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The Causative Relationship Between Periodontal Disease and Cardiovascular Disease

A thesis submitted in partial fulfillment
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by

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Abstract

Recent research on human health has revealed that the bacteria responsible for causing periodontal disease lead to the development of heart disease. With large amounts of bacterial species in the mouth, it is difficult to identify which specific strains reach the cardiac system and cause heart disease. A literature review will be done to see more specifically not only what bacteria cause heart disease, but also how the bacteria from the mouth actually invade the bloodstream and cause the inflammation in the heart's blood vessels. Investigating how these bacteria cause this disease will allow researchers to develop methods to prevent this pathogenic behavior.

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Introduction

As more information is studied about human health, researchers are finding that oral health directly reflects the overall systemic health of a person (Cullinan, Ford, & Seymour 2009; Joshipura, Hung, Rimm, Willet, & Ascherio, 2002; Dietrich, Sharma, Walter, Wetson, & Beck, 2013). While the mouth has traditionally been perceived as a separate part of the body to maintain, oral care has been deemed as less important than overall healthcare by much of the global population, and this attitude has had consequences on the rest of the body (Dietrich, Sharma, Walter, Wetson, & Beck, 2013). The cardiovascular system is one example of a region of the body that is directly affected by health conditions associated with the mouth. Periodontal disease, a bacterial infection that affects the tissue that support the teeth ultimately leading to their decay and loss, develops when a patient experiences the onset of gum disease (Schenkein & Loos, 2013). A continued duration of this disease worsens the health of the mouth until there is the development of periodontal disease, where the patient experiences bone loss underneath the teeth, and the teeth could fall out as well (Tonetti, D'Aiuto, Nibali, Donald, & Storry, 2007). The large amounts of bacteria in the mouth, combined with the severe inflammation of the gums, allows the bacteria to enter the bloodstream very efficiently. The bacteria enter the blood stream through the mouth's blood vessels (Schenkein & Loos, 2013). Once the bacteria are in the bloodstream, the pathogens immediately have access to all other major organs of the body.

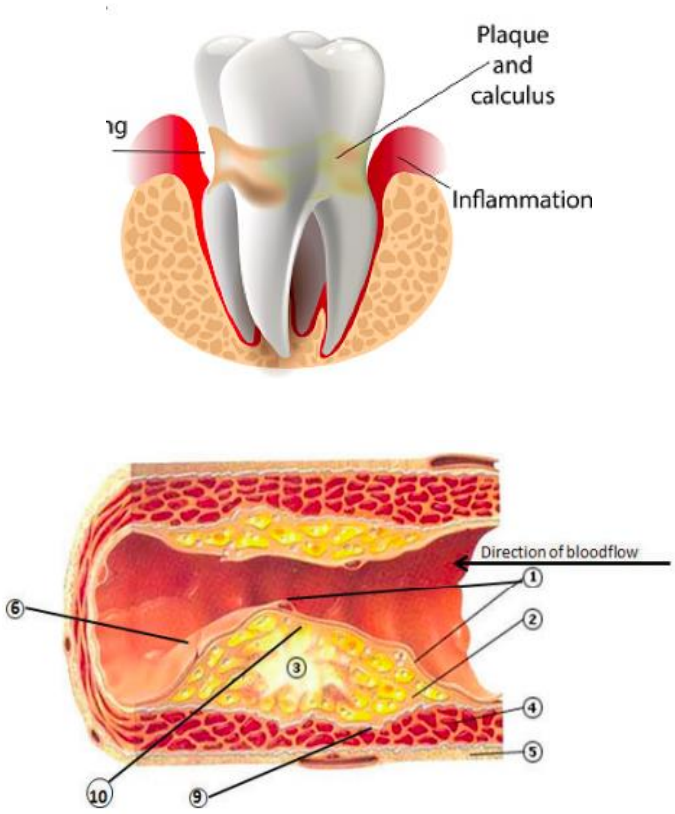

Figure 1. Comparison of the teeth's condition during periodontal disease and the blood vessel's conditions during cardiovascular disease. The tooth and blood vessel demonstrate plaque buildup.

When the bacteria make their way to the heart, they trigger inflammation of the blood vessels in and around the heart. The bacteria do this by increasing the production of cellular signals that cause specific immune cells to aggregate in the bloodstream and cause physical injury to the blood vessel. This physical injury then causes the formation of a plaque, or bacterial community buildup, which impedes the normal function of the circulatory system (Schenkein & Loos, 2013). Inflammation makes blood flow difficult throughout the body and can lead to a heart attack or stroke. For this reason, periodontal

disease has been heavily associated with causing heart disease. This discovery has potentially made it very clear that oral care is something that should be taken more seriously than it has in the past. This thesis will investigate not only which bacteria cause heart disease, but also how the bacteria from the mouth invade the bloodstream and cause the inflammation in the heart's blood vessels. This investigation will then help to answer the question of whether or not periodontal disease is not only correlative, but also causative to cardiovascular disease.

The answer to this research question will be approached by conducting a literature review of studies that examine the bacteria in the mouth that are known to be problematic with respect to causing disease. Bacteria found in the cardiac system of heart disease patients will be compared with bacterial strains found in the mouth, especially of patients suffering from periodontal disease. Once these bacterial species are identified, the pathogenic behavior of the bacteria will be investigated, to see how this behavior can promote heart disease. Along with this research, the mechanism of invasion will be investigated; more specifically, how the bacteria enter the blood stream with efficiency will be studied.

This topic is of importance in the healthcare field because identifying the root of both diseases can allow a patient with heart disease to prevent the onset of periodontal disease, and vice versa. Doing so lays the foundation on a more interconnected approach to view medicine. The body functions as a unit, and one failing component can lead to larger issues elsewhere in the body. More specifically, this finding helps incorporate the field of dentistry into the field of medicine. These two fields are often seen drastically different, with dentists sometimes referred to as "fake doctors." However, further

research into this topic could reveal that dentists have a much larger role in preventing heart disease, and potentially other systemic diseases such as diabetes, in their patients (Oberoi, Harish, Hiremath, & Puranik, 2016). Additionally, investigating this relationship would allow researchers to investigate whether there is a larger problem at hand, in that periodontal disease could cause symptoms that lead to the development of many other systemic diseases that researchers have not even considered to be connected yet.

