ORIGINAL ARTICLE

Plasma antibodies to oral bacteria and risk of pancreatic cancer in a large European prospective cohort study

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ABSTRACT

Objective Examine the relationship between antibodies to 25 oral bacteria and pancreatic cancer risk in a prospective cohort study.

Design We measured antibodies to oral bacteria in prediagnosis blood samples from 405 pancreatic cancer cases and 416 matched controls, nested within the European Prospective Investigation into Cancer and Nutrition study. Analyses were conducted using conditional logistic regression and additionally adjusted for smoking status and body mass index.

Results Individuals with high levels of antibodies against *Porphyromonas gingivalis* ATTC 53978, a pathogenic periodontal bacteria, had a twofold higher risk of pancreatic cancer than individuals with lower levels of these antibodies (OR 2.14; 95% CI 1.05 to 4.36; >200 ng/ml vs ≤200 ng/ml). To explore the association with commensal (non-pathogenic) oral bacteria, we performed a cluster analysis and identified two groups of individuals, based on their antibody profiles. A cluster with overall higher levels of antibodies had a 45% lower risk of pancreatic cancer than a cluster with overall lower levels of antibodies (OR 0.55; 95% CI 0.36 to 0.83).

Conclusions Periodontal disease might increase the risk for pancreatic cancer. Moreover, increased levels of antibodies against specific commensal oral bacteria, which can inhibit growth of pathogenic bacteria, might reduce the risk of pancreatic cancer. Studies are needed to determine whether oral bacteria have direct effects on pancreatic cancer pathogenesis or serve as markers of the immune response.

Significance of this study

What is already known about this subject?

- Pancreatitis increases the risk of pancreatic cancer.
- Bacteria can disseminate into the pancreas.
- Periodontal disease has been associated with pancreatic cancer.

What are the new findings?

- First study to prospectively examine the relationship between oral bacteria and pancreatic cancer risk.
- *Porphyromonas gingivalis*, a periodontal pathogen, may increase pancreatic cancer.
- Oral commensal bacteria may decrease risk of pancreatic cancer.

How might it impact on clinical practice in the foreseeable future?

- Provide opportunity to better understand which bacteria are related to risk of pancreatic cancer and lead to improvement in early detection of disease.

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INTRODUCTION

Each year, approximately 40 000 Americans die of cancer of the pancreas, making pancreatic cancer the fourth most common cause of cancer-related mortality.[1] Pancreatic cancer is one of the most rapidly fatal diseases, with fewer than half of patients surviving past 6 months from diagnosis. Detection of pancreatic cancer at early stages could increase survival; however, no biomarker currently has sufficient sensitivity and specificity for screening of pancreatic cancer at the population level. Consequently, primary prevention of pancreatic cancer is of particular importance in reducing the burden of this malignancy.

A positive association between periodontal disease and pancreatic cancer was observed in a prospective cohort study of men health professionals.[2] In this study, men reporting a positive history of periodontal disease had a 64% higher risk of pancreatic cancer compared with those reporting no periodontal disease; among never smokers, a twofold increase in pancreatic cancer risk was observed (RR=2.09, 95% CI 1.18 to 3.71), ruling out the possibility that the overall association was confounded by smoking. Other studies reported similar findings between periodontal disease[3 4] or tooth loss[5] and pancreatic cancer.

The role of bacteria in pancreatic diseases aetiology or tumour development has been suggested by multiple approaches. Using molecular methods, *Helicobacter* genus-specific DNA (but not *Helicobacter pylori* species-specific DNA) was identified in pancreatic cancer tissues,[6] while a positive association between *H pylori* infection and pancreatic cancer has been reported in several studies.[7] Using culture methods, the microbiota isolated from the pancreas had similarities to oral microbiota, particularly in the case of pancreatitis.[8–11] Bacteria reaching the pancreatic tissues by dissemination has been documented in both animal models and human subjects.[9 12 13] Additionally, multiple observations have shown that oral microbiota overlap with the digestive tract microbiota, providing multiple avenues for dissemination in dysbiosis.[14–17] In a recent retrospective case-control study, oral bacteria measured in saliva were associated with pancreatic cancer.[18]

