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The Use of Different Irrigation Techniques to Decrease Bacterial Loads in Healthy and Diabetic Patients with Asymptomatic Apical Periodontitis

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Abstract

BACKGROUND: Diabetes mellitus is a multisystem disease which weakens the human's immunity. Subsequently, it worsens the sequelae of apical periodontitis by raising a fierce bacterial trait due to the impaired host response.

AIM: This study aimed to estimate bacterial reduction after using different irrigation techniques in systemically healthy and diabetic patients with asymptomatic apical periodontitis.

MATERIAL AND METHODS: *Enterococcus faecalis, Peptostreptococcus micros*, and *Fusobacterium necleatum* bacteria were chosen, as they are the most common and prevailing strains found in periodontitis. Bacterial samples were retrieved from necrotic root canals of systemically healthy and diabetic patients, before and after endodontic cleaning and shaping by using two different irrigation techniques; the conventional one and the EndoVac system. Quantitive polymerase chain reaction (qPCR) was utilised to detect the reduction in the bacterial count.

RESULTS: The EndoVac irrigation system was effective in reducing bacteria, especially *Peptostreptococcus micros* in the diabetic group when compared to conventional irrigation technique with a statistically significant difference.

CONCLUSION: The EndoVac can be considered as a promising tool in combination with irrigant solution to defeat the bacterial colonies living in the root canal system. Additional studies ought to be done to improve the means of bacterial clearance mainly in immune-compromised individuals.

Introduction

Apical periodontitis (AP) is viewed as a provocative procedure that happens around the apex of a tooth. Inflammation is caused by the ingress of bacteria from an infected pulp canal system. Extension of the periradicular lesions causing bone destruction is a sequel resulting from the coexistence of polymicrobial irritants from the diseased root [1].

Diabetes mellitus (DM) is an assembly of complex multisystem metabolic disorders which has a direct influence on the functions of the immune system which leads to delayed healing and affected immune responses. Diabetes mellitus may act as a precursor for inducing pulp necrosis and successive periapical lesions and failed endodontic treatment cases due to altered wound repair, immune and vascular functions [1, 2].

It was known that root canal treated teeth showing apical periodontitis have decreased success rate when compared with teeth with no apical disease which may end by endodontic failure [3]. An intimate noteworthy link between an increased incidence of apical periodontitis and diabetes mellitus was noticed. Moreover, when cases with preoperative periradicular lesions were investigated, diabetics had lower successful outcomes when compared with non-diabetics' patients preoperatively [4].

To overcome the restrictions of culture

techniques, molecular analysis have been agreed for invading the microbial world. The advantages of molecular tests are the detection of uncultivable bacteria in diseased root canals, given the chance to obtain definite and detailed new evidence on the endodontic microbial field. However, the differentiation between living and dead organisms was still questionable and impossible [5]. The intervention of qPCR, with new advancement process by using propidium monoazide (PMA), permits quantitative distinguish between viable and non-viable cells [6].

The good prognosis of endodontic treatment depends mainly on the efficient eradication of co-existed bacterial biofilms and their end products from the affected canal by using required cleaning and shaping means. The agitation of irrigant used is mandatory during filing to eradicate debris and bacteria from root canal system. To improve the flow and distribution of irrigating solution various techniques and devices should be introduced [7]. The EndoVac System is considered as negative pressure alternating devices safely used in debris removal from the working length without causing their extrusion into the periapical area [8].

This study aimed at estimating bacterial reduction after using different irrigation techniques in systemically healthy and diabetic patients with asymptomatic apical periodontitis.

Materials and Methods

The study protocol was approved by the ethical committees of Faculty of dentistry, Ain Shams University, Cairo, Egypt and the National Research Center, Cairo, Egypt (protocol number 15/026). All patients included in the study signed an informed consent.

Forty samples retrieved from single rooted single canaled lower premolars. Teeth were collected from 20 healthy and 20 diabetic patients. Patients were recruited from the Endodontic Department, Faculty of Dentistry, Ain Shams University, and from the Dental Clinic at the Diabetes Institute, Cairo, Egypt. Patients were divided into two main groups according to health condition being systemically normal (Group A) or diabetic (Group B), then they were subdivided according to irrigation methods used during cleaning and shaping to conventional syringe groups (Micro-Mega, Besancon, France) A1 & B1 and EndoVac groups (Discus Dental, Culver City, CA) to A2 & B2. The patients included were chosen to have pulp necrosis and infected root canals with asymptomatic apical periodontitis confirmed by vitality pulp testing and radiographic examination. The only systemic disease accepted in selection criteria was controlled type 2 Diabetes Mellitus based on a range of glycosylated haemoglobin (HbA1c) [9].

