ORIGINAL RESEARCH ARTICLE

Scardovia wiggsiae: Role in Periodontal Health and Disease – A Pilot Study

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ABSTRACT

Introduction: *Scardovia wiggsiae* is an anaerobic, pleomorphic Gram-positive bacillus isolated from the human oral cavity. It has been found to be strongly associated with severe early childhood caries, and a significantly increased prevalence of gingivitis was demonstrated in these patients. *S. wiggsiae* was also identified among caries-free children. In addition, this bacillus has also been isolated in periodontal pockets of adults. At present, the role of this organism in periodontal health and disease is ambiguous at best. **Objectives:** The objectives of the present study were to assess the level of *S. wiggsiae* in subgingival plaque in periodontally healthy subjects and patients with gingivitis and periodontitis and to compare the levels of *S. wiggsiae* in subgingival plaque in periodontally healthy subjects and patients with gingivitis and periodontitis. **Methodology:** Patients were categorized, based on clinical parameters, into periodontally healthy, gingivitis, and periodontitis groups, with five patients in each. Subgingival plaque samples were collected for all groups. *S. wiggsiae* level in the samples was determined by real-time polymerase chain reaction (rt-PCR) method. Data were analyzed statistically by the Kruskal–Wallis test, followed by Mann–Whitney U-test and ANOVA with *post hoc* analysis. **Results:** rt-PCR analysis demonstrated the decreased prevalence of *S. wiggsiae* among the gingivitis and periodontitis groups as compared to the periodontally healthy subjects. **Conclusion**: The above-mentioned results indicate some role of this bacteria in the progression of periodontal disease. Larger-scale and interventional studies are required for a better understanding of the exact nature of its role.

Key words: ANOVA, clinical parameters, novel bacillus, periodontitis, plaque, real-time polymerase chain reaction, *Scardovia wiggsiae*

INTRODUCTION

The role of oral microbiota in the etiology of periodontal disease has been well established.[1] Initially, it was thought to consist of five main microbial complexes.[2] However, in recent years, a number of studies using molecular techniques have been published, thus confirming the presence of new micro-organisms responsible for periodontal disease.[3-6]

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Scardovia wiggsiae is a novel anaerobic, pleomorphic Gram-positive bacillus, isolated from the human oral cavity and an infected arm wound.[7] *S. wiggsiae* not only displayed moderate associations with gingivitis among children undergoing fixed orthodontic therapy but also was also subsequently isolated in periodontal pockets of adults undergoing the same treatment.[8-10]

The precise etiological and pathological role of this organism in periodontal health and disease are thus, still ambiguous. To the best of our knowledge, no pre-existing literature on the quantification of *S. wiggsiae* in various periodontal health and disease states is available. Therefore, the aim of the present study was to detect and compare the level of *S. wiggsiae* in the subgingival plaque of patients with gingivitis and chronic periodontitis.

Objectives

The objectives are as follows

1. To assess the levels of *S. wiggsiae* in subgingival plaque in patients with healthy periodontium, gingivitis, and chronic periodontitis.
2. To compare the levels of *S. wiggsiae* in subgingival plaque in patients with healthy periodontium, gingivitis, and chronic periodontitis.

MATERIALS AND METHODS

Study Population

Individuals visiting the Out Patient Department of Periodontics, College of Dental Sciences, Davangere, seeking dental treatment.

Sampling Technique

This was a random sampling technique.

Sample Size

For the study, 15 individuals in the age group of 20–55 years were selected and divided into three groups (five patients in each group):

1. Group 1 – Periodontally healthy subjects.
2. Group 2 – Patients with gingivitis.
3. Group 3 – Patients with chronic periodontitis.

Inclusion Criteria

All the outpatients reporting for treatment at the dental hospital who had visited a total minimum number of twice were included in the study until the sample size is achieved.

Exclusion Criteria

The following criteria were excluded from the study:

1. Individuals with systemic conditions.
2. Individuals who have had periodontal therapy in the past 6 months.
3. Pregnant and lactating females.
4. Individuals who are smokers.
5. Individuals who consume alcohol.

Clinical Parameters

The following clinical parameters were recorded:

1. Plaque Index (Silness and Loe, 1964).
2. Gingival bleeding index (Ainamo and Bay, 1975).
3. Probing Pocket Depth with UNC 15 probe.

Sample Collection

After recording the clinical parameters, the selection of the sample site and assignment of patients to the appropriate study group were done. Pooled plaque samples from each subject were collected with sterile curettes and placed in labeled Eppendorf Safe-Lock Tubes Tris-EDTA (TE) buffer solution. Said tubes were transported to the laboratory for the real-time polymerase chain reaction (rt-PCR) procedure.