We undertook this study to further investigate the association between periodontal bacteria and pancreatic cancer risk. Our a priori hypothesis (NIH R21 grant) was that antibodies to three periodontal pathogens (*Porphyromonas gingivalis, Tannerella forsythia* and *Aggregatibacter actinomycetemcomitans*) are positively associated with pancreatic cancer risk. We measured antibody levels to 25 oral bacteria, including strains that are elevated in patients with chronic periodontitis, in plasma samples collected prior to disease onset in a large European cohort study. This is the first study to examine antibodies to oral bacteria in relation to pancreatic cancer risk.

MATERIALS AND METHODS

Study population

The European Prospective Investigation into Cancer and Nutrition (EPIC) includes 519 978 participants, mostly aged 35–70 years, who were recruited in 23 centres within 10 European countries (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden and the UK) between 1992 and 2000. Detailed descriptions of the study design, population and baseline data collection of the cohort can be found in the previous report.[19] Each participant provided informed consent. The local ethical review committees approved the EPIC cohort study as well as the current project.

Blood sample collection and storage

Collection of blood samples was obtained from 385 747 EPIC study participants. In all but three countries (Denmark, Norway and Sweden), blood samples were collected based on a standardised protocol and aliquoted in plastic straws (plasma, serum, erythrocytes and buffy coat for DNA). The aliquoted specimens were then stored in a central biorepository (IARC, France) in liquid nitrogen (−196°C). In Norway the biological samples were collected in 20 0.5 ml plastic straws; 12 of the 16 plasma and two of the four buffy coat samples were shipped to IARC for storage in the central repository. In Sweden, all samples were stored locally in freezers at −80°C and in Denmark in nitrogen vapour (−150°C).

Pancreatic cancer ascertainment

In seven of the participating countries (Denmark, Italy, the Netherlands, Norway, Spain, Sweden and the UK), follow-up of cancer cases is based on population cancer registries. In France, Germany, Greece and Naples (one centre in Italy), a combination of methods are used for follow-up and cancer ascertainment, including health insurance records, cancer and pathology registries, and active follow-up through study subjects and their next-of-kin. In all EPIC centres, data on vital status were collected from mortality registries at the regional or national level, and combined with health insurance data (France) or data collected by active follow-up (Greece). The percentage of subjects lost to follow-up was 1.6% (loss is due to emigration, study withdrawal or non-response in active follow-up centres). Cancer incidence data are coded according to International Classification of Diseases-Oncology second edition and mortality data according to the International Classification of Diseases tenth edition (ICD-10). Incidence cases of exocrine pancreatic cancer reported during the study period were eligible for selection into this study. We excluded participants who had other malignant tumours preceding the diagnosis of pancreatic cancer, except for non-melanoma skin cancer. We identified 578 incident cases of pancreas cancer that were coded according to ICD-10 (C25.0–25.3, 25.7–25.9); 468 of these cases had blood specimens available. For each case, one control subject was selected (alive and free of cancer at the time that the index case was diagnosed) using an incident density sampling procedure. Matching characteristics consisted of: study centre, sex, age at blood collection, date and time of blood collection, fasting and exogenous hormone use (women only). For efficiency purposes matching was conducted for several planned cohort analyses simultaneously; matching factors were selected with consideration of all analyses. Due to insufficient volume or assay failure, the final dataset consisted of 405 cases and 416 control subjects.

Antibody detection

All samples were de-identified and blinded to case-control status. Samples were sent to the laboratory in matched pairs so that assays were consistently conducted on the same day for each pair; however, the pairs were not kept in the same order (eg, case-control; case-control; control-case) to maintain blinding. The presence of antibodies in the plasma samples against a preselected panel of whole-cell formalin fixed bacterial antigen was tested using an immunoblot array.[20] This method has the advantage of using a very small amount of primary sample (less than 10 µl). Bacterial strain selection was based on prior detection in pancreatic tissues[8–11] and known serotypes for *P gingivalis* (ATCC 33277 (also known as strain 381), serotype a; ATCC 53978 (also known as the capsulated strain W50), serotype b),[21 22] and *A actinomycetemcomitans* (ATCC 29523, serotype a; ATCC 43718, serotype b)[23] (see table 2 for full list).

