk factors and are characterized by a chronic inflammatory process. This candidate-gene association study identified an association of a genetic susceptibility locus shared

by both periodontitis and CHD. The researchers of this study confirmed the known association of two neighboring linkages disequilibrium regions on human chromosome *9p21.3* with coronary heart disease and showed the additional strong association of the loci with the risk of aggressive periodontitis.

The researchers in this study were careful to avoid error in their results, for example they matched the controls for an age older than the general age of onset of mild forms of periodontitis (60 years) because the severe form of aggressive periodontitis is suspected to be determined by genetic risk factors. Since less severe forms of periodontitis can be difficult to diagnose at a young age, the matching of an older age seemed appropriate. The controls in the replication panel were matched to an age that better mirrored the gene pool of the average population, this was done in order to avoid stratification which could have been caused by the older control participants being representative of "survivors," which would not comprise the complete gene pool of the population.

The overall results of this study demonstrate that coronary heart disease and periodontitis are genetically related by at least one susceptibility locus. Further findings from this research offer promise of new insight into the underlying shared pathogenic mechanisms of these complex common diseases.

Conclusion

Of the three studies that were analyzed for this thesis there was one that was lacking in the number of participants, this was the study "Periodontal Disease and Mortality in Type 2 Diabetes." There were only 628 total participants in this study, a low number for a population-

based study. One of the benefits mentioned about population-based studies is that if they are

done properly they can depict the target population, however for such a study to fulfill this goal

there must be enough participants. 628 participants is typically not an adequate number from

which to draw formal results. The other two studies, "Identification of a Shared Genetic

Susceptibility Locus for Coronary Heart Disease" and "Periodontitis and Gender Differences in

the Relationship Between Periodontal Disease, Tooth Loss, and Atherosclerosis," both had over

one thousand participants which, although two to three thousand is recommended, is likely a

sufficient study size for meaningful results.

The first study included participants with periodontal disease but no history of

myocardial infarction or stroke. This study also included a control group, which, as mentioned,

increases the value of the study. Although the concept of this study was potentially interesting, it

is uncertain to what degree its results can be generalized to the population as a whole. This

experiment purports to demonstrate that atherosclerosis was related to attachment loss and tooth

loss in males but not females. The study found that females had tooth attachment loss but it was

likely resulting from independent reasons. Although their analysis may be accurate I find it to be

too little information to say with confidence that males and females are affected completely

differently by periodontal disease. It would be interesting, however, for future studies

concerning periodontal disease to research the effects on how osteoporosis might affect women.

In the second study all of the participants suffered from type two diabetes, and this study

examined how periodontal disease and diabetes could affect CVD. The idea of this study was

interesting, however it does not seem adequate in the overall design. Being a longitudinal study,

it was appropriate for examining the effects of disease, but there was not a control group which

would better clarify the obtained results. Aside from the perhaps questionable aspects of the

design, the results, suggesting that periodontal disease may lead to other systemic disease but other systemic disease does not typically lead to periodontal disease, does seem probable.

The third study was worked with participants who all had coronary heart disease as well as periodontal disease. Some participants CHD was worse than others, with about fifty percent of whom had suffered a myocardial infarction. There were also control participants included in this study. The results of this candidate gene association study found that CHD and PD are genetically related by at least one locus. Of the three studies I found this one to be the most assuring in its results. I particularly liked this study because I have a difficult time with self-assessment of the participants in the other studies, creating some measure of uncertainty and possible bias about disclosed data. The design of this third study drew on advanced technology and a great deal of relatively recent knowledge about the human genome.

From this thesis I have become aware of the diversity of medical research, and the fact that such research is always open to improvement. It is great to receive grants to fund research but research will be neither efficient nor effective if its methodology is not up to date or well-designed. From the three research methodologies that were analyzed in this study I have learned that there are wholly different ways to go about research, and that these different approaches are important and necessary. Population-based studies, for example, are critical for understanding a target population. Laboratory experiments may have a better accuracy potential than surveys and might be chosen first depending on the research question and the availability of tools.

Understanding the proper use of research methodology is important to practitioners as well. As a practitioner one should be alert that potential areas for future research may be identified in regular treatment experiences. The practitioner's regular work with patients creates first hand familiarity and the motivation to address treatment problems with immediacy. Thus, it

is crucial for practitioners to consider themselves part of the research process, to understand research, and make intelligent decisions about research results based on an understanding of the forms of research methodology techniques. Undertaking this study I have become increasingly excited to start into my future as a dental practitioner, as well as a researcher! I feel I have a start toward the knowledge and problem-solving mindset I will need in my intended career.

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