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An analysis of caries rates, biofilm composition, socioeconomic status and fluoride

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Abstract

Caries remains a pandemic disease that is resistant to a cure. Despite over one hundred years of scientific effort, we still do not fully understand the disease process and are left with symptomatic therapies that do little more than damage control. Recent reports on the epidemiology of caries in various populations suggest that the disease is making a comeback. New data is available which suggests that caries is a very complex poly microbial phenomenon which is best understood in ecological terms. Early caries researchers such a W.D. M iller and G. V. Black were frustrated by the primitive tools of microbiological investigation that were available during their era. Recent advances in the application of molecular methods to biofilm analysis provide the caries investigator with powerful tools to probe the mysteries of the disease. This study was developed to use molecular tools to investigate biofilm composition in populations with high caries rates (Medicaid) and lower caries rates (NonMedicaid). The same study was able to evaluate biofilm profiles between populations living in fluoridated and non fluoridated communities. The purpose of the study is simply to identify if differences exist between biofilm profiles in the study groups. Such information may lead to a better understanding of the disease and hence more effective therapeutic opportunities in the future.

Introduction and literature review

Dental caries has been a disease with many faces over time. Prior to 1800 it was a disease of affluence largely linked to the price of sugar. Only the rich could afford sugar once known as white gold. As production costs came down, sugar became more prevalent in the diets of people of all demographics. Caries began to move into new populations During the 1920's and 30's Cincinnati Ohio dentist Dr. Weston Price traveled the world Indiana Jones style studying diets and caries rates in indigenous populations. He showed conclusively that caries is a disease of high sugar content western diets. Between 1930 and 1980 a great deal of research was conducted to identify the "pathogen" responsible for caries the disease. The medical model of "pathogen specific" disease had been well established by Robert Koch and Louis Pasteur in the previous century. With improved laboratory techniques and equipment oral microbiologists pursued the holy grail of dental disease. Several potential candidates for the caries pathogen

appeared as high acid producing bacteria grown in appropriate media. Mutans Streptococci and Lactobacillus acidophilus became the likely candidates of the day. Remarkable progress was made in the understanding of the biochemistry of these particular pathogens. The enzymatic systems that convert simple sugars into organic acids were worked out in laboratory conditions. An explanation for the differences in caries rates between populations demonstrated by Weston Price began to be understood at the biochemical level. Risk factors also manifested in advance of the disease. During the 1950's topical and systemic fluorides were introduced with widespread success. Caries rates were dramatically reduced across wide demographics. Students entering dental schools during the 1970's and 80's wondered if they would be able to support themselves in a population less in need of their restorative services. Seven US dental schools closed their doors, including the authors (Emory 83). Then in the year 2000 Dr. David Satcher issued the first surgeon general's report on the oral health of Americans. This report identified a "growing epidemic of dental disease" in certain populations. These included persons living in lower socioeconomic situations, persons with disabilities, seniors and emigrant populations. Caries had become a disease of poverty. This report represented a wakeup call to the profession. While success against caries had been made, we were losing ground in some areas. The epidemiology of caries was once again on the move.

Also published in 2000 was a landmark paper by Professor Phillip Marsh titled "Are dental diseases examples of ecological catastrophes?" Dr. Marsh argued that both caries and periodontal diseases are classic plaque or biofilm mediated processes. The profession had argued for decades over the value of a specific pathogen plaque model or a non specific plaque model. Clinicians have noticed that some patients develop cavities without obvious plaque and other patients with lots of plaque remain disease free. The ecological plaque model introduced by Marsh overcame the limitations of previous hypotheses and produced a working model of disease based on current knowledge of biofilm physiology. A recent paper by Takahashi and Nyvad builds on the ecologic plaque hypothesis and supports the view that a biofilm should be considered pathologic or not pathologic based on the behavior of the ecosystem as a polymicrobial whole. As it turns out, all of the work done by oral microbiologists using mono culture invitro methods produced data that described bacterial behavior far removed from the oral environment. As molecular methods were being applied to the identification of oral biofilm inhabitants, it became obvious that only a minority of oral bacterial species had ever been successfully cultured in the laboratory, hence little to nothing was known about the physiology and biochemistry of the majority of oral biofilm inhabitants. Medical microbiologist Dr. William Costerton suggested that microbiologists may need to "reboot their hard drive" in order to understand bacterial mediated disease in the new biofilm paradigm. Then in 2007 the Centers for Disease Control released a major report demonstrating that after fifty years of progress, caries rates were increasing in our youngest children ages 2-5. Also in the year 2007 the state of Oregon Department of Human Services released the Oregon Smile Survey. This report showed dramatically increasing caries rates in children 5-8. In 2009 Dr. Robert Bagramian published "The Global Increase In dental caries. A Pending Public health Crisis. This paper documents increasing caries rates across the globe. The disease appears to be making a