DNA Extraction Procedure (Modified Proteinase-K Method)

Samples were vortexed (Bio-Rad, California, USA) for few seconds, followed by centrifugation at 5000 rpm for 5 min. The supernatant was discarded, and 500 µl of fresh TE buffer was added and centrifuged again for 3–4 min. This procedure was repeated another 3–4 times with fresh TE buffer following which the supernatant was discarded. 50 µl of Lysis Buffer I solution was added, vortexed, and kept for 5 min. The same procedure was repeated with 50 µl of Lysis Buffer II solution and 10 µl of proteinase – K (100 µg/ml) (Chromous Biotech, Bangalore, India). It was kept in a water bath for 2 h then kept in boiling water bath (Bio-bee Tech, Bangalore, India) for 10 min, and the DNA was stored at 20 C [Figure 1].

TE Buffer

0.5 ml 1M Tris Buffer, 100 µl 0.5 M, and distilled water made to 50 ml.

Lysis Buffer I

500 µl 1M Tris buffer, 500 µl Triton X-100, 100 µl 0.5M EDTA, and distilled water made to 50 ml.

Lysis Buffer II

50 mM Tris HCl (pH 8.0), 50 mM KCl, 2.5 mM $MgCl_2$, 0.45% Tween 20, 0.45% Nodient P-40.

rt-PCR Procedure

Reagents

PCR primers (Specific for S. wiggsiae), rt-PCR master mix (Syber green based), molecular grade water, and DNA extracts (template).

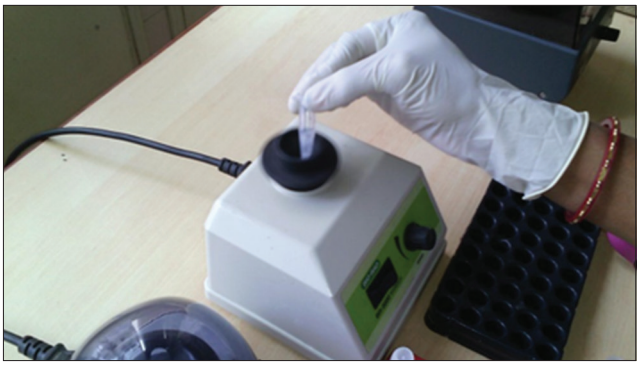

Figure 1: DNA extraction procedure

S. wiggsiae primers

The following set of PCR primers were used in the study (Bioserve India pvt Ltd, Hyderabad, India):

- *S. wiggsiae* - F 5'- GTG GAC TTT ATG AAT AAG C-3'.
- *S. wiggsiae* - R 5'- CTA CCG TTA AGC AGT AAG-3'.

rt-PCR master mix

Roche's FastStart Universal SYBR green PCR master mix (Roche, Basel, Switzerland), which is a double-concentrated master mix that contains FastStart Taq DNA Polymerase, reaction Buffer, nucleotides (dATP, dCTP, dGTP, and dUTP), and SYBR Green I. SYBR Green I is a DNA double-strand-specific dye. During each phase of DNA synthesis, the SYBR Green I dye, which is included in the reaction mix, binds to the amplified PCR products; the amplicon can be detected by its fluorescence.

Procedure

A premixture with a volume of 20 μl was prepared and aliquoted into each tube. The PCR reaction mixture was prepared by gently vortexing and briefly centrifuging PCR master mix after thawing. A thin-walled PCR tube was placed on ice, and the following components were added for each 20 μl reaction:

10 μl FastStart Universal SYBR green master mix, 1 μl (10 pmole) *S. wiggsiae* (forward primer), 1 μl (10 pmole) *S. wiggsiae* (reverse primer), 2 μl (<1 μg/reaction) template DNA, and water was added to make final volume to 20 μl.

The samples were gently vortexed and span down. Finally, the tubes were placed in real-time thermal cycler (Mastercycler ep, Eppendorf). Initial denaturation was performed at 95 C for 5 min followed by 40 cycles of denaturation at 94 C for 20 s, annealing at 51 C for 20 s, and finally extension 72 C for 20 s [Figure 2].

Analysis

The SYBR Green dye binds with double-stranded DNAs, which were specifically amplified by *S. wiggsiae* specific primers. The fluorescence was captured by the detector, which generates a graph in the Realplex software as the amount of fluorescence against the number of cycle. The dye emits fluorescence in the form of peaks. The cycle number at which fluorescence has initiated is called as "ct value."