On a subset of the case and control subjects (n=532) replicate measurements of each bacterial strain were performed (see online supplementary table S1). These were averaged for the overall analysis and per cent concordance was calculated among this subset of subjects for each bacterial strain, in the following ranges of human IgG (ng/ml) antibody levels: 0–7.5; 7.6–50; 50–200; >200 (respectively: no signal detected and to the lower detection limit of 7.5; (>7.5 to <50 ng/ml) lower range of the fitted reference curve; within the reference curve; and higher end of the fitted reference curve to saturation). Percent

concordance was found to be good for all bacterial strains, ranging from 0.67 to 0.84 (see online supplementary table S1).

Statistical analysis

Differences between cases and controls across baseline characteristics were assessed by paired t-tests (continuous variables) or by McNemar's test (categorical variables). Continuous measurements of the IgG antibody levels were log transformed to achieve approximate normality.

To assess the association between individual bacterial strains suspected to be periodontal pathogens and pancreatic cancer, we created four categories for the human IgG (ng/ml) based on the quantitative results from the immunoassays (ranges of human IgG (ng/ml) antibody levels: no signal detected and to the lower detection limit of 7.5; lower range of the fitted reference curve (>7.5 to <50 ng/ml); within the reference curve (50–200 ng/ml); higher end of the fitted reference curve to saturation >200 ng/ml). We considered values above 200 ng/ml as seropositive and conducted the main analysis for the pathogens of interest as a dichotomous variable, comparing values above to below 200 ng/ml.

Potential confounding effects of factors other than those controlled for by matching (ie, body mass index (BMI), waist circumference, current and past tobacco smoking and diabetes) were examined by assessing the association of these factors with pancreatic cancer risk. We retained smoking and BMI in all multivariate models; none of the other variables changed the logistic β estimate by more than 10% (individually or when included simultaneously). Subjects were defined as diabetics if they self-reported the condition in the baseline questionnaire at recruitment. Analyses using unconditional regression models controlling for matching factors led to similar results; we present results for the conditional regression analyses.

To avoid multiple comparison issues when examining the measured oral bacteria antibodies for which we did not have strong a priori hypotheses (ie, the non-pathogenic periodontal strains), we used an exploratory analysis to identify groups of people with similar levels of oral antibodies (using all 25 measured strains). The cluster analysis was performed in R using the MCLUST procedure.[24] We retained two clusters based on optimal clustering examining the Bayesian Information Criterion scores (for more details on cluster analysis refer to online supplementary method).[25] We examined the association between the two identified clusters and the risk of pancreatic cancer using conditional logistic regression.

For the oral pathogens, we performed subgroup analyses to assess possible effect modifications by smoking status and age (median, 62 years); tests for interaction were conducted by including a product term of the antibodies by smoking and age in the regression models. In sensitivity analyses, we removed the first 2 years of follow-up after blood collection to address reverse causality, and separately, removed diabetics for the analysis as these individuals are at higher risk for periodontal disease. For these subanalyses we used unconditional logistic regression to retain power.

All statistical analyses were conducted using the Statistical Analysis System (SAS) software package, V.9.2 (SAS Institute Inc., Cary, North Carolina, USA) except for the cluster analysis which was conducted in R (using RStudio, Inc., V.0.94.110).

