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Research Article

Prevalence of *Scardovia wiggsiae* among a Pediatric Orthodontic Patient Population

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Abstract

Orthodontic treatment has been associated with changes in oral microbial flora, particularly among pediatric populations. Many studies have focused on the alterations in the prevalence of cariogenic pathogens, such as *Streptococcus mutans*. Recent evidence has revealed a newly discovered Gram-positive cariogenic pathogen, *Scardovia wiggsiae* – although few studies exist that explore prevalence among Orthodontic patients. Based upon this information, the primary objective of this study is to determine the prevalence of *S. wiggsiae* among pediatric Orthodontic patients from an existing saliva repository.

This retrospective screening of the existing saliva sample repository revealed n = 156 pediatric (< 18) samples taken from the Orthodontic clinic that were not previously screened for the presence of S. wiggsiae. DNA isolation was performed on n = 107 samples and successfully isolated from n = 72 samples, yielding a recovery rate of 67.2%. Following DNA isolation, samples with sufficient quality and quantity were screened using qPCR with primers specific for S. wiggsiae. This analysis revealed the presence of Scardovia in n = 32/72 or 44.4% of successfully screened pediatric Orthodontic patient samples, which were almost evenly distributed among Males and Females.

Although few previous studies exist to evaluate the prevalence of *Scardovia*, a previous study from this group demonstrated prevalence among pediatric patients of 26% and adult patients of 19%. Past studies also revealed the prevalence of *S. wiggsiae* in adult Orthodontic patients to be 14%. The data from this current study suggest significantly higher prevalence among pediatric Orthodontic patients, which provides new information regarding the potential changes in pathogen levels among this population. Although inference from this study is limited by the retrospective nature of this study, it may be among the first to report significant differences in *S. wiggsiae* prevalence among pediatric Orthodontic patients that may improve our understanding of cariogenic pathogens and risk among this population.

Keywords: *Scardovia wiggsiae; Pediatric; Orthodontic*

Background and Introduction

Cariogenic bacteria are an important focus of research as almost 50% of children and most adults in the United States (US) are affected by dental caries [1,2]. Orthodontic appliances increase the risk of developing white spot lesions, an early stage of carious lesion development [3,4]. A major reason behind the increased development of pre-carious and carious lesions is due to increased difficulty in effectively removing plaque on all tooth surfaces when orthodontic appliances are present [5,6].

Fixed orthodontic appliances may also inhibit the oral environment's innate ability to cleanse itself using salivary flow and soft tissue movements, two of the major mechanisms that help clear the mouth of food [7,8]. Plaque is one of the major niches for cariogenic bacterial growth and will contribute to the formation of white spot lesions, and eventually carious lesions [9-11]. Many studies have evaluated dental plaque to identify the major cariogenic organisms, which include the *Streptococcus mutans* and *sobrinus*, *Lactobacillus acidophilus*, *Actinomyces* spp. and *Nocardia* spp [12,13].

Recent evidence has revealed the presence of a novel cariogenic bacterium *Scardovia wiggsiae* (SW), which was originally isolated from children with severe early childhood caries (SECC) [14,15]. More recent studies have demonstrated the presence of *Scardovia* among other children without SECC, although there is not sufficient evidence to determine the overall prevalence of this oral bacterium [16-18]. In addition, only two studies to date have sought to evaluate the presence and cariogenic potential of SW among orthodontic patients [19,20].

A recent pilot study at this institution determined that pediatric orthodontic patients may have increased probability of harboring SW, compared with adult orthodontic patients or pediatric patients without orthodontic brackets [17,20,21]. Due to the paucity of evidence regarding the prevalence of SW and the increased risk of carious lesions with orthodontic treatment, the overall goal of this project was to more thoroughly investigate the prevalence of SW among pediatric orthodontic patients within the patient population of the public dental school in Nevada. Since it has been well established that the oral environment changes because of patient related factors, a firm understanding of how the oral microbiome is changed during orthodontic treatment is imperative in developing strategies to ensure successful risk management among patients during orthodontic treatment.. More accurate assessment of the oral microbiome and prevalence of cariogenic risk would allow for more accurate determination of cariogenic bacteria and effective patient management and treatment with more predictable treatment results.

