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A Novel Approach for Instant Quantification of Bacterial Load During Root Canal Therapy Using Quantitative Light Induced Fluorescence - A Pilot Study

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Abstract

Background: Root canal treatments are undertaken to eradicate bacterial infections from root canals and retain the natural tooth. The persistence of microorganisms in the root canal space even after chemo-mechanical preparation is one of the common reasons for root canal failure. The current methods to evaluate the presence of bacteria after therapy is not widely practiced because of the lack of a rapid chairside method. The present study demonstrates the use of QLF in this field.

Methods: Root canal sample was collected from the patient's infected tooth with a history of pain and swelling. Exudate samples were taken using sterile paper points after access opening and extirpation in the canal (S1) and after biomechanical preparation and irrigation (S2). Intracanal medicament was given and recalled after one week, again exudate samples were collected (S3). QLF images were obtained with QLF-D Biluminator™ for all samples.

Result: The analysis was done using QA3 version 1.26 Software. The extent of redness in the blue light image depicted the bacterial load instantly and the analysis depicted the reduction of the Mean Bacterial Load based on SPS scoring and ΔR values objectively.

Conclusion: It is possible to state that QLF is a highly precise and reliable tool for the instant prediction of bacterial load in the root canal system based on the presence of porphyrins. Our concept of QLF in endodontics instantly help us to visualize the bacterial load before and after treatment.

Keywords: QLF; Endodontic therapy; Rapid chairside method; Bacterial load

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Introduction

The aim of root canal therapy is to eliminate the pathogenic bacteria in the root canal system of the teeth (BYSTROM 1987). Methods to analyze the extent bacteria persisted in the root canal system during treatment includes culturing techniques, subjective observations, DNA-based Polymerase Chain Reaction (PCR) and reverse-transcriptase PCR. Culture methods are laborious to conduct, and it takes several days to weeks to identify anaerobic bacterial species. PCR suffers from high false-positive readings. In the recent past relatively faster methods like fluorescent staining method and ATP essay method were discussed; which again required multiple processing steps and substantial laboratory instrumentation making them unfeasible for routine intraoperative application. Therefore, there is a need for a rapid chairside method to assess the bacterial load during root canal treatment [1].

Quantitative Light-induced Fluorescence (QLF) technology is a representative example of a multifunctional device used in dentistry. QLF technology is mainly used for diagnosing early caries and monitoring remineralization. Recently it has been used not only for detecting cracks and bacterial deposits such as dental plaque and calculus but also for antimicrobial treatment [2]. Quantitative Light-induced Fluorescence (QLF) technology based on the optical phenomenon has been used for detecting and quantifying bacterial deposits and bacteria-related lesions by using blue and white Light-Emitting Diode (LED) lights with a peak wavelength of 405 ± 7 nm and a modified filter set. This optical phenomenon can be explained by endogenous metal-free fluorescent porphyrin, such as protoporphyrin IX as a main component, produced by certain oral microorganisms exhibiting a strong fluorescence in the red spectral region when excited with violet light ranging from 400 nm to 420 nm [3].

Based on the scientific theoretical knowledge it can be hypothesized that QLF can be used to detect the root canal bacterial load instantly. Thus, it can provide as an adjunct chairside diagnostic aid for rapid detection during the course of root canal treatment. Present pilot study aims the application of QLF chairside to instantly detect and quantify the bacterial load in the root canal system [4].

Materials and Methods

The present study was an observational pilot type. The participants were collected in Department of Pediatric & Preventive Dentistry, JSS Dental Collage and Hospital, Mysuru, India. The Ethics Committee of JSSAHER (Reference no. 78/2020) approved this study. The parents/guardians of the participants signed individual informed consent forms containing information about the aim of the study and the treatment procedures. Children between the ages of 6 to 8 years were recruited and data concerning QLF sampling were collected. The criteria for case selection were irreversible pulpitis with necrotic pulp [5-7].