RESULTS

Age at blood collection and sex were similar for cases and controls (controls were matched to cases on these factors). Mean follow-up time was 5.0 years for cases (from time of blood draw until date of diagnosis); controls had to be alive and free of cancer at the time the matched case was diagnosed. Cases were more likely to be current smokers or diabetics than controls, and had slightly higher BMIs (BMI; table 1). Alcohol intake and height were similar among cases and controls (table 1). Characteristics among cases who gave blood were similar to overall pancreatic cancer cases in the EPIC cohort (blood cases vs total cases[26]: eg, diabetes 7.0% vs 7.1%; BMI 26.7 vs 26.2; males 48% vs 40%; age 57.8 vs 58.1); although current smoking was somewhat higher in this study (30% blood cases vs 25% all cases).

Table 1 Characteristics of pancreatic cancer cases and control subjects selected from the European Prospective Investigation into Cancer and Nutrition cohort for this analysis

	Cases	Controls	p Values*
Numbers	405	416	
Males (%)	48.4	47.8	
Mean (SD) age at blood collection (y)	57.8 (8.0)	57.8 (7.9)	
Smoking status (%)			0.09
Never	36.4	43.0	
Past	32.4	34.4	
Current	30.0	21.4	
Unknown	1.2	1.2	
Mean (SD) body mass index (kg/m^2)	26.7 (4.3)	25.9 (3.9)	0.01
Mean (SD) height (cm)	168.5 (9.1)	168.2 (9.9)	0.43
Mean (SD) waist circumference (cm)	90.2 (12.5)	88.8 (12.9)	0.11
Mean (SD) alcohol at baseline (g/d)	15.8 (22.2)	15.1 (24.0)	0.88
Diabetes (%)	7.0	4.6	0.17
Postmenopausal (%, among women)	70.3	73.3	0.40

*Paired t-test for continuous variables and McNemar's test for categorical variables; p values for variables that were used to match cases and controls are not shown (ie, sex, age, length of follow-up).

Plasma antibody detection against 25 oral bacteria was performed. Very high correlations were observed for the two strains of *P gingivalis* (ATTC strains 33277 and 53978, r=0.91), and *A actinomycetemcomitans* (ATTC strains 29523 and 43718, r=0.94). A high correlation was also noted for the two species of *Veillonella* tested (*Veillonella atypica and Veillonella parvula*, r=0.88); those species are relatively genetically distant.[27] Most antibodies were correlated to each other, although strength of correlations varied (see online supplementary table S2). High antibodies levels (>200 ng/ml) of *P gingivalis* ATTC 53978 were more common in cases than controls (p=0.05, table 2), but not for the other *P gingivalis* strain.

Given our a priori hypothesis that periodontal pathogens are associated with higher risk for pancreatic cancer, we examined individual antibodies to those bacteria which have been previously associated with periodontal tissue destruction, which include *P gingivalis, A actinomycetemcomitans* and *T forsythia* (ie, five pathogens in our assay). Of these, the highest concentration of *P gingivalis* ATTC 53978 (>200 ng/ml) was associated with a twofold increased risk of pancreatic cancer (OR=2.14, 95% CI 1.05 to 4.36, compared with levels ≤200 ng/ml; table 3). The association was similar after removing cases that were diagnosed within 2 years of blood collection, although it was no longer statistically significant (table 3). Adding a 5 year or 7 year lag did not weaken the association with *P gingivalis*, if anything the association became stronger over time (OR=2.56, 95% CI 0.75 to 8.7 for the 7 year lag). Furthermore, associations were positive for all three categories of smoking status (never: OR=2.1, 95% CI 0.9 to 4.9; former: OR=3.2, 95% CI 0.5 to

Table 2 Percentage of samples with oral bacteria levels above 200 ng/ml by case and control subjects