Material and Methods

Human Subjects

This study was reviewed and approved by the Office for the Protection of Research Subjects (OPRS) Institutional Review Board (IRB) on March 7, 2016 (Protocol#880427-1 "Retrospective investigation of Prevalence of *Scardovia wiggsiae* in pediatric orthodontic patients") at the University of Nevada, Las Vegas. The original protocol for the screening of saliva samples was approved on February 6, 2015 (Protocol#1502-5068M "The Prevalence of Oral Microbes in Saliva from the University of Nevada Las Vegas (UNLV) School of Dental Medicine (SDM) pediatric and adult clinical population"). Original saliva collections took place between July 2010 and July 2016.

Study Design

This retrospective study involved previously collected saliva samples derived from a convenience sample of pediatric and adult patients recruited from the UNLV-SDM clinics. As with all clinical studies, adult participants were required to provide Informed Consent prior to collection of demographic information and saliva samples. Exclusion criteria included patients (or their appointed guardian) who declined to participate. Pediatric and Orthodontic dental residents recruited UNLV pediatric subjects between the ages of 3 and 17 years after receiving informed consent from parents or guardians for their children to participate in the study. Although children under 18 years

of age are not able to give informed consent, in Nevada, children aged 7 years and older who are able to read, comprehend, and write are asked to provide "pediatric assent," which is an agreement to voluntarily participate in research. Pediatric assent from each patient was also obtained prior to collection of demographic data and saliva samples. Patients whose parents or guardians declined to let them participate were excluded, as were patients who themselves declined to participate. Also, any child who was not a patient of record at the UNLV School of Dental Medicine clinics was excluded.

Saliva Collection

In the original study protocol, consented dental patients were given a sterile saliva 50 mL collection container for one sample. Samples were stored on ice until transfer to a biomedical laboratory for screening and analysis. Each of these samples was given a unique, randomly generated number to prevent research bias and any identifying information from being disclosed. The patient demographic and health information was also concurrently collected and given the matching randomly generated number for analytical purposes, but no patient-specific identifying information was subsequently available to any research team member.

DNA isolation

DNA was isolated from each saliva sample using the GenomicPrep DNA isolation kit from Amersham Biosciences (Buckinghamshire, United Kingdom) and the procedure recommended by the manufacturer, as previously described [17,20-22]. DNA was suspended and stored in 50 µL DNA Hydration Solution from Amersham Biosciences (Buckinghamshire, United Kingdom) at 4C. DNA purity was calculated using ratio measurements of absorbance at 260 and 280 nm (A260/A280 ratio).

Polymerase chain reaction (PCR) primers

qPCR specifications included an initial incubation at 50C for two minutes, denaturation at 95C for 10 minutes and 40 cycles at 95C for 15 seconds and 60C for one minute [23]. Positive DNA controls were derived from previously identified SW-positive samples [17,20,21]. Primers synthesize from Eurofins MWG Operon (Huntsville, AL) were used with TaqMan universal PCR master mix, with final probe concentration at 0.2 uM using 5 uL of template (sample) DNA per reaction. The 5'-end of the *Scardovia wiggsiae* probe (SwP) was labeled with 6-carboxyfluorescein (FAM) and the 3'-end with tetramethyl-6-carboxyrhodamine (TAMRA). Nuclease-free, sterile water from Promega (Madison, WI) was added to increase the final reaction volume to 25 uL. Screenings were each performed in duplicate.

Forward primer-SW, GTGGACTTTATGAATAAGC (19 bp)

Reverse primer- SW, CTACCGTTAAGCAGTAAG(18 bp)

SwP[6 ~ FAM] 5'-AGCGTTGTCCGGATTTATT-3'G [TAMRA]

Statistical Analysis

Information regarding the basic demographics of the study sample were analyzed as simple descriptive statistics (counts and percentages). The basic composition of the study sample was compared with the overall composition of the clinics from which the samples were drawn to determine any significant differences in demographics between the sample group and the clinic population using GraphPad (San Diego, CA) Chi Square (χ^2) analysis online software.

To determine the appropriate sample size for this type of PCR screening for microbial composition using DNA extracted from saliva, the recovery rate from the sample-limited step of DNA extraction was used (90 - 95%) to establish the minimum expected difference of 0.10 or 10% . Using a significance level of α = 0.05 and a power p = 0.80, a minimum sample size of fifty (N = 50) was calculated [24].