The *Correlation* Between Periodontal Disease and Heart Disease

Effects Seen on Heart Following Periodontal Symptoms

The initial question that needs to be answered is the relationship, if there is one, between the periodontal disease and heart disease. Inflammation in the blood vessels surrounding the heart has been correlated with inflammation in the mouth of patients with periodontal disease, as will be seen in the following studies (Amar, Gokce, Morgan, Loukideli, Dyke, & Vita, 2003; Dave & Van, 2008). A correlative relationship in this case means that patients showing symptoms for periodontal disease also show symptoms for the onset of cardiovascular disease (CVD). This correlative relationship does not represent whether or not one causes the other but sets the foundation onto whether or not periodontal disease and heart disease are connected. Supporting this correlative relationship are studies with statistics that emphasize the complications following the neglect of oral health. These studies focus on why it is important to prevent the onset of periodontal disease. Periodontal disease and CVD affect a large portion of the population, with CVD accounting for half of all medical-related deaths in the United States each year, and periodontal disease affecting 75% of all individuals (Dave & Van, 2008). With

such a large portion of the US population being affected by these diseases, it becomes more important to investigate whether periodontal disease is a risk factor for cardiovascular disease. If periodontal disease can be identified as an independent risk factor for cardiovascular disease, then proper oral care could reduce the percentage of the American population with heart disease, and thus save lives.

In one dental longitudinal study done on 1,203 men, whole-mouth radiographic tests were conducted. X-rays that were performed in order to assess any serious complications such as bone loss or decay in the mouth. Special attention was paid to the alveolar bone which is the thickened area of bone in the mouth where the teeth reside. Researchers found that with every 20% increase in alveolar bone loss, the risk of death by cardiovascular disease increased by 51% (Goulart, Arman, Arap, Nejm, Andrade, Bufarah, & Dezen, 2017). This statistical finding indicates that with more progressive bone decay, causes by bacterial plaques, comes a greater risk of developing cardiovascular disease. The exact mechanism of this correlation was not investigated in the paper, however, there was a clear connection that patients with a more advanced form of periodontal disease saw a greater risk of having systemic issues involving their heart. From this longitudinal study, the researchers concluded that individuals below the age of 60 showed this correlation more strongly than individuals older than 60. They speculated that it is more difficult to assess the independent risk factors for cardiovascular disease as the individuals age. For example, it is normal for a person's heart to display signs of decreased performance past the age of 60, but it is difficult to determine whether this decreased performance is due to oral complications, or from regular daily-life activities. Additionally, the oral health of older individuals can decrease quickly due to the

accumulation of many years of decay, which ultimately lead to bone loss and the progression of periodontal disease. The statistics from the longitudinal study found that the older group of patients were the ones who were generally found with severe decay as would be expected from this gradually degenerative condition in older adults (Goulart, Arman, Arap, Nejm, Andrade, Bufarah, & Dezen, 2017). To properly assess the effects of periodontal disease on patients older than 60, more studies should be conducted on individuals older than 60 who have no major existing health conditions involving their heart. Studying the function and overall health of these younger individuals, and comparing the body's functionality with that of individuals older than 60 with heart conditions, allows doctors and researchers to see what differences there are in the heart's performance in the two groups. Researchers could then see what effects on the heart are due to normal use, and what damages of the heart are due to heart disease and the age of the individuals. This experiment would be beneficial for the age group older than 60 because that age group was difficult to assess in the previously mentioned longitudinal study.

The previously discussed studies still demonstrate a correlative finding, as no specific mechanism of causation has been addressed or uncovered from these statistics. However, the study by Goulart and colleagues also suggests that dental screenings are a helpful way of determining the susceptibility and risk that an individual is at for developing cardiovascular disease. A correlative relationship suggests that even if a mechanism of causation is not fully understood, the data is there to back up the statement that patients with periodontal disease are at a higher risk of cardiovascular disease. This screening strategy is especially useful for patients who display some risk factors for

cardiovascular disease such as obesity and diabetes. If patients are showing signs of obesity, are smokers, or have a poor diet, then they are already more at risk for developing heart disease. Therefore, to see if the patient is already approaching serious symptoms of heart complications, the quality of both the oral bacteria and the alveolar bone underneath the teeth can be screened to determine if the oral health is adding another independent risk factor to causing heart disease. The finding that poor oral health is seen in patients with higher risks of heart disease is not enough to conclude that oral bacteria can cause heart disease, therefore other journal articles and experiments must be addressed before making other conclusions pertaining to a causative relationship.

The occurrence of heart disease, strokes, and plaque buildup following the onset of periodontal disease, researchers are now confident that there is a correlative relationship between periodontal disease and cardiovascular disease. With a correlative relationship now established, attention is being directed towards there being a causative relationship between periodontal disease and cardiovascular disease. A causative relationship would provide more insight into how either disease could be prevented, because it would provide more specific details regarding the exact ways that periodontal disease could affect the body and induce the onset of heart disease.

The *Causative* Relationship Between Periodontal Disease and Heart Disease

Assessing Periodontal Disease as an Independent Risk Factor for Cardiovascular Disease

The inflammation in the blood vessels surrounding the heart demonstrates a correlative relationship between periodontitis and cardiovascular disease, but studies have

found a more causative relationship between the two (Humphrey, Fu, Buckley, Freeman, & Helfand, 2008; Joshipura, Hung, Rimm, Willet, & Ascherio, 2002).. There is not only a relationship between periodontal disease and heart disease, but periodontal disease serves as a risk factor for CVD that is independent of other risk factors associated with CVD, such as socioeconomic status, which places people at higher risk of developing heart disease (Humphrey, Fu, Buckley, Freeman, & Helfand, 2008). Other reported risk factors for periodontal disease include older age, smoking, ethnicity, high blood pressure, diabetes, and obesity, which were also considered when establishing the relationship between periodontitis and cardiovascular disease (Joshipura, Hung, Rimm, Willet, & Ascherio, 2002). Suggesting that periodontal disease is an independent risk factor means that in an otherwise healthy individual, periodontal disease alone causes measurable periodontal symptoms related to cardiovascular disease in that individual. The finding that periodontal disease is an independent risk factor makes it easier to see what risk factors of CVD are due mainly to periodontal disease.

