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Screening for Selenomonas Noxia and Akkermansia Muciniphila from the Oral Cavity of Pediatric Patients

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The human digestive tract is composed of an immense variety of microorganisms, which have been linked to many health problems such as obesity. There are many studies that demonstrate the association of cariogenic pathogens in dental patients and overall health. *Selenomonas noxia*, a gram negative anaerobe that is unable to metabolize sucrose and *Akkermansia muciniphila*, a gram negative anaerobe known to metabolize fats are two examples of pathogens that play a role in overall health.
Objectives: Few studies have investigated both *Selenomonas noxia* (SN) and *Akkermansia muciniphila* (AM) with the same patient samples. The objective of this study was to analyze and evaluate the prevalence of both these organisms in a dental school-based setting.
Methods: Saliva was collected from pediatric patients using an IRB-approved protocol. DNA was isolated for PCR screening and quality tested using the nanodrop. Gel electrophoresis was used for

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visualization. Forty seven (n=47) patients ranging from five to fifteen years of age (average 10.3) were screened. Nearly two-thirds of patients were male (64.6%) with the vast majority identified as Hispanic (72.9%) or other minority (25.0%)

Results: DNA was successfully isolated with an overall average DNA concentration of 1.43 ug/uL and overall purity (A260:A80 ratio) of 1.88. Of the forty seven patients, four patients were positive for SN. There was no presence of AM in the samples.

Conclusions: Research regarding AM and SN may suggest they inhabit different niches in the microbial community. The preliminary data of this pilot study suggests that SN could be found in pediatric patients while AM is not likely prevalent. However, due to the small patient sample size and large differences observed from these samples further research and analysis would need to be conducted to validate the findings.

Keywords: Selenomonas noxia; akkermansia muciniphila; oral prevalence; microbiome; saliva screening.

ABBREVIATIONS

SN : Selenomonas noxia
AM : Akkermansia muciniphila
qPCR: quantitative polymerase chain reaction
DNA : Deoxyribonucleic acid; IRB: Institutional Review Board;
UNLV: University of Nevada Las Vegas
SDM : School of Dental Medicine

1. INTRODUCTION

Health problems in the human population have been found to be related to many factors in the body including genetics and environmental variables [1]. Obesity, high body mass index and diabetes are some of the most common variables that have been correlated to many diseases [2,3]. More recent considerations have been placed on the microbiome of the body including the oral cavity and its abundance of bacteria [4].

Obesity in childhood and adolescence is the most prevalent nutritional disorder, it has been suggested that oral bacteria could have a possible role in obesity development and even in taste perception [5]. Two of the organisms that have been studied in the past are *Selenomonas noxia* (SN) and *Akkermansa muciniphila* (AM) [6-8] Both of these gram negative obligate anaerobes have been investigated and thought to affect the body fat index and total adipose tissue of the host in opposite ways. Periodontal disease has been linked to obesity [9,10].

SN has been implicated in converting periodontal health to disease, and been found in gastric ulcers as well (6). Increasing numbers of studies have demonstrated a relationship between SN and obesity, with greater numbers of overweight individuals exhibiting increased abundance of oral SN [11]. In mouse studies, AM played a causative role in lowering the body fat index and decreasing adipose tissue in general [12]. AM has even been touted as a promising probiotic [13,14].

This investigation aims to observe the presence of these species and correlate them with body-fat index in pediatric patients at the UNLV school of Dental Medicine. Saliva samples were taken from pediatric patients and purified using conventional methods. These samples were then screened for the presence of SN and AM using qPCR.

2. METHODOLOGY

2.1 Study Approval

The protocol for this study was reviewed and approved by the Office for the Protection of Research Subjects (OPRS) and the Institutional Review Board (IRB) at the University of Nevada, Las Vegas (UNLV) under [1619329-1] Retrospective analysis of Oral Health Status of Dental Population in July 2020. The original study for the collection of saliva samples was reviewed and approved under protocol OPRS#1305-4466M “The Prevalence of Oral Microbes in Saliva from the UNLV School of Dental Medicine Pediatric and Adult Clinical Population” .

2.2 Study Design

In brief, this was a retrospective study of an existing saliva repository. Patient samples were originally collected following protocol for voluntary participation in the study. Patients (or parents/legal guardians for patients under the age of 18) that agreed to participate provided informed consent. Patients that declined to

participate were not included in the study. The patients were not provided with additional services or money for their participation.