Clinical characteristics, defined as spontaneous pain and the presence of a deep carious lesion with necrotic pulp with gingival abscesses or fistula openings absent or present.

Radiographic evaluation revealing that the maxillary incisors had no internal resorption, with or without periapical radiolucency and no physiological root resorption.

Teeth with severely broken-down coronal tooth structures that could jeopardize leakage-free sampling conditions, teeth with prior root canal fillings, teeth with root canals filled with calcium hydroxide, and teeth presenting with vital or inflamed pulp tissues upon access were excluded [8].

The total sample size of was calculated considering standard deviation of 4 based on pilot work with a margin error of 5%, confidence level of 95%, a power of 80%. The sample size found was 20 children. Single pediatric dentist treated each incisor involved in two visits. At the first appointment, after disinfecting the operative field the incisor was isolated with a rubber dam following local anesthesia. The pulp chamber was accessed after removal of all carious tooth structures. Before any root canal instrumentation, first exudate sample (S1) was collected (Figure 1) using sterile paper point (size 25) was left inside the canal for 15 sec. Pulpal debris were removed with barbed broaches. The working length was determined by superimposing an endodontic instrument over the preoperative radiograph and keeping it 1 mm to 2 mm short of the radiographic apex. Cleaning and shaping of the root canals were carried out using k files (MANI Inc., Tochigi, Japan). The files were used sequentially in a pullback direction up to a maximum size of 35 to 40. Continuous irrigation with 2.5% sodium hypochlorite was carried out throughout the procedure. Post-cleaning and shaping second exudate sample (S2) was collected using sterile paper point (size 25). Following which sterile paper points were used to dry the root canals. Calcium hydroxide (RC-Cal, Prime Dental Products PVT LTD) was injected into the root canal and a sterile cotton ball was placed in the pulp chamber and sealed with Zinc-oxide Eugenol (3M ESPE, St. Paul, MN, USA) as temporary sealing material. At the second visit, which was planned one weeks later, the tooth was isolated with a rubber dam, and the operative field was disinfected as described above. The temporary cement was removed. The tooth was rinsed out of the intracanal medicament with sterile saline solution and third exudate sample (S3) was collected using sterile paper point (size 25). Then the root canal was obturated and restored.

QLF imaging

QLF images of the exudate samples collected at three-time interval (S1, S2 and S3) were obtained by a single trained examiner using QLF-D Biluminator™ device (Inspektor Research Systems BV, Netherlands) (Figure 2) under class 1 ASA darkroom conditions at the following setting. For white light images shutter speed of 1/30 s, aperture value of 20.0, and ISO speed of 1600 and for fluorescent light images shutter speed of 1/10 s, aperture value 10.0, and ISO speed of 1600 for blue light images were used. The distance between the specimen and the QLF-D camera was standardized at 1 cm and at 90-degree angulation. Figure 3 shows blue light image of a single exudate sample obtained from QLF at different intervals [9].

QLF analysis

The QLF images were then analyzed using the QLF software (QA2 v 1.26, Inspektor Research Systems BV, Amsterdam, The Netherlands). Figure 4 shows analysis of a single exudate sample using QA2 at different intervals.

The Simple Plaque Score (SPS) was used for quantitative and qualitative assessment of exudate samples, and scores ranging from 0 to 5 points were assigned according to the attached area of smear using QA2 v 1.23, a QLF-D analysis program [10].

QLF-D ΔR score

ΔR score gives the percentage of increase of the ratio of red and green component to the sound tissue which is related to the presence of porphyrins and therefore indirectly to the bacterial activity. A strong red fluorescence can be seen with a greater degree of active bacterial metabolism. In this study, the exudate samples were assessed with sub-scores of ΔR30, ΔR70, and ΔR120 according to the fluorescence intensity. Higher ΔR values indicate areas with more active bacterial metabolism within the sample [11-22].

Statistical analysis

The data obtained were analyzed with the help of SPSS-PC v 24. Arithmetic means and Standard Deviation (S.D) were used for descriptive statistics.