Bacteria	Cases % with IgG levels >200 ng/ml	Controls % with IgG levels >200 ng/ml	p Value*
Oral pathogens† (Gram negative)			
Porphyromonas gingivalis ATCC 33277	17.3	17.3	1.0
P gingivalis ATCC 53978	7.2	4.6	0.05
Aggregatibacter actinomycetemcomitans ATCC 29523	19.8	21.9	0.45
A actinomycetemcomitans ATCC 43718	20.0	23.8	0.15
Tannerella forsythia ATCC 43037	2.2	2.2	1.0
Oral bacterial species of the human microbiome (Gram negative)			
Captonocytophaga ochracea ATCC 33596	30.9	31.3	0.82
Eikenella corrodens ATCC 23834	10.6	12.7	0.31
Fusobacterium nucleatum ATCC 25586	4.4	4.6	0.86
Fusobacterium periodonticum ATCC 33693	0.49	0.24	0.56
Fusobacterium polymorphum ATCC 10953	0.25	0.24	1.0
Prevotella intermedia ATCC 25611	4.7	4.6	0.86
Prevotella melaninogenica ATCC 25845	10.6	11.5	0.51
Prevotella nigrescens ATCC 33563	10.4	12.0	0.30
Veillonella atypica ATCC 17744	0.25	0.25	1.0
Veillonella parvula ATCC 10790	1.0	1.9	0.25
Oral bacterial species of the human microbiome (Gram positive)			
Actinomyces naeslundii ATCC 12104	77.5	80.5	0.22
Bifidobacterium dentium ATCC 27534	0.99	0.48	0.41
Corynebacterium matruchotii ATCC 14266	0.74	1.9	0.13
Enterococcus faecalis ATCC 29212	0.25	0.75	0.32
Finegoldia magna ATCC 29328	0.25	0	
Parvimonas micra ATCC 33270	47.2	52.2	0.09
Peptostreptococcus anaerobius ATCC 27337	0	0	
Streptococcus intermedius ATCC 27335	5.4	5.8	0.86
Streptococcus mitis ATCC 49456	43.7	46.9	0.25
Streptococcus salivarius ATCC 7073	10.6	11.3	0.69

*McNemar's test.
†Oral bacterial pathogens which have been previously associated with periodontal disease.

21; current: OR=2.2, 95% CI 0.5 to 9.4) or after removing diabetics (OR=1.7, 95% CI 0.9 to 3.1). Tests for interaction for smoking, age and diabetes were not statistically significant. No associations were observed for the five oral pathogens when using four categories of antibody levels (see online supplementary table S3).

Given our lack of a priori hypothesis on individual oral bacteria that are not considered oral pathogens, and given the high correlations observed between these bacteria, we performed clustering analysis to aggregate individuals depending on their antibody profiles. Two clusters were retained for analysis based on the model with the lowest BIC score (see online supplementary methods for more details on cluster analysis). A strong statistically significant inverse association was observed in cluster 2 which identified individuals with consistently higher levels of commensal oral bacteria antibodies (table 4) compared with cluster 1 (correlations between individual bacteria and clusters are provided in online supplementary table S4). In addition, frequencies for individual commensal oral bacteria by four categories of antibody levels are provided in online supplementary table S3; the majority of these associations were inverse, indicating that antibodies to commensal bacteria are higher in controls than cases.

DISCUSSION

We observed a twofold increase in pancreatic cancer among individuals who had high levels (>200 ng/ml) of antibodies to the periodontal pathogen *P gingivalis* ATTC 53978 compared with those with lower levels (≤200 ng/ml). In addition, we

Table 3 OR and 95% CI for pancreatic cancer associated with antibody levels to *Porphyromonas gingivalis* ATTC 53978

Antibody level to *P gingivalis* ATTC 53978	Case/controls	OR (95% CI)*	MV OR (95% CI)†
Low levels (≤200 ng/ml)	376/397	1.0 (referent)	1.0 (referent)
High levels (>200 ng/ml)	29/19	2.00 (1.00 to 4.00)	2.14 (1.05 to 4.36)
Removing cases diagnosed within 2 years of blood collection			
Low levels (≤200 ng/ml)	304/397	1.0 (referent)	1.0 (referent)
High levels (>200 ng/ml)	25/19	2.10 (0.99 to 4.46)	2.11 (0.97 to 4.59)

*OR and 95% CI were obtained using conditional logistic regression (cases were matched to controls on centre, sex, follow-up time, age at blood collection, date and time of blood collection, fasting status and use of exogenous hormones among women).
†Multivariate ORs (MV OR) were obtained using conditional logistic regression and additionally adjusting for body mass index (continuous) and smoking status (never, past, current).

