PANCREATIC CANCER AND THE ORAL MICROBIOME

An Undergraduate Research Scholars Thesis

by

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We, Joyce Atkens and Vianec Olvera, certify that all research compliance requirements related to this Undergraduate Research Scholars thesis have been addressed with my Research Faculty Advisors prior to the collection of any data used in this final thesis submission.

This project did not require approval from the Texas A&M University Research Compliance & Biosafety office.

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ABSTRACT

Pancreatic Cancer and the Oral Microbiome

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Pancreatic Cancer (PC) is among the most difficult cancers to detect in its earliest stages when treatment is most effective. Factors that may contribute to PC include periodontal disease, smoking, chronic diabetes, obesity, immune factors, poor diet, chronic inflammation of the pancreas, gender and age. Understanding the relationship of oral pathogens due to periodontal disease may help reduce the risk for PC. In this research, we focused on bacteria that is associated with periodontal disease. Periodontal disease is one of the most common inflammatory diseases that affects the human body and is possibly correlated to PC. The oral cavity contains numerous bacteria including *Porphyromonas gingivalis* (*P. gingivalis*),

Aggregatibacter actinomycetemcomitans (*Aa*) and *Fusobacterium Nucleatum* (*F. Nucleatum*). *P. gingivalis* and *Aa* have the ability to penetrate in two different pathways into the pancreas. Firstly, oral bacteria can penetrate the gingival and alveolar tissue and reach the bloodstream. Lastly, oral bacteria that is swallowed can travel via gastrointestinal tract to the pancreas. These bacteria work in conjunction with each other and initiate an inflammatory response in the pancreas leading to the increased risk of PC. It is essential for dental healthcare professionals (DHP) to educate the public regarding oral risk factors such as periodontal disease that may increase the risk for PC. Preventing oral disease by encouraging good oral hygiene and the management of periodontal disease through non-surgical periodontal therapy by DHP may reduce oral pathogens and decrease the risk for PC. The DHP can make a difference by educating the public about the risk factors correlating to PCs. Further research into better early screening methods should include salivary markers and oral microbiome population as possible indication of early PC.

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INTRODUCTION

What will it take to end cancer? The American Cancer Society employs many biomedical scientists and spends billions of dollars on research to answer this question. Pancreatic cancer (PC) is among the most difficult cancers to detect in its earliest stages when treatment is most effective. Cancer research, notably research into testing the oral microbiome, is of particular interest to dental healthcare professionals (DHP's). The oral systemic link is a well-known area of research, particularly among dental healthcare professionals. How the oral microbiome may influence the risk for PC is the focus of our research. This subject aligns with the ADHA research objectives interprofessional education and oral health prevention.

The intent of this research is to educate the DHP about PC, discover oral bacteria that may be related to PC and lastly, identify risk factors and oral health intervention that may be performed by the DHP to reduce PC risk.

1. RECOGNIZE PANCREATIC CANCER SIGNS AND SYMPTOMS

Pancreatic Cancer (PC) begins in the pancreas, an organ lying posterior to the lower part of the stomach.[1] The pancreas is responsible for releasing enzymes that aid digestion and hormones such as insulin that help regulate the human metabolism of sugars.[1] A clear sign of cancer is when the cells in the body mutate in their DNA, and this leads to an uncontrolled growth resulting in tumor formation.[1] There are two categories of PC, the exocrine and the endocrine. Exocrine PC affects the cells that line the duct of the pancreas; it is recognized as pancreatic adenocarcinoma.[1] The pancreatic endocrine type which is known as pancreatic neuroendocrine is the hormone producing cells of the pancreas. Each of these categories has its own types, symptoms, and prognosis. Adenocarcinoma, the exocrine PC, is the most common type of PC and is usually detected in the late stages of the disease.[1] This research will focus more on the common exocrine type of PC.

The American Cancer Society estimated in 2019 that 57,000 people were diagnosed with PC in 2020, and about 47,000 died, of the same disease.[2] The average lifetime risk of PC in the United States is about 1 in 64.[2] In the United States, exocrine PC accounts for three percent of all cancers and seven percent of all cancer-related deaths.[2] This type of cancer is more common in men than in women and is seldomly discovered in the early stages when it is most curable.[2] The disease slowly metastasizes to other organs, and that is when the cancer signs and symptoms are revealed.