Results

Intra group comparison of ΔR values from QLF

Group comparison of means at different time intervals was done by one-way ANOVA.

Table 1 shows the Mean and Deviations of Area ΔR of different samples at multiple intervals.

ΔR values which indicate red fluorescence produced as a result of the presence of porphyrins on the paper points also showed a reduction from Immediately after access interval (S1) to one week follow up after intracanal medicament (S3). And the difference was highly significant.

Table 2 shows Mean differences between intervals for ΔR values. Statistically significant mean differences between all intervals were observed for ΔR values.

SPS scoring

At S1(After Access) interval 85% of samples had SPS scoring of 5. During S2 (After BMP) SPS score reduced with 50% sample showing score 4 or less. At S3 interval (After intra canal medicament) SPS

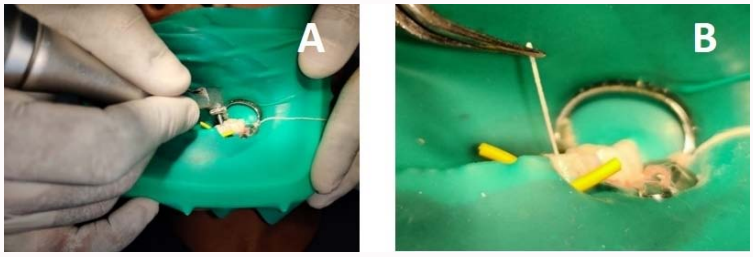

Figure 1: Access opening using contra angle hand piece (B) Exudate sample collection with the help of paper points.

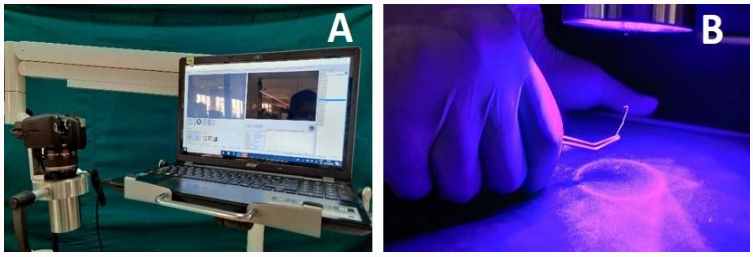

Figure 2: QLF-D Biluminator 2 Equipment (B) Blue light imaging of exudate samples.

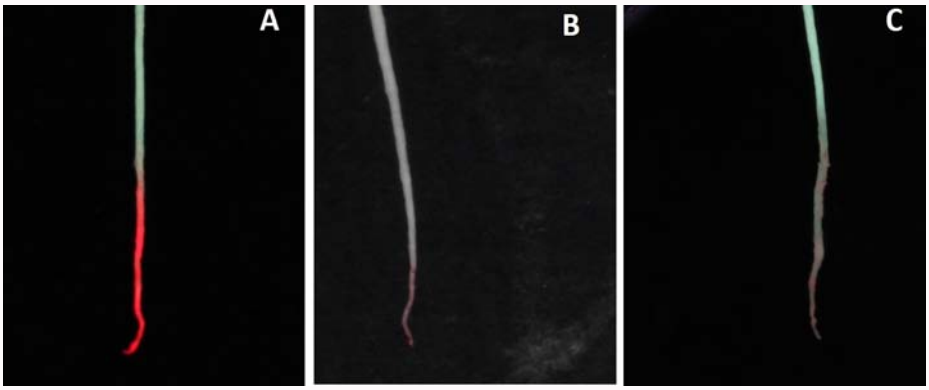

Figure 3: Blue Light of exudate samples obtained (A) After access opening (B) After Irrigation (C) After Intracanal medicament placement.

scoring further reduced with 80% of samples showing a score of 1 Figure 5.

