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Microbial alterations of oral cavity and their association with Pancreatic Cancer

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Abstract

Introduction: Pancreatic Cancer (PC), while relatively infrequent, remains one of the deadliest cancers as a result of late diagnosis and absence of preventive measures. Over 50 % of patients diagnosed with PC already suffer metastasis. Combined with lack of effective treatment, the need arises for universal biomarkers specific to PC. In the last decades the importance of human microbiota in its upholding of the body homeostasis has been under radar, in special regards to its association to cancer. Several species of oral microbiome have been linked to increased or decreased risk of developing pancreatic cancer, most notably *P.gingivalis* and *Fusobacteria.*

Purpose of the study: The aim of this study is to collect and summarize existing evidence on connection of oral microbiome and pancreatic cancer, as well as to assess their potential use in diagnostics of PC .

Material and method: Literature review was performed, in English databases, using keywords : cancer, pancreatic cancer, pancreatic cancer prevention, oral microbiome, microbe variation, microbial biomarkers.

Results and conclusions: In our systematic review, evident differences between microbial architecture of PC patients and healthy individuals were observed. The data on association between microbiota and risk of developing cancer is limited, however it still provides evidence for relationship between microbial composition and incidence of pancreatic cancer.

Keywords : cancer, pancreatic cancer, pancreatic cancer prevention, oral microbiome, microbe variation, microbial biomarkers.

Introduction and purpose

Pancreatic Cancer (PC) is 7th most common cause of cancer death worldwide for both sexes, whilst ranked as the 14th most common cancer in the world, as of 2020 [1] . This discrepancy between incidence and mortality highlights poor survival rates. Furthermore increased incidence of PC over the past few years, paints a grim picture for patients worldwide. PC is prone to show little to none specific symptoms in early stages of development, up until it grows beyond control and spreads around the body. The term 'pancreatic cancer' is an umbrella term which pertains to several pancreatic tumors, although it is most commonly used to describe pancreatic ductal adenocarcinoma (PDAC). PDAC represents 85 % of all cancers located in the pancreas[2] .The main obstacle in the treatment of PC is late diagnosis, with more than a half of the patients being diagnosed while already suffering metastasis, whereby cancer cells are more invasive. At present, 5-year survival rate for patients with PC is below 10%[3], although on average patients die within 5-8 months of a diagnosis. The only chance for curative treatment is surgical removal of the tumor, for which only a small percentage of patients qualify (10%- 20 %). It is also not rid of long period of recovery, post-operative morbidity and cure rate of 25 % [2]. Following this curative scheme does little to improve survival of patients, given that 80 % of individuals after resection eventually die of disease. Other forms of therapy, such as radiotherapy or chemotherapy can be beneficial after resection of the tumor, however pancreatic cancer has shown resistance to both[4]. Combined with lack of specific early symptoms, retroperitoneal location of the pancreas protects developing cancer from detection.

At this time the efforts taken on increasing efficacy of treatment of PC are focused on prevention, screening and early detection [4]. In addition to non-modifiable risk factors (age, sex, family history, genetic susceptibility, blood group,) associated with PC, there is a number of modifiable factors such as smoking, alcohol consumption, type 2 diabetes and oral health, of which natural microflora of the oral cavity especially fell under the scrutiny of researchers[4].

Human microbiota consists of various forms of microorganisms, colonizing vast surfaces of the human body such as skin, oral cavity, respiratory tract, gastrointestinal tract and urogenital tract[3]. Composition of microbiome is varied depending on age, diet and dietary habits. Oral cavity (OC) is a habitat for over 700 species of bacteria, which are collectively referred to as oral microbiome. Microbiome population varies depending on inhabited surface of oral cavity, and includes both pathogenic and mutualistic bacteria[5]. Various habitats of OC include periodontal pockets, surface of teeth, buccal mucosa and tongue, which displays the highest diversity of microorganisms[6]. Any change in environmental conditions of OC facilitates the growth of pathogenic bacteria and increases their potential to create oral diseases. Dysbiosis of OC has been associated with local and systemic diseases including periodontal disease, dental caries and oral cancer, diabetes, rheumatoid arthritis and pulmonary disease[5]. Recent data shows a link between alterations in oral microbiome and development of cancer, most notably of cancers located in the gastrointestinal tract and oral cavity [5]. There is also compelling evidence from various studies on the correlation between oral bacteria and etiology of colorectal and pancreatic cancer[5, 6, 7, 8]. Out of numerous diagnostic markers of cancer, none is sufficiently specific to facilitate early diagnosis of PC. Lack of effective means of diagnosis paired with expeditious development of this disease is

reflected in morbidity of this cancer. Thus, the aim of this study is to gather recent findings and provide comprehensive summary of current state of knowledge about association of human microbiota and oral health to pancreatic cancer.