A study found that people with periodontal disease are twice as likely to develop cardiovascular disease than people who do not have signs of missing teeth or signs of deteriorating alveolar bone (Oberoi, Harish, Hiremath, & Puranik, 2016). The same study also find that patients who are treated for a stroke are also more likely to be diagnosed with periodontal disease at the same time (Oberoi, Harish, Hiremath, & Puranik, 2016). This close correlation between periodontal disease and cardiovascular suggests that periodontal disease plays a significant role as an independent risk factor in the development of cardiovascular disease in patients. More specifically, examination of the dental plaques and levels of alveolar bone loss can be used as predictors for the

development of cardiovascular disease. Although this study claimed periodontal disease to be an independent risk factor of cardiovascular disease, it also assessed the effects of periodontal disease on other systemic diseases such as obesity, with many of these other systemic diseases having similar risk factors as cardiovascular disease (Oberoi, Harish, Hiremath, & Puranik, 2016). Thus, this could lead to some confusion of the results as to whether the damaging symptoms of cardiovascular disease are solely due to the symptoms seen in periodontal disease. Further studies that assess only cardiovascular disease should be conducted. The measured variables should be ones that only apply to cardiovascular disease exclusively in order to make a more confident conclusion on what cardiovascular symptoms can be attributed to tooth and bone loss of patients.

With there being many behaviors and diets that make individuals more susceptible to developing heart disease, periodontal disease has been more confidently associated as being an independent risk factor for developing cardiovascular disease. Finding that periodontal disease is an independent risk factors showcases how medical professionals should focus on preventing the onset of periodontal disease symptoms such as gum recession and oral plaque buildup, since plaque buildup in the mouth could potentially lead to cardiovascular disease symptoms. Specific methods for how periodontal disease can cause heart disease will now be considered.

Assessing Risk Factors of Periodontal Disease

Equally important to identifying whether periodontal disease is an independent risk factor of cardiovascular disease, is the consideration of the causes of periodontal disease. Investigating the risk factors of periodontal disease helps determine if cardiovascular disease could also be responsible for causing periodontal disease, and

would result in a bigger picture of connections between the two diseases. Identifying if either disease causes the other leads to bigger problems in finding a solution or prevention of either disease, because more factors with regards to symptoms and treatment needs to be considered for both diseases.

Not every person in the world has the same susceptibility to developing periodontal disease. It is a disease that sporadically infects the general population and does not occur by chance. Researchers have discovered that a specific subset of the global adult population is infected with the disease and it is not considered a universal disease, that has equal chance of developing in any individual regardless of current health status (Genco & Borgnakke, 2013). It is important to mention that risk factors are not synonymous with causality. Recent research on the risk factors for periodontal disease has not concluded a causal relationship with the previously discussed factors. The risk factors just described in the studies by Oberoi and colleagues are strongly associated with periodontal disease and serve as predictors or symptoms for predicted or existing periodontal infections, respectively.

In one study, adults 25-75 years of age were assessed for the health of their teeth and gums. Observed factors were the levels of tooth attachment to the gums, gingival inflammation, plaque formation, and the type of bacteria that populated the mouth of each individual. X-rays were also taken of these individuals, and the level of gum recession was observed. If the gums were receding, or in other words pulling away from the teeth, then that individual was experiencing symptoms of periodontal disease due to the bacteria damaging the gums and causing their recession (Grossi, et al., 1994). Factors that are independently associated with periodontal disease are gender, smoking, alcohol

consumption, diabetes, obesity and metabolic syndrome, osteoporosis, stress, and genetic factors.

Males tend to have a lifestyle that leads them to have a higher chance of developing periodontal disease than women. The paper by Grossi et al. found that men have a 180% greater chance of developing severe periodontitis than women. With men being more at risk than women for developing periodontitis, men could serve as an ideal target when testing preventative procedures and treatments for periodontal disease (Grossi, Zambon, Ho, Koch, Dunford, Macheti, Norderyd, & Genco, 1994). When considering gender and any of the following risk factors, studies note that any causes of periodontal disease are of concern when considering the causes of cardiovascular disease. If the causative relationship between periodontal disease and cardiovascular disease is strong, then any major risk factors for periodontal disease could ultimately lead to the development of cardiovascular disease depending on how severe the complications of periodontal disease are due to that risk factor.

Smoking is a significant risk factor for both periodontal disease as well as cardiovascular disease (Grossi, Zambon, Ho, Koch, Dunford, Macheti, Norderyd, & Genco, 1994). Although it has been well established that smoking is a cause of heart disease, researchers have also made it clear that it can independently cause periodontal disease after observing the health of the gums and plaque in individuals' mouths. One problem that is encountered when assessing smoking as an independent risk factor for periodontal disease is that other variables interfere with reaching a conclusion. Some smokers have poor overall oral hygiene where they neglect brushing their teeth. Neglect of brushing causes plaque buildup on its own, so the individuals that were assessed were

picked carefully in order to test which plaque formation and gum recession was solely due to smoking. One study with a total of 2,361 participants supported the claim that cigarette smoke alone led to the development of periodontal disease (Genco & Borgnakke, 2013). This same study confirmed a positive relationship between the levels of clinical attachment loss (gum recession), and the number of pack years. The number of pack years refers to the number of packs smoked each day for a year, so two pack years would be smoking two packs of cigarettes every day for one year. As the number of pack years increased for an individual, there was more severity experienced in the amount of gum recession and tooth detachment.

Although there are few longitudinal studies on the effects of smoking on periodontal disease, it has been confirmed that smoking has detrimental effects on the healing process of the gums following periodontal treatment (Heasman, Stacey, Preshaw, McCracken, Hepburn, & Heasman, 2006). The finding that smoking prevents proper gum healing showcases how smoking can directly cause the onset of cardiovascular disease. If a patient is experiencing severe periodontal disease, and bacteria are entering the blood stream from that wound created at the gums by the bacteria, then smoking is preventing the closing of that wound and is allowing the plaques to continue building up in the arteries of the individual.