Table 4 OR and 95% CI for pancreatic cancer associated with two profiles of antibody response (clusters 1 and 2)

Cluster*	Case/ controls	MV OR (95% CI)†
1. Low antibody levels to commensal oral bacteria	192/229	1.0 (referent)
2. High antibody levels tocommensal oral bacteria	212/187	0.55 (0.36 to 0.83)

*For more details on cluster analysis approach, refer to Supplemental Methods.
†OR and 95% CI were obtained using conditional logistic regression (cases were matched to controls on centre, sex, follow-up time, age at blood collection, date and time of blood collection, fasting status and use of exogenous hormones among women) and adjusting for body mass index and smoking status.

noted that individuals with consistently high levels of antibodies to common oral bacteria had a 45% lower risk of pancreatic cancer compared to those with a profile of lower antibody levels. Antibodies were measured in blood samples that were collected up to 10 years prior to cancer diagnosis, thereby most likely minimising changes in immune response that could have occurred after pancreatic cancer development.

A recent study reported an association between microbiota and pancreatic cancer using saliva specimens collected after cancer diagnosis and comparing them to healthy controls (retrospective case-control study).[18] In this study, microbiota from 10 pancreatic cancer cases were compared to 10 healthy controls, and validated in 28 independent pancreatic cancer cases and 28 healthy controls. Two bacteria, *Neisseria elongata and Streptococcus mitis*, were found to be lower in cases than controls in both datasets. These findings are similar to ours as we also observed an inverse association with *S mitis* (we did not measure *N elongata*). Our results, however, suggest that it may be more than one or two commensal bacteria that are inversely associated with risk of pancreatic cancer.

Despite a high correlation between the two *Porphyromonas* strains measured in this study (r=0.91), high levels of antibodies to *P gingivalis* ATCC 53978 (6%) were much less prevalent than for *P gingivalis* ATCC 33277 (17%). These two strains are quite distinct as *P gingivalis* ATCC 53978 has a capsule known as a major antigen associated with pathogenicity of the strain,[28] while *P gingivalis* ATCC 33277 lacks this antigen and is minimally inflammatory.[29 30] Furthermore, these two strains also have different fimbrial and outer membrane serotypes,[21 31] and a set of common antigens shared among species based on genomic data. The association between 19 bacterial antibodies and measured clinical indices of oral health was examined in a large US population study (the third National Health and Nutrition Examination Survey).[32] Of the 19 antibodies tested, only antibody titres to *P gingivalis* (mixed suspension of ATCC strains 53978 and 33277) were significantly higher in individuals with periodontitis (moderate or severe, and separately for extensive periodontitis) compared with healthy individuals ($p<0.05$),[32] as was previously observed in smaller studies.[33–36] High levels of antibodies to *P gingivalis* ATCC 53978 may be the best antibody marker for high bacterial load and aggressive periodontal disease, which is in agreement with the third National Health and Nutrition Examination Survey data,[32] and thus may explain why it was the only suspected periodontal pathogen associated with an elevated risk of pancreatic cancer.

P gingivalis ATCC 53978 levels were also elevated in cluster 2 (which was inversely associated with risk of pancreatic cancer), as most of the other tested bacterial target were elevated in this cluster. In adults, prevalence of *P gingivalis* in subgingival plaques of patients with periodontitis has been found to be higher than in individuals with healthy gums.[36 37] While *P gingivalis* bacterial load is strongly associated with periodontal pocket depth, prevalence is otherwise similar between patients and controls with healthy gums indicating the normal presence of the species in health.[38–40] Most recently, a study from the Human Microbiome Project reported that certain bacterial genera thought to be composed of pathogenic species present in periodontal disease (eg, *Aggregatibacter, Porphyromonas, Tannerella and Treponema*) were found in at least 93% of individuals with no gum disease suggesting that these bacterial genera are also part of the normal oral microbiota and exposed to the immune system response.[41]

Oral diseases originate from changes in the ecological balance of the microbiota,[35 42 43] suggesting that there is a beneficial effect of members of the oral microbiota. *Capnocytophaga ochracea*, for example, was significantly more prevalent among healthy patients (>90%) than chronic periodontitis patients (<60%), and was found at more sites among healthy individuals.[38] In addition, this microbe has been associated with significantly less disease progression in other studies.[44 45] *Veillonella* species were, in another study, associated with periodontal health.[42] We therefore propose that the inverse associations observed in our study for cluster 2 may reflect individuals with oral microbial stability, healthy gum status, and a strong immune response toward bacteria.