Figure 2: Real-time polymerase chain reaction apparatus

Standard stain of *S. wiggsiae* (CCUG 58090) was used in the study. The standard strains were grown on a specific medium; inoculum was put into the broth and allowed to grow depending on the growth requirement. The turbidity of the broth was adjusted to 0.5 McFarland standard to obtain a quantity of 10^8 CFU/ml. DNA was extracted from this standard sample. Serial dilutions of this DNA sample were made, ranging from 10^8 to 10^2 CFU/ml. PCR was run for the standard samples to obtain a standard curve. The standard graph was plotted; ct values versus quantity. Test samples were run and cycle threshold ("ct" – cycle number at which peak has generated) values for respective samples were obtained. Quantities were obtained by plotting "ct" value of each sample on the standard curve.

Statistical Analysis

The data obtained from three groups were analyzed using the following statistical analyses:

1. ANOVA.
2. *Post hoc* Tukey analysis.

RESULTS

On analysis with rt-PCR, a standard curve ("ct" values vs. fluorescence) was obtained, as shown in Figure 3.

The number of *S. wiggsiae* cells per sample is tabulated below, where "H" represents periodontally healthy group, "G" represents the

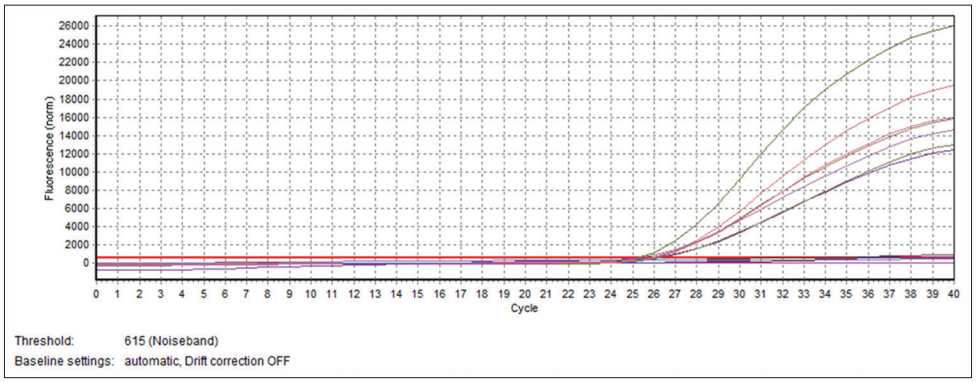

Figure 3: Standard curve: Green → Periodontally healthy, Blue → Gingivitis, Red → Chronic periodontitis

gingivitis patients, and "P" represents the chronic periodontitis patients [Table 1].

The samples collected from periodontally healthy patients, numbered H1-H5, on rt-PCR quantification revealed *S. wiggsiae* counts of 8.49 $\times 10^{10}$ –4.68 10^{11}. Similarly, the gingivitis patients (G1-G5) were 6.72 $\times$ 10^{9}–9.02 $\times$ 10^{10} and chronic periodontitis patients (P1-P5) were 4.21 $\times$ 10^{9}–3.75 $\times 10^{10}$.

ANOVA test reveals a significant difference between the groups (P = 0.14) and is tabulated in Table 2

A *post hoc* Tukey's analysis was done, and the results are tabulated in Table 3.

The analysis reveals significant differences between the *S. wiggsiae* counts in healthy (H) and chronic periodontitis (P) groups. Gingivitis group has a statistically significant difference to healthy group at 20% confidence level.

DISCUSSION

The oral cavity has one of the largest and most diverse microbiota, harboring over 700 species of bacteria. The mouth, with its various niches, is an exceptionally complex habitat where microbes colonize the hard surfaces of the teeth and the soft tissues of the oral mucosa.[11] Many patients with poor oral health often harbor multiple disease-causing organisms that may contribute to one or more pathologies within the oral cavity. Recently, a new oral pathogen *S. wiggsiae* was discovered in patients with poor oral health.[12-15] Although some screenings for *Scardovia* are now beginning to emerge, much remains to be discovered about the oral ecology and microbial interactions that facilitate or inhibit the growth of this organism.