Selected teeth (n=40) received no prior endodontic treatment. Subjects who received antibiotic treatment within the preceding three months, teeth with periodontal probing depth greater than 4 mm, teeth had pain on palpation or percussion or had swelling, any multi-rooted tooth, non-restorable tooth, with root fractured tooth, or canal communicated with oral cavity were not included in the study. In addition, any chronic systemic diseases other than type 2 diabetes mellitus were excluded from the study.

After determining the provisional working length, complete teeth isolation and disinfection protocols were performed to avoid any field contamination. Strictly stuck to aseptic conditions, an appropriate access cavity was done; the canal was filled with sterile saline solution, and then introduced by a sterile #15k file one mm short of the root apex. The pre-operative microbiological sample was taken by four sterile paper points with a size compatible with the root canals anatomic diameter for 60 seconds. Then, the paper points were immediately placed in sterile 1.5 ml labelled tubes containing 500 μl of sterile phosphate buffered saline (PBS) solution, transported to the microbiological laboratory and frozen -70°C until quantitative real-time polymerase chain reaction (qPCR) analysis.

Cleaning and shaping were started using a #k-file of size 10 or 15 put to the full length of the root canal. Canal preparation was completed with one shape file system. According to subgroups classification, in groups A1 & B1, the root canals were irrigated with 5.25% NaOCL aided with side vented needle gauges 30 (Micro-Mega, Besancon, France) whilst groups A2 and B2 were treated by 5.25% NaOCL using EndoVac irrigating device (Discus Dental, Culver City, CA). The working time for the chemo-mechanical procedure was established at 15 minutes for all teeth. All canals were temporised using reinforced glass ionomer as coronal restoration for the next appointment after 48 hours to inhibit the degrading action of NaOCL on DNA amplicons. Post-instrumentation sampling in next visit followed the same aseptic conditions and same sample taking steps. Finally, all canals were obturated with gutta-percha points using lateral condensation technique. Coronal portion of the tooth was restored using composite resin.

Bacterial culturing and DNA Extraction

The positive control was settled by choosing *E. faecalis* because it contains four copies of the 16S rRNA gene covering almost the DNA sequence of most known endodontic bacteria which helps in drawing the standard curve for the bacterial comparative template. *Enterococcus faecalis* were cultured on trypticase soya broth media overnight. Once, the black colonies specific for the bacterial

strains appeared 100 colony forming units up to 10^8 CFU/μl were used for DNA extraction. Quantification of total bacteria levels for each sample was performed using a standard curve made off known concentrations of genomic DNA extracted from *Enterococcus faecalis* [10].

At room temperature, clinical samples were thawed, vortexed vigorously, and centrifuged at 8,000 x g for 5 minutes. The pellets were used for DNA extraction. The DNA was extracted and purified with a Qiamp DNA Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Using enzymatic extraction method, DNA from both Gram-positive and Gram-negative bacteria was retrieved with no apparent discrimination against either bacterial group [11].

Quantitative Real-time polymerase chain reaction procedures (qPCR)

The primers (forward & reverse) and probes designed for the *E. faecalis* were (CGCTTCTTTCCTCCCGAGT), (GCCATGCGGCATAAACTG) and (CAATTGGAAAGAGGAGTGGCGGACG) [10]. While for *Peptostreptococcus micros* (AAACGACGATTAATACCACATGAGAC), (ACTGCTGCCTCCCGTAGGA), and (TCAAAGATTTATCGGTGTAAGAAGGGCTCGC) [12], and for *Fusobacterium nucleatum* (AAAGTAGCG GCGAGCGAAATGG), (TGGTCCTCACTGATTCACACAGA), and (ACTTTGCTCCCAAGT AACATGGAACACGAG), respectively [13].