comeback. Why? I designed this study as a step toward understanding the possible role of biofilm composition in high and low caries rate populations. This is the first study to use molecular methods to identify oral biofilm profiles in Medicaid and non Medicaid populations. Since the sample population also lives in fluoridated and non fluoridated communities, this represented an opportunity to examine the possibility of differences in biofilm composition based on this parameter, also a first effort.

Methods and Materials

Plaque Sample collection protocol

The decision was made to collect plaque samples from multiple oral sites from each patient. The strategy was to obtain an oral biofilm profile rather than a site specific perspective of the micro biota. Micro brushes were sterilized and placed in sterile collection bags. 1.5 ml of sterile molecular grade water was placed into a sterile ependorf tube and added to the collection bag. 160 plaque collection kits were prepared in this manner. The plaque collection protocol was to apply the first microbrush against the occlusal surface of posterior molars, place the brush into the solution in the ependorf tube and spin to dislodge bacteria into the medium. This process was repeated for posterior interproximal spaces with the second brush and finally on anterior teeth facial surfaces with the third brush. The ependorf tube was then placed into a freezer bag together with the patient survey/consent form and frozen at the clinic site. A video was made of the collection protocol and placed on YouTube so that all offices participating in the study could easily view and duplicate the plaque and patient date collection protocol.

Patient selection and data collection

Dental practitioners were contacted throughout Oregon for willingness to participate in this study. Cities from Medford in southern Oregon to Portland in the north were included in the study. A majority of patients were obtained through the cooperation of Capitol Dental Care a large Medicaid managed care organization in the state. Patients were to be selected out of regular preventive and restorative treatment schedules. A randomization protocol was developed that identifies as a study candidate every fifth patient age 2-12 in regular daily schedules and to ask parents or guardians for interest in study participation. Appropriate consents were obtained and the following data recorded. Name, age, clinic location, DMFT, a subjective analysis of plaque presence, oral hygiene practices and diet were recorded on a scale of 1 to 10. 1 being good and 10 being bad. Patient samples were segregated into the following four groups.

1. Medicaid living in a non fluoridated community
2. Medicaid living in a fluoridated community
3. Non Medicaid living a non fluoridated community
4. Non Medicaid living in a fluoridated community

Species Identification

All samples were transported in a frozen state to my laboratory in Portland Oregon. Arrangements were made to accomplish species identification from the samples through the Forsyth Institute Human Oral Microbe Microarray Identification service. This service employs a 16S RNA molecular identification protocol from DNA extracted from plaque samples. Dr. Bruce Paster leads this lab and is a pioneer in the use of molecular techniques to investigate the role of oral biofilm in states of disease and health.

Samples were thawed and pooled together based on the four patient categories. Economic factors dictated the approach of pooling the samples versus running individual biofilm profiles and then collapsing the data. DNA was extracted from each of the four patient groups using the Microbial Ultra Clean kit produced by MOBIO. Standard steps for sample preperation and DNA extraction were conducted. Assistance was provided at this step from Marco Gutierrez, D.D.S. and Lynn Yu D.D.S.,Ph.D. The final four DNA samples were packaged with dry ice and sent overnight to the Forsyth Institute. A spreadsheet was returned which identified the presence, absence and relative quantity of 425 individual oral bacterial species in each sample. The patient category data of DMFT, plaque, oral hygiene and diet scores were averaged across the four patient groups and combined with the biofilm composition to produce data sets for analysis.

Results

Discussion

Conclusions