After assessing smoking as an important independent risk factor for both periodontal disease and cardiovascular disease, treatment options can be better developed for the prevention and treatment of both diseases. In searching for literature regarding the risk factors for periodontal disease, there were no journal articles that suggested cardiovascular disease could be a cause or risk factor for periodontal disease. Current

research only suggests that periodontal disease can lead to cardiovascular disease and not vice versa. More research must be conducted on whether heart disease can cause the onset of periodontal disease. The causative relationship between the two diseases may be that only one disease can lead to the other and it is not a two-way street. If the causative relationship is strictly described as periodontal disease causing heart disease, then prevention and treatment to both diseases must begin in preventing the development of periodontal disease, since periodontal disease can lead to individuals experiencing both diseases if left untreated.

Diabetes is another condition that makes individuals more susceptible to developing periodontal disease. Periodontal disease is one condition that is commonly associated with diabetes. Patients with uncontrolled chronic diabetes were found to have an increased risk for periodontal disease, however, this relationship was concluded to be two-way in that either periodontal disease could lead to diabetes or vice versa. Periodontal disease has been found to lower sugar uptake by skeletal muscle, thus promoting insulin resistance which is known to be a characteristic of diabetes. This insulin resistance is also due to the release of inflammatory signals causes by oral bacteria. In patients that already have diabetes, the development of periodontal disease could worsen their overall health because it would promote higher sugar levels and a decreased ability of the body to store the sugar taken up from the diet (Oberoi, Harish, Hiremath, & Puranik, 2016).

Since periodontal disease has been identified as an independent risk factor, the risk factors for periodontal disease were assessed to see what symptoms should be avoided in the mouth that could eventually lead to the onset of cardiovascular disease

symptoms. In other words, if periodontal disease is a cause of heart disease, then preventing the development of periodontal disease is important in preventing some of the symptoms seen in heart disease patients. Heart disease symptoms that could be avoided include widespread inflammation throughout the entire body as well as bacterial plaque buildup within the arteries surrounding the heart.

Specific Oral Bacteria in Vascular Plaques

To successfully identify the specific bacterial species responsible for periodontal disease and the resulting systemic plaque formation, researchers first grow the strains harvested from the patients. Growth of the bacteria from patients on artificial media such as a petri dish allows researchers to have a better understanding of the role of the bacteria, both on the environment and on pathogenesis in infected individuals. The bacteria grows since it is given nutrients in the petri dish. The bacteria can be observed more accurately on a petri dish because the behavior of the bacteria allows researchers to observe the behavior that the bacteria have on the media, as opposed to what can be seen directly in the patient. The behavior resembles what is happening inside of a patient, and the observed behavior allows doctors and researchers to better assess how to prevent the damaging behavior of the bacteria. If there is any pathogenic behavior displayed by the bacteria on the petri dish, then the researchers can conclude that the pathogenic behavior on the petri dish is replicated within a living host. By observing the bacterial behavior outside of the host, researchers can examine what characteristics of the bacteria cause the symptoms of periodontal disease such as gum recession, and the symptoms of cardiovascular disease such as plaque buildup. Researchers today have been able to manipulate synthetic petri dishes to grow the abundant bacterial species found in the

mouth and blood vessel plaques of patients with both periodontal disease and heart disease (Davis, Bull, Horsfall, Morley, & Harris, 2014). Without these petri dishes, researchers would only be able to assume what kind of damage the bacteria are causing inside the blood vessels around the heart. Before the advancement of highly specialized microscopes and media, identifying the relationship between periodontal disease and heart disease would be a great endeavor. With petri dishes now a well-established research practice, bacteria in vitro (in laboratory setting) can be more readily analyzed so that bacterial growth within patients can be stopped before the bacterial colonies cause excessive irreversible damage. Not only can the specific strains inside the mouth and vascular blood vessels be identified, but the activity of the bacteria found in these locations can be studied in a controlled setting.

One bacterial strain identified using specialized media on petri dishes, *Porphyromonas gingivalis*, is the bacteria found in the mouth of patients with periodontal disease, as well as the strain found colonizing the plaque formations (atherosclerosis) found in blood vessels. This bacteria is found in all patients with periodontal disease (Cullinan, Ford, & Seymour 2009). Researchers in some studies extracted plaque samples from patients with cardiovascular disease, and grew the bacteria found within the plaque on a petri dish. A sample of spit was also placed on a petri dish to observe which bacterial colonies grew. When analyzing the two petri dishes, and after comparing the prevalent bacteria in each sample, researchers found that the *P. ginigvalis* strain was prevalent in both samples (Cullinan, Ford, & Seymour 2009). The finding that the bacterial strain was found in both samples suggests that *P. gingivalis*, which is known to populate peoples' mouths, has a method of entering peoples' systemic circulation and

eventually arriving at the blood vessels around the heart. It is at the heart where the bacteria can then cause the inflammation characteristic of cardiovascular disease. That *P. gingivalis* is found in the blood vessels suggests a causative relationship existing between periodontal disease and heart disease; however, questions remain as to whether or not the bacteria are directly responsible for causing the symptoms associated with cardiovascular disease. For example, more research must be conducted to conclude whether *P. gingivalis* causes the plaques found in the blood vessels, or whether the bacterial strain colonizes plaque buildup that was previously established from a long-term case of heart disease. These suggested experiments could be limited to the technology currently available to track bacteria within a living system such as the petri dishes used to grow them. New technology must also be used in a safe and ethical way. One way that experimental methods could be tested first in a humane way is on model animals such as mice.

Assessing the *P. gingivalis* in the mouth and in the vascular plaques helps researchers identify how closely tied periodontal disease and cardiovascular disease are. Finding the bacteria in the mouth and in the heart not only supports the correlative relationship between cardiovascular disease and periodontal disease, but it also suggests that there is causative relationship between these two diseases. The ability of these bacteria to create plaques in the mouth supports the mechanism, explained in greater detail later on, of how the bacteria can enter the bloodstream following damage to the gums and alveolar bones of the mouth.