Exocrine PC may exhibit symptoms that include but are not limited to fatigue, blood clots, the skin becomes yellow, and the eyes turn white (also known as jaundice) light colored stools and dark colored urine, abdominal pain that radiates to the back, loss of appetite that

causes weight loss, and pruritis.[1] Confounding symptoms may result in a misdiagnosis of diabetes or an inability to maintain a current diabetic condition. As the disease progresses many complications can appear, such as weight loss. This happens because as the tumor grows it puts pressure in the stomach and effects the eating process. Also, the digestion of enzymes is disturbed, making it difficult for the body to process the food nutrients.[1] Another complication is jaundice, and this occurs when the cancer cells grow and obstruct the liver's bile duct.[1] Furthermore, as PC grows it puts pressure on the nerves that innervate the abdominal region, resulting in severe pain.[1]

The Mayo Clinic recognizes several risk factors for PC. Modifiable risk factors include smoking, chronic diabetes, obesity, and chronic inflammation of the pancreas. Non-modifiable risk factors include gender and age.[1] According to the American Cancer Society, men are twice as likely to be diagnosed with PC than women, and there is an increased risk for people over the age of sixty-five.[2] Generally, physicians agree that any combination of these modifiable and non-modifiable risk factors may increase the risk for PC.[2]

The oral cavity contains numerous species of bacteria, and the presence of different types of bacteria in the oral cavity is associated with periodontal disease.[3] Due to the inflammatory process of periodontitis, systemic conditions such as PC has been linked. Periodontitis is among one of the most common inflammatory diseases that affects the human body.[4] According to the Center for Disease Control, 47.2% of adults over 30 years suffer from some type of periodontal disease.[4] Also, with age, the risk increases to 70.1% of adults over 65 are diagnosed with periodontal disease.[4] Similar to PC, periodontitis mainly affects men (56.4%) rather than women (38.4%) and smokers (64.2%).[4]

It has been reported that periodontal disease affects 90% of the world population.[5] This shows that periodontitis is a very common disease and understanding its association with PC may impact a patient's overall health. For many years, the pancreas was considered to be free of pathogens because it contained alkaline solution.[6] In 2013, Michaud found that microorganisms may reach the pancreas through blood and the digestive system.[6] The presence of oral pathogens in the pancreas may disturb the alkaline environment and initiate an inflammatory response within the pancreas.[6]

The body's inflammatory response to periodontal pathogens may influence the pancreas regulation of blood sugar and increase the risk for PC. Diagnosing PC in its earliest stages is particularly challenging. Diagnosis and treatment of periodontal disease and removal of periodontal pathogens may assist in reducing the risk for PC.[7, 8] In order to help with a better outcome of this disease, oral health habits based on patient knowledge, attitudes, and practices needs to be promoted.[8]

2. DISCUSS PERIODONTAL PATHOGENS ASSOCIATED WITH PANCREATIC CANCER

Biofilm accumulation of microorganisms is what results in the formation of pellicle on the teeth and serves as a source to initiate inflammation in the mouth. Progression of gingivitis, which is an inflammation of the gingival tissue, is what leads to periodontitis. In the Journal of Oral Microbiology by Olsen et. al study showed there is a relationship between inflammation and the incidence of pancreatic cancer.[10] Research indicates there are specific oral microbial bacterium that activates an immune system response.[7] Oral microbial bacterium has the ability to travel through GI tract and the oral cavity by penetrating into the alveolar bone reaching the bloodstream.[7] The oral environment is very important when it comes to human health. Oral microbial bacterial attach to epithelial cells within the gingiva and disrupts their metabolism.[7] It is very important that the number of oral pathogens are reduced in order to maintain oral environmental balance.[7] An imbalance of the oral environment could potentially change the body's immune response to stimulate and secrets the inflammatory mediators which could initiate PC.[7]

There are several oral microbiome bacteria associated with periodontal disease, but this research will mainly focus on *Porphyromonas gingivalis (P. gingivalis)* and *Aggregabacter actinomycetemcomitans* (Aa). The presence of *P. gingivalis* and *Aa.* destroys the periodontium, and this may initiate the risk of PC. In a 2013 study conducted by Michaud et al., 405 individuals with pancreatic cancer levels of antibodies were tested against *P. gingivalis*, and these levels are higher compared to the healthy individual antibody levels.[7,9]