Methods:
We carried out literature search using English based databases (PubMed, Springer, The Lancet) looking for articles referring to pancreatic cancer, oral health and microbiota, favoring more recent works. Keywords used : "microbiome", " oral bacteria", "oral microbiota", "mouth diseases", "pancreatic cancer", "cancer". Recent Studies (from 2010 to 2022) matching this criteria were reviewed, and those most relevant were picked for our summary.

Results:
Human microbiome is presumed to trigger various immune responses associated with cancer growth, as it was observed in numerous studies, most notably with Helicobacter pylori playing a role in pathology of gastric cancer[9]. Similar connection has been observed between carriage of populations of *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* and *Fusobacterium nucleatum* in the oral cavity and incidence and development of PC [5,7,10]. *P.gingivalis* and *A. actinomycetemcomitans* are pathogens primarily involved in triggering of periodontal disease and tooth loss, and those two afflictions are directly linked to development of PC[5].

Study performed by Gnanasekaran *et al.* 2020 demonstrated that direct exposure of *P.gingivalis* on PDAC cells, induced their proliferation [11]. They established that those pathogens thrive in hypoxic condition and infection of cancer cells with *P.gingivalis* was related to enhanced growth of PDAC cells in vivo.
Most of existing clinical studies tend to substantiate the association between the state of oral microflora and a risk of pancreatic cancer. In 2018 study performed by Fan *et al.* oral wash samples carrying *P.gingivalis* and *A.actinomycetemcomitans* were linked to increased risk of pancreatic cancer[5]. It was also evaluated that increased carriage of *Fusobacteria* and its genus *Leptotrichia* is connected to decreased risk of PC. It is worth mentioning that the study was adjusted for risk factors for pancreatic cancer such as age, race, sex, BMI, history of diabetes, smoking and alcohol consumption.

Correspondingly, risk of developing PC was also observed by Michaud *et al.* to increase two-fold in population with high levels of antibodies against *P.gingivalis* [10] in comparison to individuals with low levels of those antibodies . Furthermore, they observed 45 % lower risk of developing PC in patients with consistently high levels of antibodies to commensal oral bacteria in the serum.

It is worth mentioning that 2020 comparative study conducted by Wei *et al.* provided evidence for correlation between high abundance of *Streptococcus* and *Leptotrichina* and the incidence of PDAC, while simultaneously *Veillonella*, *Fusobacteria* and *Neisseria* populations increased in healthy control group[13]. This is contradictionary to findings of Fan *et al.* which found Leptotrichia to be elevated in control group.
Furthermore, Vogtmann *et al.* Found *Enterobacteriaceae*, *Lachnospiraceae G7*, *Bacterioidaceae* and *Staphylococcaceae* to be associated with increased risk of PC in Iranian population [14]. Those results differ from the ones obtained on Western and Chinese populations, which may point to existing variability in microbial population depending on specific place of residence of microbial host.

Torres *et al.* in the study of 108 patients (8 with diagnosed PC, 78 diagnosed with other diseases including cancer, and 22 healthy control group) observed high levels of *Leptotrichia* in pancreatic cancer group [15] similarly to Weit et al, and on the other hand lower levels of *Porphyromonas* and *Neisseria* in patients with PC. However, clear distinction between patients with PC and the other participants was observed, in contrary to similarities between microbial populations of healthy patients and patients suffering from different diseases than PC [15] . This highlights the possible importance of oral microbiota in regards to detection of pancreatic cancer.
Significant distinction between the architecture of oral microbiome of healthy patients and PC afflicted group was observed by Ferrel et al. In their study elevated risk of pancreatic cancer was associated to *Gemella adiacens*, while *Neisseria elongata* and *Streptococcus mitis* were vice versa decreased in PC patients compared to healthy control [16].

Table 1. Association between alterations of oral microbiome with Pancreatic cancer, summary of clinical trials

Author	Participants	Control group	Results	Location, year
Fan *et al.*	I cohort : 170 PC, 170 controls II cohort: 191 PC, 201 controls	Matched, Consisted of healthy participants	High abundance of *P.gingivalis* and *A.actinomycetemcomitans* were associated with increased risk of PC, *Fusobacteria* connected to decreased risk of pancreatic cancer	United States,(2018)
Michaud *et al.*	405 PC and 416 controls	Matched	Patients with high level of antibodies against *P.gingivalis* had double the risk of pancreatic cancer, than patients with low levels of these antibodies	Europe, (2013)
Wei *et al.*	41 PC, 69 controls	Matched, Consisted of healthy patients	High levels of *Streptococcus* and *Leptotrichina* were associated with PC, Control group had increased population of *Veillonella* and *Neisseria*	China, 2020
Vogtmann *et al.*	273 PC, 285 controls	Control group was clear of PC	*Enterobacteriaceae, Lachnospiraceae* G7, *Bacterioidaceae* and *Staphylococcaceae* were linked to PC	Iran, 2020
Torres *et al.*	108 PC, 100 controls	78 participants of the had other diseases, 22 were healthy controls	Ratio of *Leptotrichia* to *Porphyromonas* is increased in PC	United States, 2015
Petrick *et al.*	122 PC and 354 controls	Participants were African-American only, controls were clear of diseases	No association between any microbial taxa and risk of PC	United States, 2021
Farrel *et al.*	38 PC, 27 chronic pancreatitis, 38 healthy	Double control group, healthy group was matched with PC; some samples were selected for validation phase	*N. elongata* and *S.mitis* are decreased in PC, while *G.adiacens* is increased	United States, 2012
Sun *et al.*	10 PC, 17 benign pancreatic disease, 10 healthy	Double control group	High concentrations of *Fusobacteria* and low concentrations of *Neissera* associated with elevated risk for PC	China, 2020