Research Shown on Animal Models

Animal models are used to further investigate the causative relationship between the oral bacteria and heart disease. Animal models are ideal for research purposes

because models allow scientists to see the direct effects of the bacteria by manipulating not only the bacterial load, or concentration of the bacteria, but also the route of entry of the bacteria in animals. The manipulation of the bacterial administration is done on model animals such as mice. The bacterial activity observed accurately represents what would occur in humans who are either healthy, or have active periodontitis or cardiovascular disease. To assess how significantly the concentration of *P. ginigvalis* affects the onset of cardiovascular disease, mice are injected with *P. gingivalis*. The injection of this periodontal pathogen induces heart disease followed by a heart attack in the mice. Research provides evidence that signal molecules circulate within the blood of individuals, whether human or animal, and show signs of an inflammation (Joshipura, Wand, Merchant, & Rimm, 2004). Since periodontal disease and cardiovascular disease are associated with systemic inflammation, mice are also tested for inflammatory signaling molecules. Mice tested positive for many of the inflammatory signals such as C-reactive protein ,discussed later, associated with both periodontitis and heart disease (Schenkein & Loos, 2013).

More comprehensive research done on lab mice has found that endothelial dysfunction, or an abnormal health status of the inner lining of blood vessels, can lead to the development of plaque formation in the blood vessel of these mice. Healthy blood vessels have a lining that minimizes the passage of bacteria through the blood vessel walls. The blood flowing through the body stays free from pathogens and transport efficiently. However, inflammation from periodontal disease causes the lining to allow the infiltration of bacteria into the surrounding tissue. In other words, bacteria that have already entered the blood stream from the mouth then access other tissues that blood

vessels run through because the bacteria "leak out" out of the blood vessels. (Pereira, Vasquez, Stefanon, & Meyrelles, 2011).

In one experiment, several cultures of *P. gingivalis* were grown on petri dishes in preparation for an experiment on lab mice. Mice that were 18 weeks old were orally infected with the bacteria, and it was not until 30 weeks later that the mice were euthanized and their hearts harvested. The hearts and surrounding blood vessels were harvested to image the amount of plaque formation that was present. The amount of bone loss in the mouth was also assessed. Samples of the bone that supports the molar teeth (teeth most commonly subjected to the effects of periodontal disease), harvested from the mice in order to assess how much of that bone was lost during the periodontal infection period. Results confirmed that mice infected with *P. gingivalis* had greater bone loss, and greater signs of systemic inflammation than control mice that were not infected with bacteria. However, infected and noninfected mice did not show a significant difference in the size of any plaques found in the heart (Pereira, Vasquez, Stefanon, & Meyrelles, 2011).

Although there was not a significant difference in the plaque size of the two groups of mice, several variables could account for this discrepancy in different plaque sizes. The experimentally infected mice were only allowed 12 weeks for the inoculated bacteria to run their course. The bacteria may need more time to establish a high concentration of inflammatory signals within the host before a significant plaque can be detected by the imaging techniques used by these researchers. Another explanation as to why the plaques in both the experimental group and control group were of comparable size could be due to the bacteria not causing the plaque. Perhaps blood vessel plaque

formation is independent of the concentration of bacterial load, and the blood vessel plaque formation is dependent on the severity of the heart disease. If the severity of the heart disease case determines how large the plaque is, then the bacteria (regardless of the concentration) would have to take up whatever plaque-mass exists without having an influence on how much more the plaque will grow. Molecular research has found support against this possibility, but until there is an unfalsifiable connection made with regards to *P. ginigvalis* causing the plaque, the possibility that the bacteria do not cause blood vessel plaque still stands (Schenkein & Loos, 2013; Pereira, Vasquez, Stefanon, & Meyrelles, 2011).

The tests run by the researchers confirmed there was significant inflammatory signal levels within the mice; however, the concentration of inflammatory signals may need to be higher. Some inflammatory signals did not increase in concentration while others did. More research should be conducted to test the relative importance of certain inflammatory signals over others. While some signals may have a negligible effect on the disruption of the blood vessel lining, other inflammatory mediators such as C-reactive protein may have a more drastic effect on the pathogenesis of *P.* gingivalis. Testing which inflammatory signals are more important would point researchesr in the right direction with regards to which signals they should test for when determining the severity of either periodontal disease or cardiovascular disease. The appropriate species of bacteria were used, as demonstrated by the deteriorating mouth conditions of the mice. Future researchers did not doubt whether the species of the bacteria is the cause for the lack of the plaque formation in the mice. Perhaps in future experiments, the mice should be allowed a longer life-span to observe what effects the *P. gingivalis* can cause in mice

in the long term. By allowing the mice to live longer, a more accurate representation of what can happen in humans will be observed since the bacteria will be given more time to develop a potential plaque or bring inflammatory signals to lethal concentrations within the host. The formation of plaques in human blood vessels takes a significantly longer time than 12 weeks, so more realistic testing conditions will allow researchers to more confidently confirm whether or not oral inoculation of *P. gingivalis* will not only cause periodontal disease, but also cardiovascular disease. This specific research study helps to support the claim that *P. gingivalis* is responsible for the onset of cardiovascular disease, after being identified as the primary microbe responsible for causing periodontal disease (Pereira, Vasquez, Stefanon, & Meyrelles, 2011). This microbe is thus partly responsible for tying the two diseases together, and assessing the behavior and effects of *P. gingivalis* more closely may reveal more on how periodontal disease causes cardiovascular disease. A better understanding of this mechanism will further research in the both the oral care field and in the overall healthcare field.

Animal models such as mice prove beneficial due to bacteria being able to grow in this animal in a way similar to that of humans. *P. gingivalis* is able to create plaques in mice just like in humans, so researchers have successfully been able to see what happens when this bacteria creates plaques in mice, and then translate the effects onto humans in order to assess how likely it is that periodontal disease causes heart disease.

Mechanism of Pathogenesis

Further research into the mechanisms relates to how the bacteria cross from the mouth to the heart, or how bacteria in the heart makes its way to the mouth. This research

provides arguably the most direct evidence towards an existing causative relationship between periodontal disease and cardiovascular disease. Explaining, on the molecular level, how oral bacteria cross from the mouth into the blood stream and cause heart disease provides information that cannot be seen with the naked eye, and is the closest humans can currently get to directly observe bacterial behavior in a live organism. The large picture of how bacteria can cause heart disease is drawn from many studies that track, collect, and decipher data that provides a clear image of the how the blood vessels undergo inflammation and long-term health problems (Zoukel, Khouchaf, Martino, & Ruch, 2012; Dave & Van, 2008; Howell, Ridker, Ajani, Christen, & Hennekens, 2001). Actual pictures cannot be taken of the bacteria directly inflicting damage due to limits of existing technology. For example, the most powerful microscopes are only capable of taking pictures on dead tissue or cells due to the powerful yet damaging methods they use to take the image (Zoukel, Khouchaf, Martino, & Ruch, 2012). Articles on the actual mechanism of pathogenicity have been published, suggesting that there is progressive work being completed in this area and deserves further attention.