Discussion

Even though the goal of Root canal treatment is to eliminate bacteria from the root canal system of infected teeth in endodontic therapy, assessment of the degree of disinfection is currently not being performed routinely because of the lack of an instant chairside method. The present study showed that the fluorescence-based QLF detection method can be used for this purpose and can help clinicians to determine the degree of disinfection during the course of treatment. The QLF method serves as a convenient tool compared to the time-consuming and laborious bacterial culture method.

SPS scores and ΔR values obtained from QLF showed a significant reduction of bacterial load pre- and post-chemo-mechanical preparation of canals and subsequently after intracanal medicament placement. Marked reduction in SPS scores were obtained from S1 interval to S2 and subsequently to S3 interval. The ΔR scores which indicate red fluorescence was also observed to decrease from S1 to S3 which indicates in subsequent intervals the bacterial load has reduced significantly.

The limitation of the method illustrated in the present study is that it does not allow the identification of endodontic microbiota present in an infected root canal and another limitation of this method will be that QLF can detect only porphyrins producing bacteria. Bacterial strains like *E. faecalis* commonly found in reinfected root canals might not exhibit red fluorescence under QLF. However, such information is of limited value because endodontic infections are polymicrobial in nature, could possess heterogeneous etiology, and may vary between individuals.

The sampling method is, of course, critical, and the current study has applied the method designed in a previous study. Sterility checks during the sampling process was done to prevent potential contamination. To ensure comparable microbial load reductions among samples before and after, chemo-mechanical debridement and then further chemical disinfection using an interappointment dressing were performed.

Because paper point sampling is limited to the pathway created by endodontic instruments, detection within inaccessible structures (e.g., lateral canals) is impossible. However, endodontic paper points have the benefit of being highly absorbent and flexible, enhancing

Table 1: Mean and standard deviations of area ΔR of different samples according to the intervals evaluated (n=20).

	Interval	Mean	SD	F	Sig.
Area ΔR30	After access opening (S1)	58.60	7.92		
	After BMP(S2)	39.10	4.14	611.89	0.001***
	After intra canal Medicament (S3)	2.30	0.47		
Area ΔR70	After access opening (S1)	50.98	6.67		
	After BMP(S2)	32.07	4.42	592.31	0.001***
	After intra canal Medicament (S3)	1.16	0.11		
Area ΔR120	After access opening (S1)	48.10	9.57		
	After BMP(S2)	22.72	3.77	326.29	0.001***
	After intra canal Medicament (S3)	0.14	0.07		

p>0.5 highly significant

Table 2: Mean differences between intervals for ΔR values.

	Time interval	Time interval	Mean Difference	Std. Error	Sig.
Area ΔR30	After access opening (S1)	After BMP (S2)	19.5*	1.63	0.001***
	After access opening (S1)	After intra canal Medicament (S3)	56.3*	1.63	0.001***
	After BMP(S2)	After intra canal Medicament (S3)	36.8*	1.63	0.001***
Area ΔR70	After access opening (S1)	After BMP (S2)	18.91*	1.46	0.001***
	After access opening (S1)	After intra canal Medicament (S3)	49.82*	1.46	0.001***
	After BMP(S2)	After intra canal Medicament (S3)	30.91*	1.46	0.001***
Area ΔR120	After access opening (S1)		25.38*	1.87	0.001***
	After access opening (S1)	After intra canal Medicament (S3)	47.96*	1.87	0.001***
	After BMP(S2)	After intra canal Medicament (S3)	22.58*	1.87	0.001***