Conversely, Petrick *et al.* noted lack of significant association between carriage of bacterial microbes and PC in the study on African Americans. Nonetheless elevated PC risk was noted amongst never smokers, participating in the study[17]. Authors have pointed to differences between characteristics of population of their study and other studies performed previously by other researchers. While results for African-American populations didn't prove any connection between architecture of oral microbiota and risk of PC, these same connections were found for white population of their own pool of participants, for this instance corresponding with findings of Fan *et al.* as well as Michaud et al. [5,10].

Similarly Kabwe et al. summarized latest literature regarding possible use of oral microbiomes as cancer markers, and found that it might be arduous to single out a specific bacteria universally linked to PC risks[18]. They also pointed out that comparing studies with different methodology is crucial to distinguish the connection of oral microbiome and PC, other than results drafted from oral wash samples only. Proposed role of oral bacteria in cancer development of distant organs consists of pathogens traversing the blood vessels and via circulatory system travel to distant tissues. It was observed by Abed at el. that component of oral microbiota such as *Fusobacterium nucleatum*, may reach the colon and be directly responsible for local cancer growth [19].

Sun et al. found striking differences in oral bacterium architecture of patients with PC in comparison to patients suffering from benign pancreatic disease as well as healthy group. Participants with PC were observed to have higher abundances of *Bacterioides*, *Firmicutes* and Fusobacteria[20].

In 2017 meta-analysis provided by Michaud et al. conclusion was drawn that there is consistency of positive association between periodontal disease and pancreatic cancer in various studies[21] . It highlighted at least 50 % growth in possible risk of PC for patients with periodontitis, which was supported by at least 3 studies with statistically significant results[21] .

Variety of results of the above mentioned studies is reflected in variety of oral microbiome, that occurs in different populations. It is known that there are multiple factors impacting the composition of oral microflora, such as temperature, pH, oxidation-reduction potential, salinity, saliva, as well as the state of oral hygiene of the host. At the same time, the composition of the oral microflora is documented to present similarly between populations of different regions of the world [22]. As Stasiewicz *et al.* points out in their 2021 article, most of the studies took an approach on singular microorganisms, sparing little attention to factors like mycobiome and its possible influence on the connection of oral microbiome and pancreatic cancer.

So far, the findings on possible efficacy of microbial organisms as cancer markers are limited and sometimes conflicting. Future studies should be conducted with larger population sizes and more thorough adjustment for other external risks. With that in mind, existing evidence points to a link between oral microbiome and the development of PC . Identification of microbial species connected to pancreatic cancer can result in development of easily accessible biomarkers, with practical use in screening of larger populations , as well as oriented preventive approach to PC, with use of antibiotics and probiotics. The use of microbial biomarker in detection of cancer, has been evaluated by Guo et al, proving valuable as possible marker of colorectal cancer[24] . Thus, further exploration of these microbial associations may prove crucial in earlier diagnosis, before the cancer reaches metastatic phase of growth, and in the establishment of new possibilities of treatment.

Conclusions :

1. Pancreatic Cancer is a multifactorial disease, with many risk factors. With its late diagnosis often at metastatic phase, staggeringly low 5-year survival rate and lack of effective therapies it becomes an urgent problem for healthcare professionals. Finding new reliable markers of PC would play a huge role in the management of the disease.
2. There is established connection between poor oral health (as reflected in periodontitis and edentulism [25]) and the risk of pancreatic cancer.
3. *P.gingivalis* is notoriously proven to be connected to increased risk of PC, adjusted for external risk factors such as smoking and alcohol consumption [10,11].
4. There is distinguishable difference between the composition of oral microbiota of patients with PC in comparison to both healthy patients and those suffering from different diseases. The exact mechanisms behind those differences remain elusive so far, and require further research. Future studies may provide us with more clues on how to utilize the composition of oral microflora in prevention of pancreatic cancer.

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