For example, one study found that rather than bacteria in the mouth causing direct damage to invade the bloodstream, the bacteria induced an inflammatory response in the body that caused the gums to be damaged, thus making it easier for the bacteria to cross the tissue and enter systemic circulation (Dave & Van, 2008). Researchers found that the endotoxin, a toxin released by oral bacteria, accumulates in the mouth and enters the bloodstream from the mouth (Howell, Ridker, Ajani, Christen, & Hennekens, 2001). In this case, it is the metabolic byproduct, rather than the actual bacteria, that enters the systemic circulation and causes inflammation, which, in turn, causes vascular injury.

It has also been found that *P.* ginigivalis can activate cells of the vascular tissues to create adhesion components that promote plaque formation around the heart (Amar, Gokce, Morgan, & Loukideli, 2003). Adhesion components are proteins that allow for the sticking and accumulation of molecules in the blood vessels. This strain of bacteria is also known to increase cholesterol in the body, and promote the deposit of fat onto the inner linings of arteries (Takahashi, Omae, Uridiales, Banci, & Oliveira, 2017). These findings are relatively brief and require the discovery of more specific details in order to find an efficient way to treat both diseases. Once a clear mechanism can be identified, steps can be taken to prevent or reverse the damage caused by the hosts' inflammatory response.

One more specific mechanism regarding the upregulation of adhesion molecules presents a clearer understanding of what happens in blood vessels of patients with periodontal disease that can lead to cardiovascular disease, or increase the risk of developing cardiovascular disease. The large amount of bacteria in the mouth of patients with periodontitis leads to systemic inflammation that upregulates the expression of the adhesion molecules in the blood vessels. Adhesion molecules allow lipids (fats) to attach to the inner lining of the blood vessel. Cells specialized to the immune system are then recruited to the surface inner layer of the artery. These immune cells have the role of engulfing, or "eating," any substance in the body that serves as a risk to the health of the host. In the case of inflammation of the sticking of fats to the arteries, the immune cells engulf the fatty area that has attached to the vessel. The ensuing reaction creates complications to the host because the cell becomes what is known as a "foam cell," which begin to calcify within the person's arteries and release even more molecules that

trigger further inflammation. Thus, the body is undergoing a cascade of inflammation that creates a larger blockage of the artery over time as more immune cells calcify into a larger mass (Schenkein & Loos, 2013; Pereira, Vasquez, Stefanon, & Meyrelles, 2011). The oral bacteria of periodontal patients have the ability to trigger an inflammatory immune response throughout the body, therefore, controlling the bacterial communities in the mouth from proliferating and prevent one risk factor for developing the plaques characteristic of heart disease.

Difficulty arises in assessing a causative relationship because there are many probable ways that a person could develop plaques in blood vessels. Genetic variation among individuals prevents the establishment of one standard way that the bacteria can induce heart disease. Although there are many potential mechanisms that depend on the individuals' genetic predisposition, multiple mechanisms can be at play simultaneously ultimately leading to formation of a plaque within the blood vessels.

To fully understand what the mechanism could be for the onset of either periodontal or cardiovascular disease, different scenarios need to be assessed. Preexisting health conditions in patients help to fully understand how the bacteria take advantage of the body to achieve maximum pathogenicity, or an ability to cause disease. In a study that involved the oral conditions of HIV positive patients, periodontal disease was assessed over a time period of about two years. In these patients, there were complications developing in the arteries of the cardiovascular system. The level of *P. gingivalis* bacteria in the mouth was also assessed. However, in patients that saw some resolution in their periodontal disease, researchers noticed an improvement in the health of their arteries and improved blood flow to and from the heart were noted (Vernon, Babineau, Demko,

Lederman, Wang, & Toossi 2011). There is still research being conducted on whether or not this causative relationship can be associated to increased virulence of bacteria in HIV positive individuals. Even if the bacteria were able to cause this damage due to their increased virulence, the pathogenic mechanism of the bacteria are more apparent due to the bactiera's behavior being amplified and easier to observe.

Treatment of periodontal disease affects the physiology of patients with CVD. In patients affected with periodontal disease, treatment therapy over a six-month period heavily improved the health and function of the tissue lining the blood vessels and arteries, suggesting that preventing and treating periodontal disease are key components in lowering the mortality rate of people due to CVD (Tonetti, D'Aiuto, Nibali, Donald, & Storry, 2007). The exact methodology of the periodontal treatment was not discussed, however it was effective at reversing some damages caused by periodontal disease. However, in patients that saw some resolution in their periodontal disease, they noticed an improvement in the health of their arteries and improved blood flow to and from the heart (Vernon, Babineau, Demko, Lederman, Wang, & Toossi 2011). There is still research being conducted on whether or not this causative relationship can be associated to increased virulence of bacteria in HIV positive individuals. Even if the bacteria were able to cause this damage to their increased virulence, it would make the pathogenic mechanism of the bacteria more apparent due to their behavior being amplified and easier to observe.

Other methods to prevent periodontal disease is by preventing *P. ginigvalis* from acquiring iron. Researchers found that by altering the ability of the bacteria to acquire iron from the environment, the bacteria became less virulent, and proliferated at a slower

rate than normal bacteria that utilize the surrounding iron with maximum efficiency (Bergman, Rosato, & Lewis, 2014). Since these bacteria cause damage to the teeth and gums by forming colonies called biofilms, treatment using specific types of light have been used to disinfect the mouth and get rid of these colonies to prevent widespread inflammation (Pourhajibagher, Chiniforush, Raoofian, Ghorbanzadeh, Shahabi, & Bahador, 2016).