2.3 Saliva Collection

Due to its non-invasive nature, saliva collection has been used to isolate and collect DNA from saliva samples for microbial screening [15-17]. The original protocol for sample collection involved patients providing up to five mL of unstimulated saliva in sterile saliva tubes from each patient. As noted previously [18], the samples were stored in a freezer (-80C) in a biomedical safety level 2 (BSL-2) laboratory for the purpose of longevity and analysis of the samples. The saliva samples were provided with random, non-repetitive numbers to remove any patient identification factors from the origin of the samples. Only the information relevant to the study was recorded for each sample: ethnicity/race, age, sex and BMI.

2.4 DNA Isolation

The DNA in the saliva was isolated using the phenol:chloroform extraction method with Invitrogen (TRIzol reagent) known to isolate high-quality DNA/RNA from samples in fluids and tissues, as previously described [18]. For analysis, 100 µL of saliva was removed and 300 µL of TRIzol reagent was added and mixed. 200 µL of chloroform was added to the mixture after five minutes. The sample was then incubated for five minutes and then centrifuged for fifteen minutes at 4 ◦C using relative centrifugal force (RCF) of 12,000x g.

100% isopropanol was subsequently added to a new microcentrifuge tube with the transferred DNA-containing aqueous phase. For five minutes, each sample was centrifuged to pellet the DNA. After aspirating the supernatant, 100% ethanol was used to wash the pellet, which was then centrifuged for five additional minutes. After aspirating the ethanol, 100 µL of DNA rehydration solution was added to the DNA pellet for analysis.

2.5 Polymerase Chain Reaction (PCR)

A qPCR assay has potential to be used as a routine diagnostic method in clinical microbiology laboratories [19]. Polymerase chain reaction was performed to screen the patient saliva samples for microbial and human DNA using GoTaq polymerase and the following forward and reverse primers for AM and SN [6]. The specifications were set with an initial incubation at 94 °C for 2 minutes, denaturation at 94°C for 20 seconds and 35 cycles, denaturation at 60°C for 20 seconds and 35 cycles, denaturation at 72°C for 20 seconds and 35 cycles, with annealing at 72°C for 5 minutes. The reaction included: 10 µL GoTaq PCR Mastermix, 1µL forward primer, 1µL reverse primer, 3 µL sterile H2O, and 5 µL patient saliva DNA. The final reaction volume was 20 µL.

The primers synthesized from Fisher Scientific Company LLC were [6,20]:

Forward primer- SN, 5'-TCT GGG CTA CAC ACG TAC TAC AAT G-3', 25 nt

Reverse primer- SN, 5'-GCC TGC AAT CCG AAC TGA GA-3', 20 nt

Forward primer- AM, 5'-CAG CAC GTG AAG GTG GGG-3', 18 nt

Reverse primer- AM, 5'CCT TGG GGT TGG CTT CAG AT'-3', 2

2.6 Statistical Analysis

Patient demographics were compiled and presented as both raw numbers and percentages of the overall study population. Comparisons were made to the overall clinic percentages using Chi Square analysis to determine if the study sample demographics were representative of the overall clinical population. An alpha level of 0.05 was used to determine statistical significance.

3. RESULTS

Forty seven (n=47) patients were screened, ages ranging from five to fifteen years of age (average 10.3). Nearly two-thirds of patients were male (64.6%) with the vast majority identified as Hispanic (72.9%) or other minority (25%). DNA was isolated from each of the 235 sampled sites with an overall average DNA concentration of 1.43 ug/uL and nanodrop found overall purity (A260:A80 ratio) of 1.88 [21]. We found no presence of AM in these pediatric patient samples, while SN on the other hand was found to be present in 4 out of 42 total samples (Fig.1). No correlation was found between SN-positive samples and BMI.

In addition, the patient demographics were analyzed to determine whether the sample was representative of the overall clinic population (Table 1) [18]. These data demonstrated that