p>0.5 highly significant

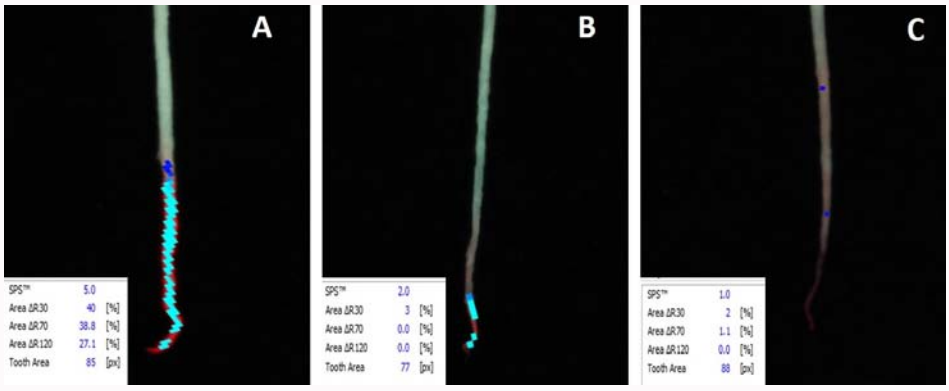

Figure 4: Analysis using QA3 version 1.26 (A) After access opening (B) After Irrigation (C) After Intracanal medicament placement.

the detection of vital cells in the root canal, as well as at the entrance to the lateral canals, which remain untouched by mechanical instrumentation.

An alternative method for instant detection and quantification of root canal bacterial load was demonstrated by Sato et al. developed in Japan. The method was able to quantify the bacteria in a sample of infected root canals in about 20 min, indicating that it is useful for clinical bacterial examination during the course of the treatment in infected root canals when evaluating the outcomes of the treatment.

Another approach demonstrated by Herzog et al. relies on the fluorescence emission from biofilm by-products such as porphyrins and cannot therefore indicate the presence of vital cells and bacteria in particular. Furthermore, it remains unclear whether the sensitivity of such autofluorescence measurements would be sufficient to detect very low quantities of bacteria.

However, the lack of feasibility and methods being technique sensitive makes it impractical to perform these as a chairside technique on a daily basis. On the other hand, QLF is a simple, user-friendly, and non-invasive method for detecting the bacterial load during the course of the treatment in infected root canals which does not require any separate laboratory procedures.

Conclusion

This is the first clinical approach testing the use of QLF on root canal samples as a rapid tool to predict bacterial load based on presence of porphyrins. Thus, this study envisions that the availability of QLF as a rapid chairside diagnostic test will be useful as an adjunct in root canal treatment. However, further research is required validating present technique with the gold standard cultural methods.

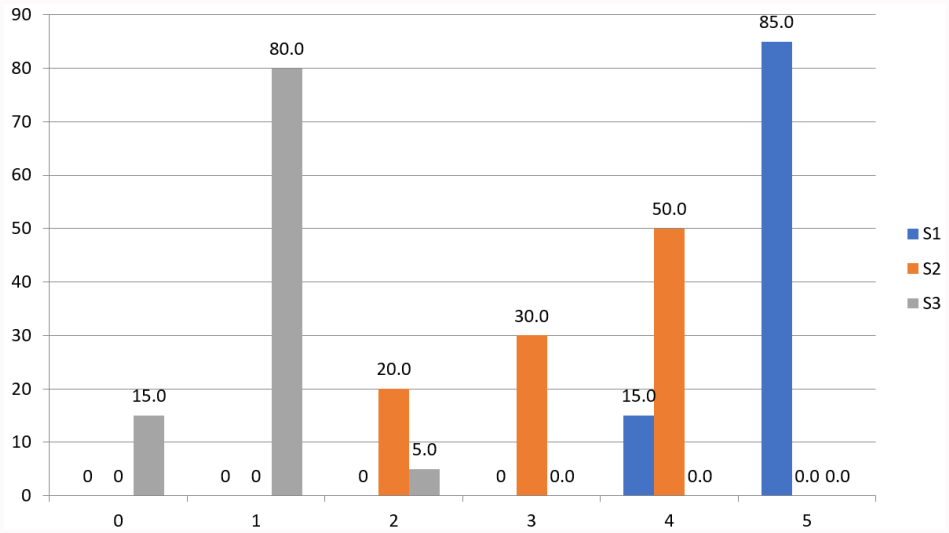

Figure 5: Graphical representation of SPS scoring during 3 different intervals (S1, S2, S3).

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