According to Henderson et al., 2010. *Aa.,* a gram-negative non-motile facultative anaerobe bacterium, has the potential to travel into the host cells by endocytosis. In the host cell, it releases phospholipase C, which damages the membrane vesicles and let go of themselves ending up in the cytoplasm.[6] There is a growth and division after this vigorous process, whereby anchoring to the host cell membrane.[6] *Aa,* then infiltrates the adjacent epithelia cells or deep cells through microtubules.[6] This process enables *Aa.* to infect deep cells and escape from the immune system.[6] *Aa*. is capable of releasing a toxin known as Leukotoxin (LtxA), a lipoprotein that attaches to neutrophils, monocytes, and lymphocytes.[6] The end results is forming pores on the cell membrane, thereby changing and altering its function of osmotic homeostasis, leading to cell death.[6]

According to Fan et. al., *P. gingivalis* was considered as having the ability to invade the immune system and damage the signaling pathways through cytokine and receptor degradation. *Aa*. on the other hand, is capable of initiating the Toll-like receptor (TLR) signaling pathways, which is crucial in promoting PC.[1,7] In 2018, a prospective experimental study conducted by Fan et al., demonstrated the relationship between PC and oral microbes by testing saliva mouth wash samples collected from participants. Oral mouth wash samples were collected from 361 men and women with PC and compared with 371 healthy individuals.[10] The mouth wash samples were characterized with *16S rRNA* gene sequencing. A group of organisms were evaluated based on the phylogenetic similarity of the bacteria abundance, the presence or absence using operational taxonomic units (OTUs). The participants were monitored very closely for nearly a decade between subjects to determine who developed pancreatic cancer.[7,10] Participants with *P. gingivalis* in their oral microbiome had a 59% higher risk of developing PC than those

without.[7,10] Individuals with *Aa.* had at least 50% higher risk of developing PC.[7,10] The results indicated *P. gingivalis* and *Aa.* are associated with an increased risk of PC.[7,10]

Also *P. gingivalis* and *Aa*. may work in concert to increase the risk of PC. The process known as the bacteria driver passenger model explains the pathogenic process. Initial bacteria, in this case *Aa*., damages the DNA of the host cell. The rapid changes in the host cells microenvironment created by *Aa*. result in an increase for *F. nucleatum* to create a more stable ecosystem for the pathogens. Consequently, the formation of non-pathogenic "passenger" bacteria like *F. nucleatum* acts as a bridging organism between cancer cells and *Aa. Aa.* is active in the early inflammation response, and as the disease progress, *P. gingivalis* hinders the cancer cells apoptosis and promotes tumor development.[6]

This combination of periodontal pathogens may play a role in increasing the risk of PC.[6] Thus, the different microbial ecosystem is stable and more toxic in their environment.[6] This could be one of the significant factors in potentially resulting in PC.[6] Early detection of *P. gingivalis* and *Aa*. along with treatment of periodontal disease may help reduce a patient's risk for PC.[6]

3. IDENTIFY DENTAL HEALTH PROFESSIONAL'S ROLE IN REDUCING ORAL HEALTH RISK FOR PANCREATIC CANCER

Dental Health Professional's (DHP's) role in preventing disease in the oral environment is by conducting a non-surgical periodontal therapy. This therapy consists of the removal of dental biofilm and calculus from the oral cavity by scaling with hand scalers, curettes and/or the use of ultrasonic instrumentation.[12] These instruments will help in the removal of supragingival and subgingival biofilm and calculus and altering oral microbiota. Patients who suffer from chronic periodontal disease might require surgical periodontal therapy by DHP's. This consists of an open flap debridement in which part of the gingival is surgically separated to visibly access subgingival and debride lesions by reducing biofilm and calculus accumulation in deep periodontal pockets.[12] The American Dental Association recommends periodontal disease to be treated by reducing inflammation, arrest disease progression, and provide patient with oral hygiene instructions such as brushing two times daily for 2 minutes with a soft bristle brush, cleaning interproximal with dental floss, using toothpaste that contains fluoride and professional prophylaxis at follow up visits this will help in maintaining good oral hygiene.[12]

Preventing oral disease by encouraging good oral hygiene and the management of periodontal disease by reducing oral pathogens such as *P. gingivalis* and *Aa* will mitigate the risk for PC.[11] Since the absence of oral pathogens will not initiate an immune response, this will reduce the risk for the development of inflammation in other parts of the body, such as the pancreas.