Host genetic susceptibility related to immune function could explain our observations. Recent data from Genome-Wide Association Studies examining genetic susceptibility and pancreatic cancer risk reported that individuals with non-O blood groups had a higher risk of pancreatic cancer than those with blood type O.[46] Although it is not clear how these findings translate to carcinogenesis, SNPs at the *ABO* gene locus were found to be determinants of circulating levels of molecules that are important mediators of chronic inflammation and immune cell recruitment.[47–50] The best-established connection between local inflammation and pancreatic cancer comes from studies on chronic pancreatitis.[51] It has been proposed that the prolonged inflammation observed in chronic pancreatitis patients is what initiates or aids the progression of a pancreatic tumour.[52] Culture based studies of pancreatitis have shown a bacterial colonisation of the tissues.[8–10]

Genetic determinants of immune surveillance clearly play a critical role in pancreatic cancer development. Consequently, it is plausible that elevated levels of antibodies to oral bacteria in individuals serve as a marker for a genetically stronger immune response, providing protection against carcinogenesis. In a study of periodontal disease and cancer among twins, the associations with periodontal disease could be partially explained by shared genetic risk factors.[4] As periodontitis is a complex disease of polymicrobial origins which is influenced by genetic susceptibility, host response and environmental factors, deciphering the genetic component of the disease is still under study.[35 53–55]

The strength of this study includes a large sample size, prediagnostic bloods and a methodology that enabled us to measure a large number of antibodies using small volumes of plasma (as these are valuable resources). By using prediagnostic bloods, we were able to minimise reverse causation and examine the association with antibodies many years prior to diagnosis of cancer. With 405 cases and 416 controls, we had adequate power to examine a large number of antibodies. Furthermore, we had detailed data on smoking history and other known risk factors of pancreatic cancer and conducted multivariate analyses to rule out potential confounding by these factors.

The two main limitations of this study were lack of gold standard measurements for the antibodies and having only one blood measure per subject at one point in time. We could not conduct ELISA tests to measure antibodies, which are considered the gold standard measurement, because we did not have sufficient blood volume in the EPIC samples; however, the methods we used have been validated in previous studies where blood product amounts were not a limiting factor. While we only had one measurement per individual, antibodies to periodontal pathogens have been shown to be fairly stable over time,[33 56] which suggests that our findings would be similar if we had more than one measurement over time. Another limitation of this study was that we did not have any data on drug use (eg, antibiotics or NSAID use) that may have influenced bacteria antibody levels. Similarly, we were not able to control for chronic pancreatitis as these outcomes were not ascertained in the EPIC cohort; it is plausible that the association observed is mediated through pancreatitis, in which case we would not want to control for this factor. More research is needed to examine the pathways and mechanisms that may explain our findings.

In summary, this is the first study to examine antibodies to oral bacteria and risk of pancreatic cancer. Our findings suggest that individuals who have high levels of antibodies to *P gingivalis* ATTC 53978, a bacterium strongly associated with periodontal tissue destruction, are at higher risk of pancreatic cancer, while a distinct cluster of individuals with elevated antibodies to oral bacteria were associated with a lower risk of pancreatic cancer. Given that this is the first study to examine these associations, they will need to be confirmed in other studies. If confirmed, our findings may open new avenues to investigating the role of the oral bacteria and periodontal disease in pancreatic carcinogenesis and provide exciting opportunities to improve our understanding of the development of this fatal disease.

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