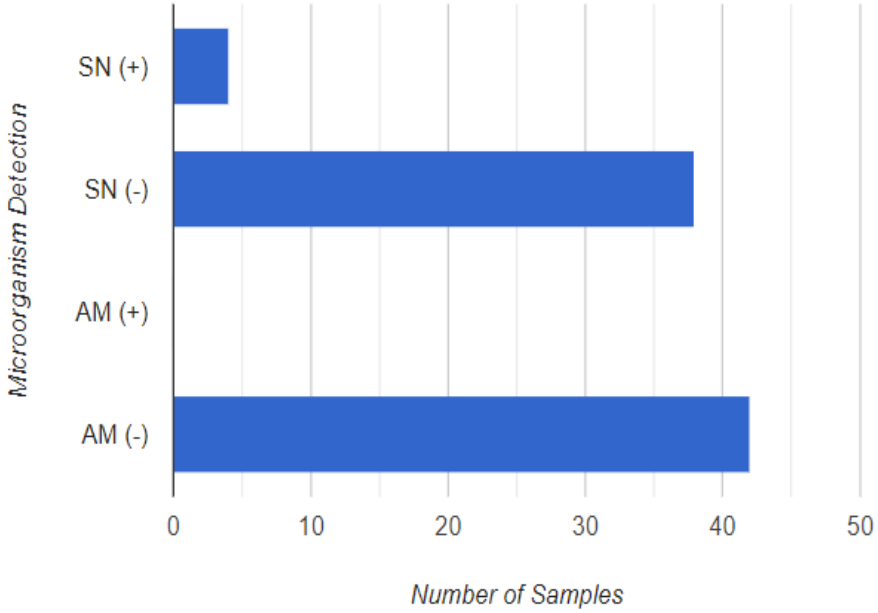

Fig. 1. Microorganism Presence in Saliva Samples

Table 1. Demographic analysis of study sample

Demographic	Study Sample	Clinic	Statistical Analysis
Sex			
Female	n = 17/47 (36.2%)	52.8%	X^2 = 110.571, d.f.=1
Male	n = 30/47 (63.8%)	47.2%	p - 0.0012
Race/Ethnicity			
White (non-Minority)	n = 2/47 (4.25%)	24.7%	X^2 = 223.753, d.f.=1
Minority	n = 45/47 (95.75%)	75.3%	p=0.00018
Hispanic	n = 35/47 (74.5%)	52.1%	
Black	n = 8/47 (17%)	11.8%	
Asian/Other	n = 2/47 (4.25%)	11.4%	
Age			
Average age	10.26 year	11.34 years	
Age range	5 - 15 years	0 - 18 years	

there were significantly more females in the study sample than in the clinic population, which may indicate this sample represents a significantly higher proportion of one sex versus the other. In addition, this study sample was almost exclusively composed of minority patients, which was significantly higher than the overall percentages of minorities in the clinic, which may suggest oversampling of this demographic.

4. DISCUSSION

The goal of this study was to perform high quality qPCR of patient saliva samples screening for both AM and SN. AM was not present in the pediatric patient saliva samples we analyzed. This result suggests that AM may not be prevalent in the mouths of children and young adults. This is in agreement with the determined role of this mucin-degrading bacteria (causative role in lowering body fat index) [22]. Children are constantly adding weight during periods of rapid growth and development and would not typically lower their body fat. Previous studies have further exemplified the mucin-degrading role used in the treatment of AM in high-fat-diet-induced metabolic disorders such as insulin resistance from type II diabetes mellitus, adipose tissue inflammation, fat-mass gain, and metabolic endotoxemia [23]. Treatment was found to be advantageous in support of the

negative association of AM concentrations to hypertension, overweight conditions, obesity, untreated type II diabetes mellitus [7].

Conversely, SN was found in four of our samples. SN levels in saliva have been discovered to be indicative of obesity in patients and could affect the pathology of increased adipose storage. Pediatric patients would likely have more use for microorganisms in their gastrointestinal tract that help with fat storage, which could explain the higher prevalence SN. Although we did not find any correlation with SN and BMI, further analysis with a larger sample size and more age diversity in samples is recommended. In addition to having higher prevalence in obesity, previous studies have indicated that the presence of SN contributes to the conversion of periodontal health to disease not limited to obesity [11], but also gastric ulcers [6].

Few studies have evaluated SN prevalence among pediatric patients including age, race or ethnicity and BMI, although we did not find a correlation between SN and any demographic variable. Because of the retrospective nature of this study, more investigation should be done with an increase in samples to have a better understanding of these organisms and their role in metabolic processes of their respective hosts, as previously outlined [6,11]. It is known that periodontal disease and it's microbiota are bi-directional systems when it comes to diabetes and other high inflammatory diseases [24]. SN has also been found in patients with aggressive periodontal disease [10]. Using samples collected from adult patients would also bring us a better understanding of the relationship between these organisms and could give us more information regarding the host and diabetes and other high inflammatory diseases.

5. CONCLUSION

The presence or absence of AM and SN in the pediatric patient pool as determined by qPCR screening provides a starting foundation for correlating the roles of these bacterium among set demographic parameters. Evaluation of a larger sample size would be necessary to determine further correlations to age, ethnicity, and BMI.

CONSENT

Pediatric assent and parental permission to consent for voluntary participation were obtained at the time of study enrollment.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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