Results

The existing saliva sample repository was screened for samples from pediatric patients that were undergoing orthodontic treatment, which revealed a total potential study sample size of n = 156 (Table 1). The analysis of the demographic information regarding these samples revealed that slightly more than half were derived from female patients (56.4%), which is not significantly different from the overall orthodontic clinic population. However, the overwhelming majority of saliva samples identified were derived from minority patients (91%), which is significantly higher than the overall percentage from the clinic population (58.6%). The average age for the patient samples identified for this study was 13.5 years, which is lower than the overall average age for all pediatric, orthodontic patients from the clinic (15.8 years) with a range between 11 and 17 years of age.

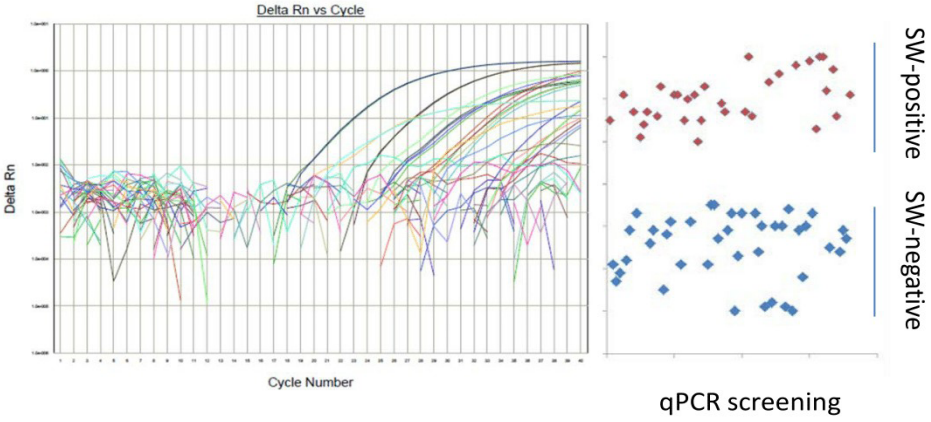
	Study sample (n = 156)	Clinic population	Statistical analysis
Sex			
Female	n = 88 (56.4%)	50.9%	$c^2 = 1.468$, d.f. = 1
Male	n = 68 (43.6%)	49.1%	$p = 0.2257$
Race/Ethnicity			
White	n = 14 (9.0%)	41.4%	$c^2 = 60.286$, d.f. = 1
Minority	n = 142 (91.0%)	58.6%	$p<0.0001$
Hispanic	n = 100 (64.1%)	35.9%	
Black	n = 10 (6.4%)	13.1%	
Asian/Other	n = 32 (20.5%)	4.2%	
Age			
Average	13.5 yrs.	Under 18 (15.8 yrs.) Over 18 (21.4 yrs.) Combined: 18.6 yrs.	
Range	11-17 yrs.	11-38 yrs.	

DNA isolation was then performed on each of the identified samples, n = 156 (Table 2). Some samples identified for this study had insufficient volume remaining to perform the DNA isolation procedure (n = 49), which represented 31.4% of the potential study sample. Although DNA was isolated from n = 102 samples, only n = 72 had sufficient DNA quantity (> 0.1 ug/mL) and sufficient DNA quality (A260:A280 ratio > 1.65) for subsequent qPCR screening. This represented only 66.4% of the previously identified samples. The percentage of samples from females and males was roughly equal at each step of the screening process (sufficient volume, successful DNA recovery), while the percentage of samples from non-minority (White) patients remained fairly constant (~10%).