The PCR primers and TaqMan probe were based on species-specific highly conserved regions from the 16S rRNA gene. qPCR amplification and detection were performed with the ABI-PRISM 7500 Sequence Detection System using a 96-well format. qPCR reaction conditions for the three different bacteria included in this study were 95°C for 15 min for initial heat activation, followed by 40 cycles of 95°C for 15 seconds for denaturation, 95°C for 30 seconds for primer annealing and 60°C for one min for an extension. Cycle threshold (CT) values were calculated using the Sequence Detection Software and compared to an *E. faecalis* standard curve generated in parallel with quantification of target DNA from clinical test samples.

Statistical analysis

The mean and standard deviation values were calculated for each group. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests and showed non-parametric distribution, while Mann–Whitney U-test was used to compare the difference between the two groups. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with *IBM® SPSS® Statistics Version 20 for Windows.

Results

Effect of different irrigation techniques on bacterial reduction by qPCR

F. nucleatum and *E. faecalis* number were reduced in healthy and diabetic individuals when using EndoVac technique compared to using the conventional syringe technique without significant difference. Regarding the effect of the health condition of the patients on the bacterial reduction**,** there was no significant difference in *F. Nucleatum, P. Micros* and *E. faecalis* count between healthy and diabetic patients, regardless of the method of irrigation used. On the other hand, *P. micros* count was reduced upon irrigation with EndoVac at a higher rate when compared to the conventional syringe method without significant difference in the healthy group; While there was a significant difference in the diabetic group (P = 0.05).

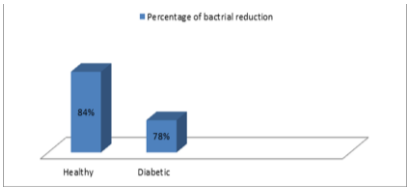

Figure 1: Column chart of percentages of overall bacterial reduction according to patient`s health status

The assessment of the health condition of the patients could not be ignored. It was observed that the overall bacterial reduction was higher in healthy population collectively when comparing with the diabetic group without statistically significant difference.

Table 1: Mean, standard deviation values and percentages of *Fusobacterium nucleatum*, *Peptostreptococcus micros* and *Enterococcus faecalis* reduction of the experimental groups

Variables	*Fusobacterium nucleatum*					*Peptostreptococcus micros*					*Enterococcus faecalis*				
	Conventional Syringe (1)		Endovac (2)			Conventional Syringe (1)		Endovac (2)			Conventional Syringe (1)		Endovac (2)		
	Reduction (Mean ± SD)	% of Reduction	Reduction (Mean ± SD)	% of Reduction	*p-value*	Reduction (Mean ± SD)	% of Reduction	Reduction (Mean ± SD)	% of Reduction	*P-value*	Reduction (Mean ± SD)	% of Reduction	Reduction (Mean ± SD)	% of Reduction	*p-value*
Healthy (A)	$2.15 \times 10^7 \pm 5.93 \times 10^{7\ a}$	81.82%	$3.34 \times 10^7 \pm 7.70 \times 10^{7\ a}$	99.6%	0.31	$5.92 \times 10^6 \pm 1.84 \times 10^{6a}$	88.02%	$5.14 \times 10^4 \pm 9.64 \times 10^{4a}$	97.40%	0.68	$1.30 \times 10^5 \pm 2.78 \times 10^{5\ a}$	49.64%	$5.66 \times 10^4 \pm 1.02 \times 10^{4\ a}$	57.80%	0.75
Diabetic (B)	$6.17 \times 10^4 \pm 1.66 \times 10^{5\ a}$	90.06%	$7.56 \times 10^4 \pm 2.00 \times 10^{5\ a}$	98.41%	0.57	$1.38 \times 10^5 \pm 2.53 \times 10^{5a}$	82.61%	$1.36 \times 10^6 \pm 2.14 \times 10^{6b}$	99.62%	0.05	$2.78 \times 10^2 \pm 3.93 \times 10^{2\ a}$	26 %	$3.44 \times 10^4 \pm 7.58 \times 10^{4\ a}$	40.00%	0.91
P-value	0.68		0.19			0.43		0.01*			0.34		0.84		

According to the present study, the EndoVac as negative pressure device was found to be more effective than conventional side vented syringe in the bacterial reduction in both groups

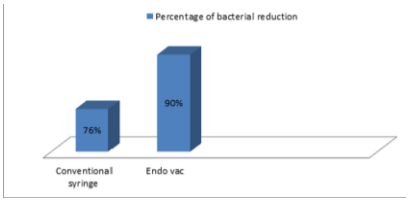

Figure 2: Column chart of percentages of overall bacterial reduction by using different irrigation techniques