Supporting the research on the bacterial invasion mechanism are experiments that track specific proteins in the body associated with inflammation. C-reactive protein (CRP) is the name of one of those proteins that increases as a sign of existing inflammation. . CRP levels increase significantly following inflammation throughout the body which allows researchers to follow the concentration of this protein in order to assess how much wide-spread inflammation is happening in the body. The levels of this protein in the blood increase dramatically following the onset of periodontal disease. This is a sign that the body is experiencing systemic inflammation that affects areas of the body beyond the mouth. Periodontal disease is related to signals that indicate blood vessels undergo inflammation, and are thus damaged (Joshipura, Wand, Merchant, & Rimm, 2004). Other findings related to CRP involve examining the number of periodontal pockets and how the periodontal pocketing severity affects the amount of CRP in the blood. Periodontal pockets are damage to the gums and underlying bones which lead to loss of tissue underneath the teeth, which ultimately leads to tooth loss if left untreated. After this examination, it was noted that an increased number of pockets resulted in higher levels of CRP, further supporting that damage in the mouth leads to an

increased level of inflammation-biomarkers in the blood (D'Aiuto, Ready, & Tonetti, 2004).

CRP is also associated with the causative relationship between periodontal and cardiovascular diseases due to the protein's ability to bind to fat molecules which are found within the blood vessel plaque. Therefore, not only is CRP's concentration significantly increased following the development of periodontal disease, but CRP is also localized within the site of vascular injury. CRP concentrations have been found elevated in periodontitis patients that are otherwise healthy, suggesting that CRP is independently associated with the decay and inflammation that follows the inflammatory response of bone loss. Along with the increase in CRP levels, there were also increased levels of proteins involved in blood clotting, and decreased levels of anti-inflammatory mediators, all of which contribute to more efficient onset of plaques in blood vessels (Schenkein & Loos, 2013).

The concluded mechanism of pathogenesis is convincing in that it specifically states how the bacteria makes its way from the mouth to the heart. The mechanism supports a clear causative relationship between periodontal disease and cardiovascular disease, and could incite other researchers to discover even more specific details of the mechanism in order to design treatment that could interfere with the establishment of *P. gingivalis* in the blood vessels of the heart, or even prevent the bacteria from establishing plaques in the mouth to prevent periodontal disease from having a chance at causing any heart disease symptoms in individuals.

Counterarguments to a Causative Relationship

Examination of counterarguments is important to assess what areas of research are either lacking, or do not support the hypothesis that periodontal disease is a causative agent to the onset of cardiovascular disease. Credible organizations and journals showcased many of the reasons that some researchers do not believe there to be a causative relationship. The International Journal of Cardiology acknowledged a claim from the American Heart Association (AHA) which stated that there was no causative relationship between the two diseases. The journal then constructed a meta-analysis of many studies in order to provide evidence against the validity of that claim. The allotted studies were performed in a way that prevented bias from the overall argument of the paper, indicating that although a major organization can make such a claim, current research is developing quickly that can quickly contradict the statement that there is no causative relationship. Symptoms of cardiovascular disease scuh as plaque formation were closely linked to the effects caused by periodontal disease. (Leng, Zeng, Kwong, & Hua, 2015). Other journals also disagree with this claim by the AHA and provide several pages of data containing measures of association that support a causative relationship between the two (Dietrich, Sharma, Walter, Wetson, & Beck, 2013). With the previously discussed journals providing a bountiful amount of information that contradicts statements by major credible organizations such as the AHA, there is clearly more attention that needs to be devoted to this research in order to make it more clear as to whether or not a causative relationship exists between the two.

Although CRP levels in the blood are elevated in patients with periodontal disease, there are some findings that point to the possibility that the high levels of CRP

may not be due to a causative relationship (Schenkein & Loos, 2013). In periodontal patients that received treatment for the disease, the level of CRP, blood clotting proteins, and anti-inflammatory mediators did not change. The lack of change between CRP, blood clotting proteins, and anti-inflammatory mediators could either be due to these protein levels being independent of the amount of decay in the patient, or it could be that the response to the protein level changes varies among individuals, and some patients will not show changes to the proteins following treatment for periodontal disease (Schenkein & Loos, 2013). However, there is substantial evidence that does point away from the statement that periodontal disease and cardiovascular disease are independent diseases that do not influence one another.

Counterarguments to their being a causative relationship between periodontal disease and cardiovascular disease help because they provide researchers with more research questions. Any claims against there being a causative relationship demonstrate what areas of research require more work in order to show organizations such as the AHA that periodontal disease can cause heart disease due to widespread inflammation.

Directions for Future Research

A More Integrated Healthcare Field

Researchers interested in the healthcare field, as well as healthcare providers would be interested in finding a causative relationship between periodontal disease and cardiovascular disease because finding a causative relationship between the two diseases would create more collaboration among medical professionals. A larger conversation

would take place between dentists and physicians as both fields provide information to the other on the potential risk factors to either periodontal disease or cardiovascular disease. Physicians would also be more aware, and thus advise patients with more effective advice on how they can take care of their bodies to prevent widespread illnesses. Finding a causative relationship between periodontal disease and heart disease would allow a more holistic approach to establishing a healthy body. Understanding that all of the body's organs are more interconnected than previously thought, allows physicians to diagnose illnesses more efficiently and accurately as warning signs for some diseases that are detectable and foreseen by preexisting illnesses. The finding of a causative relationship suggests a reason for dental and medical care to be more integrated, since these two areas of the human body really do play a larger role on each other than previously thought. If periodontal disease leads to the onset of cardiovascular disease, it may be that the systemic inflammation caused by bone loss and bacterial infiltration can lead to serious illnesses other than cardiovascular disease. Seeking the answer to the initial research question on whether there is a relationship between the two diseases, may lead researches to find that periodontal inflammation is actually much worse than causing only cardiovascular disease.

A More Educated Global Population

More educated healthcare providers can lead to a more educated general population on the importance of oral health. While today the general population may see brushing their teeth as an inconvenience with no life-threatening implications if brushing is neglected, this research could change that opinion if the public does get the education. The public's overall health would then improve if the advice is taken, due to less

systemic inflammation being present throughout the body. However, before an effort can be made to educate the public on the benefits of maintaining proper oral care, the causative relationship between periodontal disease and cardiovascular disease must be confidently established, with the research pointing towards an undeniable connection.