Table 1: Quantification of *S. wiggsiae* in different groups using rt-PCR

Sample No.	Sample codes	Result *S. wiggsiae* (Number of cells/sample)
1	H1	$8.49x10^{10}$
2	H2	$4.68x10^{11}$
3	H3	$1.44x10^{11}$
4	H4	$9.98x10^{10}$
5	H5	$3.62\ x10^{11}$
6	G1	$6.42x10^{10}$
7	G2	$9.02x10^{10}$
8	G3	$8.28x10^{10}$
9	G4	$6.72x10^{9}$
10	G5	$7.03x10^{10}$
11	P1	$3.75\ x10^{10}$
12	P2	$1.29x10^{10}$
13	P3	$2.48x10^{10}$
14	P4	$6.56x10^{9}$
15	P5	$4.21x10^{9}$

S. wiggsiae: *Scardovia wiggsiae*, rt-PCR: Real-time polymerase chain reaction

Table 2: Inter-group analysis using ANOVA

	Sum of squares	df	Mean square	F	Significance
Between groups	$1.277\ 10^{23}$	2	$6.386\ 10^{22}$	6.152	0.014
Within groups	$1.246\ 10^{23}$	12	$1.038\ 10^{22}$		
Total	$2.523\ 10^{23}$	14			

F: F statistic, df: Degree of freedom

A study was conducted by Tanner *et al.* to evaluate the microbiota of severe early childhood caries (S-ECC).[12] Although strongly associated

Table 3: *Post hoc* inter-group analysis using Tukey's test

(I) Groups	(J) Groups	Mean difference (I-J)	Standard error	Significance
H	G	1.689 10^{11}	64440962190.20943	0.054
	P	2.145 10^{11}	64440962190.20943	0.015
G	H	1.689 10^{11}	64440962190.20943	0.054
	P	4.565 10^{10}	64440962190.20943	0.763
P	H	2.145 10^{11}	64440962190.20943	0.015
	G	4.565 10^{10}	64440962190.20943	0.763

H Periodontally healthy, G Gingivitis, P Chronic periodontitis, $P \leq 0.015$ is statistically significant

with *Streptococcus mutans*, S-ECC had also been linked with a widely diverse microbiota. The results showed that *S. wiggsiae* was significantly associated with S-ECC irrespective of the presence of *S. mutans*. Children with S-ECC also displayed significantly higher Plaque and Gingival Index scores. However, it was noted that 20–40% of the children in whom *S. wiggsiae* was isolated were caries-free.

Building on their previous work regarding this bacteria, another study was conducted by the same authors to characterize the plaque microbiota of children undergoing fixed orthodontic therapy, having white spot lesions (WSL) and gingivitis.[8] The results showed *S. wiggsiae* displayed associations with the presence of WSL and moderate associations with gingivitis. The bacteria were, again, isolated in patients without WSL (detection frequencies in WSL vs. No WSL $P = 0.07$) and without gingivitis (mean count in gingivitis vs. no gingivitis $P = 0.074$).

In a separate study, saliva samples were collected from adult orthodontic patients and non-orthodontic patients by Streiff *et al.* to analyze the presence of S. wiggsiae, *S. mutans* and *Porphyromonas gingivalis*.[9] It was inferred that the prevalence of *S. wiggsiae* was significantly lower in orthodontic samples than non-orthodontic ones. It was also noted that the average pocket probing depth was higher when associated with *P. gingivalis* and S. wiggsiae. Thus, with this bacteria having been isolated in gingivitis lesions as well as in periodontal pockets, it piqued our curiosity as to whether it played any role in initiation and progression of periodontal disease.

The results acquired in our study appear to illustrate a decreasing gradient of *S. wiggsiae* as periodontal disease progresses. It has been quantified maximally in periodontally healthy patients and minimally in chronic periodontitis patients ($P = 0.015$). This leads us to speculate that *S. wiggsiae*, a well-established acidogenic and aciduric bacteria, possibly cannot tolerate the increasingly anaerobic environment which generally prevails in severe forms of periodontal disease. In the previous studies, *S. wiggsiae* has been seen to have an association with gingivitis lesions among orthodontic therapy patients. This would suggest it is present in higher levels among gingivitis patients than periodontally healthy patients. It is noteworthy, however, that in the previous studies, authors have used saliva to quantify the bacteria, whereas, in the present study, subgingival plaque has been used.

CONCLUSION

The present study demonstrated a decreasing concentration of the bacteria *S. wiggsiae* found in subgingival plaque in periodontally healthy, gingivitis, and chronic periodontitis patients respectively. The fact that this bacillus has been implicated in early childhood caries may be of some relevance to this inverse relationship. Further studies, however, are required to elucidate the role, if any, of *S. wiggsiae* in periodontal health and disease.

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