Discussion

The main objective of endodontic treatment is getting a bacteria free canal to get an optimum successful outcome. Despite, the rapid evolution in irrigating materials, devices, and tools, the persistence of bacteria remains questionable. The diversity of root canal anatomy and the organisation of microorganisms hindered in the dentinal tubules, isthmuses and ramifications complicate the complete bacterial eradication from root canal which usually ends with apical periodontitis [2, 14]. It was confirmed that there is an actual relationship between the presence of specific bacterial taxa in filled root canal and treatment failure, which suggested that some taxa, such as streptococci, *Olsenella uli, Propionibacterium acnes*, and *Fusobacterium nucleatum*, might have the potentiality to be the initiator of risk factors and cause periapical diseases [15].

Diabetes mellitus is considered a modulator disease for impaired immunity, which may have a direct influence on the severity of periodontitis, the spread of periradicular lesions, endodontic flare-ups and endodontic treatment failure. Diabetics showed the least percentages of successful outcomes compared with healthy individuals [16]. *F. nucleatum*, *P. micros,* and *Streptococcus* spp. were the most prevalent pathogenic microorganisms retrieved from diabetic and non-diabetic specimens [17–19]. The selected bacterial species in this study were chosen because they are commonly present in the two studied groups. DM may trigger variations in dental pulp tissue which promotes pulp necrosis [20].

The regular trials to overcome the limitations of culturing techniques evolved the appearance of molecular technology for bacterial detection, being more accurate with greater sensitivity to provide a mean for distinguishing between the living and dead cells. Moreover, the new technologies have the ability to analyse DNA and RNA for more specificity. The elucidation of the obscure enigma of root canal microbiology was recently clarified by using different types of PCR [21, 22]. qPCR for quantification was used in this study to give an accurate survey about bacterial reduction and realise the reliability of the work.

For achieving the goal of endodontic treatment, the irrigant solutions, and the delivery devices played a very critical role in the final outcomes. After being the magical antibacterial irrigant over the years, NaOCL is the best choice when the bacterial reduction is required [23, 24]. However, its cytotoxic effects restrict its use in certain biological experiences; efforts have been made to find alternatives [25, 26]. The traditional irrigation approaches are efficient in cleaning root canals coronally, but less effective apically [27]. So for effective irrigation, an enhanced delivery system is highly desired. The EndoVac with negative pressure promoted better cleaning of main and simulated lateral canals, consequently, it helps in reducing bacterial contamination when used [28–30]. According to the results obtained from this research, irrigation with EndoVac was highly effective than conventional syringe irrigation in all groups with no statistically significant difference as described previously [29, 31]. On the contrary, two studies proved that there is no difference in bacterial reduction between different delivery devices [32, 33]. The discrepancies in results between the studies were due to the difference methods of culturing, type of bacteria selected and the systemic conditions of the patients.

To our knowledge, our study is the first to report a statistically significant difference in the reduction of *P. micros* after irrigation with Endovac in diabetic patients when compared to healthy ones. Only one study reported higher efficacy in microbiological reduction with Endovac when compared to the positive pressure with a statistically significant difference but still the group of interest is systemically healthy [34].

This study has its own limitations. Only single rooted and single-canalled teeth were included, for an easier accomplishment of the aseptic condition in this group of teeth and the chances of taking a good representative sample from the main large root canal are allegedly increased when compared with narrow canals. However, it is likely that in molars with more complex canal anatomy or in teeth with oval canals, the magnitude of bacterial reduction might have been different, even though it is reasonable to assume that not to the point of affecting the comparison between the two instrumentation techniques. Also, the recognised limited ability of paper points to collect a representative sample from the root canal system makes the information on bacterial counts restricted to the main canal [35].

In conclusion, the negative pressure irrigating

devices (EndoVac) can be considered as a promising tool in combination with irrigant solution to defeat the bacterial colonies living in the root canal system. Further studies are needed to test a wider range of endodontic microbiological species mainly in patients with systemic diseases to expand horizons to new areas of learning. It will be highly recommended to correlate the cruelty of pathogenic microorganisms with uncontrolled diabetes. The question always remains; the available irrigating devices are efficient in bacterial clearance mainly in immune-compromised individuals or not.

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