Investigations into More Connections Between Diseases

The details of this paper that refer to there being a causative relationship between cardiovascular and periodontal disease could also incite research to find causative relationships between other diseases involving inflammation that do not necessarily affect the mouth or heart. Knowing that systemic inflammation can tie two diseases together opens the door to making connections between any diseases that are affected by inflammation, including cancer. Any useful methods for tracking the progress of inflammation that were discussed in this paper can also be translated to track the progress of inflammation in other diseases. In other words, inflammation with regards to periodontal and cardiovascular disease can be studied to see how this inflammation can affect the body in ways that causes the development of other diseases such as diabetes. Overall, the results found in this paper will support the specific expectation that the onset of periodontal disease affects the vascular health of the heart, thus promoting heart disease. However, it would also support the more generalized concept that many other diseases allow the progression of other diseases within the same person, made possible by systemic inflammation.

Studies have found that periodontal disease is linked to other systemic diseases including diabetes and pulmonary disease (Oberoi, Harish, Hiremath, & Puranik, 2016). Several patients were evaluated, each of which either had cardiovascular disease,

diabetes, or pulmonary disease. When the total amount of tooth loss and bone loss was assessed, researchers found that depending on the severity of the periodontal infection, different systemic diseases arose, with the most severe form of tooth and bone loss leading to pulmonary disease. This study indicates that with variable degenerative forms of periodontal disease, comes the possibility of developing multiple systemic diseases depending on how long the patient was experiencing the varying degrees of periodontal disease. For example, if a periodontal disease patients was missing 4 teeth, then they would be more susceptible to developing diabetes with that relatively low level of tooth and bone lone (Oberoi, Harish, Hiremath, & Puranik, 2016). If the patient was to develop diabetes, and then have more degeneration of their teeth, then the patient would be more susceptible to developing other systemic diseases such as cardiovascular disease or pulmonary disease.

Although much of the global population is not accustomed to hearing about severe cases of periodontal disease leading to the development of these systemic diseases, the possibility remains that periodontitis could lead to diseases that are more recognized as serious. It is possible that developing countries with less education on this topic could have more cases where periodontal disease is leading to the development of these diseases, however, the technology of these countries leaves them oblivious to the connection between systemic diseases. The lack of existing technology could especially be true for countries where oral health is not taken as seriously as more developed nations like the United States, because developed countries may be more focused on preventing more serious and deadly diseases such as HIV or tuberculosis. Although periodontal disease is not as terminal or serious as HIV, periodontal disease is still a disease that can

have serious long-term implications in the lives of people that neglect oral health. The mouth truly is the doorway to the rest of the body, and the health conditions of the mouth can be a direct reflection of the overall health of a person. By establishing a deeper and more confident relationship between periodontal disease and cardiovascular disease, more research will be conducted on the effects of periodontal disease on other systemic conditions, thus leading to possible treatments of periodontal disease that could prevent those other systemic diseases tied to it.

A confident conclusion that periodontal disease can cause cardiovascular disease would have many implications in the healthcare field due to its effect on the general public. Better cooperation between healthcare providers results in patients that are more educated on how to prevent the onset of cardiovascular disease by taking care of other parts of their health such as their oral health. Collaboration among healthcare professionals would therefore help doctors realize that prevention of one disease such as periodontal disease could result in many other diseases, such as heart and pulmonary disease, being prevented in patients.

Conclusion

Although there has been much emphasis on researching whether there is a causative relationship between periodontal disease and cardiovascular disease, there is still more research that must be conducted to confidently see the direct relationship between them. Some research, such as journals discussing the pathogenic mechanism of *P. gingivalis* do well in suggesting a detailed causative relationship between periodontal disease and heart disease; however, the described mechanism is not enough to fully

establish a causative relationship that researchers would be able to study in order to prevent the onset of either disease. Current research has been able to effectively describe observations following experimental manipulation, however not many have suggested a possible treatment or prevention method to. A prevention method has not been suggested by many researchers because of the uncertainty still associated between periodontitis and heart disease. The journal articles that do point to a causative relationship require more specific information to be known about the mechanisms that the bacteria in the mouth use to cause disease in the heart. This research can be tedious because it requires meticulous microbiological tools that may not be well developed yet, such as being able to directly observe the bacteria with a microscope during an active infection in a live organism. Technological limits prevent researchers from knowing what specific step in the infection cycle must be stopped or treated to maintain healthy conditions in a person.

Research methods that can tag and track specific oral bacterial communities could prove useful because then researchers will be able to see what route the bacteria are taking and at what speed they are arriving at the blood vessels. The speed at which disease can develop in the heart is important because it can demonstrate how lethal and serious the oral bacteria are in terms of their blood vessel destruction. Previously discussed articles have suggested that current experiments require a longer time-span for the research to effectively demonstrate what effects bacteria can have on the human body such as plaque formation around the heart (Pereira, Vasquez, Stefanon, & Meyrelles, 2011).

Unanticipated findings from this research include counterarguments from the American Heart Association. A credible organization like the AHA claims that there is no

causative relationship between periodontal disease and cardiovascular disease (Leng, Zeng, Kwong, & Hua, 2015), thus emphasizing how misunderstood the exact mechanism of pathogenicity is. However, studies by journals that help to invalidate some of these counterarguments are reassuring in that they provide evidence that there is a causative relationship after all. Organizations such as the American Heart Association may need more information and proof of a causative relationship before making a statement on their website suggesting it. Making a statement that lacks enough support would destroy the organizations credibility, thus this move by the AHA in making the statement, further points to the idea that more research must be performed and more data must be retrieved before the causative relationship between periodontal disease and heart disease can be seen as a fact.

Although there is a well-established correlative relationship between periodontal disease and cardiovascular disease, a definitive causative relationship is yet to exist. Previously described studies demonstrate how it is likely that there is a causative relationship between the two diseases. The studies discussed in this paper support the concluded mechanism the bacteria uses to enter the bloodstream, and supports that severe periodontal disease causes plaque buildup in the blood vessels around the heart characteristic of heart disease. However, technological and research limitations prevent researchers from arriving at an unquestionable explanation as to how oral bacteria are directly responsible for the onset of periodontal disease. The two groups of people, those that believe in a causative relationship and those that do not, are divided because there is still a lot of undiscovered information pertaining to the significant effects periodontal disease could have on cardiovascular disease. More research that investigates unexplored

ideas in this topic, or supports currently existing arguments, will only advance scientists towards fulfilling the answer to the research question on whether or not periodontal disease can directly lead to cardiovascular disease.

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