In a cross-sectional study by Farrell et al., a method that can potentially be used in the future for the detection of periodontal pathogens that can be linked to the increased risk for PC is

microbial gathering using the Human Oral Microbe Identification Microarray.[3] This study was conducted to demonstrate the variations of salivary microbiota and the association between oral disease and the increased risk of PC.[3] In this study, all subjects were gathered from the UCLA Medical Center where 103 saliva pellet samples containing 38 PC, 38 healthy control, and 27 chronic pancreatitis samples were selected.[3] This study had a discovery phase and a verification phase to an independent validation phase.[3] 20 pellet samples with salivary microflora were profiled by the HOMIM array, 10 patients with PC and 10 healthy control subjects.[3] Following, an independent sample set was used including 27 patients with chronic pancreatitis, 28 patients with PC and 28 healthy controls for the biomarker validation phase using a qPCR test.[3] The outcome of the study showed a significant variation of microflora in the saliva of patients with PC(n=10) in comparison to healthy control subjects(n=10).[3] 31 species/clusters were shown in the saliva pellets of patients who had PC in contrast healthy control subjects(n=10) 25 species/clusters were less.[3] These species/clusters shown in the saliva pellets were from the *Firmicutes, Proteobacteria, CFB group bacteria, and Actinobacteria.*[3] In the independent validation bacterial biomarkers phase, 2 microbial biomarkers, *N elongate and S mitis*, demonstrated a significant difference in patients with PC and healthy control ($p<0.05$, $n=56$).[3] This indicated that the levels of these bacterial biomarkers were less in a patient with PC(n=28)than in healthy control subject(n=56)($p<0.05$).[3] This study demonstrated that profiling saliva and the detection of biomarkers in saliva can result in a credible tool for the early detection of cancer and improve survival rates for PC.[3] In addition, DHP's can use this method in order to detect oral pathogens and inform patients about the increase risk for PC.

Modifiable risk factors such as smoking, diabetes, and obesity may also trigger PC and it is essential to inform patients of the risk for cancer if these factors are not changed or controlled

in addition to poor oral hygiene.[1] For example, DHP's can educate the patient of the increase risk for smoking and provide information about tobacco cessation which may reduce the risk for disease. Also, educating the diabetic population about the importance of having a controlled blood sugar and its association to periodontal disease can help reduce the risk for PC. Another important point is to promote healthy eating habits for the overweight population such as a diet full of fruits and vegetables and whole grain meals, which may help reduce the risk for PC.[1]

The importance of creating awareness by educating the DHP's about pancreatic cancer and its association to oral health may lead to the prevention of PC. Since periodontitis is linked to increase risk for pancreatic cancer, the dental profession should take responsibility to inform and educate patients about the risk. Further research is essential to learn about more screening methods for the early detection of pancreatic cancer.[7]

CONCLUSION

PC is a fatal disease that is hard to diagnose since cancer cells metastasizes to other organs and that is when the cancer signs and symptoms are revealed. One of the factors that contributes to increase risk to PC is periodontal disease. By understanding the relationship of oral pathogens in periodontal disease such as *P. gingivalis* and Aa this may mitigate the risk for PC. In addition, recognizing modifiable risk factors such as smoking, diabetes, periodontal disease, obesity and non-modifiable risk factors such as age and genetics may help in the prevention of the development of disease. The DHP's may play a vital role in decreasing the morbidity and mortality rate from one of the deadliest cancers by providing non-surgical periodontal therapy, using potential tools such as saliva microbial profiling, tobacco cessation, controlled diabetes's, educating and promoting good oral hygiene as well as recommending well balanced diet may reduce the risk for PC. Further research is needed to identify additional risk factors, in order to recognize high risk individuals for PC and screening methods for the early diagnosis and management of this disease.

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