	Sufficient volume	DNA recovery
Total samples (n = 156)	n = 107/156 (68.5%)	n = 72/107 (67.3%)
Female	n = 55/107 (51.4%)	n = 39/72 (54.2%)
Male	n = 52/107 (48.6%)	n = 33/72 (45.8%)
White	n = 11/107 (10.3%)	n = 6/72 (8.3%)
Minority	n = 96/107 (89.7%)	n = 66/72 (91.7%)
	[DNA] = 396.2 ng/uL	[DNA] = 335.1 ng/uL
	A260:A280: 1.12 - 2.0	A260:A280: 1.65 - 2.0

Table 2: *DNA isolation and screening.*

All DNA isolates that had sufficient DNA quantity (> 0.1 ug/mL) and purity (A260:A280 ratio > 1.65) were then screened using qPCR for the presence of *Scardovia wiggsiae* (Figure 1). These results revealed that slightly less than half of the samples (44.4% or n = 32/72) harbored DNA for this organism, with the remainder testing negative. The analysis of these data revealed that the SW-positive and SW-negative samples were nearly equally distributed among males and females, which was similar to the overall sample composition (p = 0.6877). In addition, the percentages of SW-positive and SW-negative samples that were obtained from minority patients was also similar to the overall sample composition at approximately 90% (p = 0.7124).



	SW-positive (n=32)	SW-negative (n=40)	Statistical analysis
Sex			
Female	53.1% (n=17)	55.0% (n=22)	$\chi 2$=0.162, d.f.=1
Male	46.9% (n=15)	45.0% (n=18)	p=0.6877
Race/Ethnicity			
White	9.4% (n=3)	7.5% (n=3)	$\chi 2$=0.136 d.f.=1
Minority	90.6% (n=29)	92.5% (n=37)	p=0.7124

Figure 1: *Scardovia wiggsiae qPCR saliva screening results. DNA isolates from each saliva sample were screened for of S. wiggsiae, with 44.4% (n = 32/72) testing SW-positive. These were nearly equally distributed among females and males, with correspondingly similar percentages of SW-positive and SW-negative samples coming from minorities (90.6% and 92.5%, respectively).*

Discussion

The primary objective of this study was to examine the oral prevalence of *S. wiggsiae* among pediatric patients within the patient population of the public dental school in Nevada using an existing saliva repository. Although a large number of samples were identified for inclusion in this study (n = 162), approximately one-third did not contained sufficient volume for processing, which resulted in a final sample size with sufficient DNA quality and quantity of less than half the original number (n = 72). However, this was greater than the minimum sample size needed derived from the initial sample size estimated (n = 50) from the power calculation.

Other studies from this institution have determined the prevalence of *S. wiggsiae* using adult and pediatric samples from this saliva sample repository and patient population. These studies demonstrated that only about one-fifth of adults and approximately one-fourth of pediatric patient saliva samples harbored DNA from this organism [17,20]. The results of this current study suggest that pediatric patients with orthodontic appliances may have increased prevalence of oral *S. wiggsiae*. However, a more appropriate comparison may include an analysis SW-prevalence among other orthodontic patients.

One of these previous studies included *S. wiggsiae* screening among both adult orthodontic and non-orthodontic patients, which demonstrated prevalence of 19% and 14%, respectively. These data suggested that the prevalence among adult orthodontic patients may be lower than adults without orthodontic brackets. Although a small pilot study of pediatric orthodontic patients at this institution (n = 48) revealed a somewhat higher prevalence 31.3%, the results of the current study of pediatric orthodontic patients clearly demonstrated a much higher prevalence (44.4%) of this organism than the two previous studies of non-orthodontic pediatric patients undertaken at this institution (21.3%, 26.3%), which suggests that pediatric patient populations may be at higher risk for harboring this organism while undergoing orthodontic treatment [21].

Despite the significance that this study is among the first to screen for *S. wiggsiae* among pediatric orthodontic patients, there are some limitations inherent to this study design which must also be considered. The most important of these considerations is the retrospective nature of this study, which limited the quality (and quantity) of saliva samples available for testing after long-term storage [25,26]. An additional consideration, also related to the retrospective nature of this study, is the lack of temporal information regarding *Scardovia* prevalence. For example, no longitudinal data are available to determine if the prevalence of this organism increases among the same patients after orthodontic bracket placement or if some other as yet unidentified factors may explain these results.

Based upon these factors, it is imperative that longitudinal studies of this organism be undertaken to determine if the placement of orthodontic brackets is sufficient to alter the prevalence of *S. wiggsiae* among these various groups of patients. In addition, studies that evaluate and compare these results for adults, as well as pediatric patients, are important if oral health researchers are to determine the potential for disease risks and contributions made to the oral health of orthodontic patients.

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