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Analysis of microbiota associated with root canal treatment failure cases – An in vivo study

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Abstract

Aim: The aim of this study is to detect different aerobic microorganisms present in the root canals of the teeth with failed endodontic therapy.

Objective: One of the goals of root canal treatment is to eliminate the microbes and their products from the pulp space. Therefore, in this study, analysis of the type of aerobic microbesassociated with failure has been done by collecting samples from root canal of endodontic treatment failure cases and culturing them.

Criteria for selection of subjects: 20 patients are selected on basis of history of previous root canal treatment, presence of pain and periapical radiolucency associated with maxillary and mandibular teeth.

Procedure: 20 patients are selected based on the above mentioned criteria. Removal of the coronal restoration followed by removal of GuttaPercha is done and canal is irrigated with normal saline. Sterile swab is inserted into the root canal and sample is sent for incubation.The samples are cultured and microbial colony count is done followed by identification by biochemical reactions.

Results: It was seen that streptococcus species were the most common microorganism associated with endodontic treatment failure Enterococcus faecalis (40%) followed by Klebsiella Pneumoniae (30%) and Diphtheroids, Citrobacter Freundii , Klebsiella Oxytoca and Candida Albicans found in 10% of cases.

Conclusion: In this study it was found that, usually single species of microorganisms are associated with root canal treatment failure cases and adequate care should be exhibited during root canal treatment so that no portal of entry is left for microbes to reach the canals again and cause re infection.

Keywords: Root canal retreatment, aerobic microorganisms, culture media

Introduction

Root canal treatment is associated with a proper access to the minor foramina, biomechanical preparation and a complete fluid tight seal. The pulp chamber harbors lot of

different colonies of microorganisms. Most of the time when the number becomes high, virulent pathogenic organisms can cause destruction of the pulpal tissue. Treatment will destroy a sizable number of them. However, the part of the remaining colonies of microorganisms may remain in a dormant state. When suitable environment is available they will start multiplying and cause infection again.

According to J. F. Siqueira Jr (2000), microorganisms must withstand intracanal disinfecting measures and adapt to an environment in which there are few available nutrients so that they can survive in the root-filled canal [1].Bacteria located in areas such as isthmuses, ramifications, deltas, irregularities and dentinal tubules may sometimes be unaffected by endodontic disinfection procedures and survive (Siqueira JF Jr 1996) [2].

Although it has been reported that nonmicrobial factors may be implicated in root-canal treatment failure, the literature suggests that persistent intraradicular or secondary infections are the major causes of the failure of root-canal treatment (Siqueira 2001)[1]. Other studies (Molander et al. 1998[3], Sundqvist et al. 1998[4], Peciuliene et al. 2000[5]) have revealed the composition of root-canal microbiota after failed treatment differs from that normally found in untreated teeth.

Therefore, a study to analyze these microorganisms will provide a clear picture of the cause for endodontic treatment failure and provide us guidelines to follow up cases with the same.

Study

Aim: The aim of this study is to detect different aerobic microorganisms present in the root canals of the teeth with failed endodontic therapy.

Objective: One of the goals of root canal treatment is to eliminate the microorganisms and their products from the pulp space. Therefore, it is important to know the role of microorganisms associated with failure of root canal treatment.

Twenty Patients Are Selected On The Basis Of The Following Criteria

- Symptomatic tooth with suitable radiographic findings.
- Improperly obturated root canals with suitable radiographic findings (non healing or non reducing radiolucency of more than 6 months duration)
- Incomplete treatment with suitable radiographic findings
- Tooth can be restored followed by re root canal treatment.

Figure 1

Materials Used

- NSK Para Air Airotorhandpiece
- Premier dental Gates Glidden drill
- Mani K files
- Mani H files
- Protaper Universal Re treatment rotary files
- Meta Sterile Absorbent paper points
- Sterile saline solution 0.9%
- Thioglycolate broth Transport media.
- Blood agar and Mac Conkey agar plates Culture media for bacteria.

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Figure 2

Procedure

Access cavity preparation is done under the rubber dam with a high speed handpiece in single/multi rooted teeth without water under constant supply of sterile normal saline. All the Gutta Percha is taken out of the root canal with rotary Protaper Universal Retreatment files. This is followed by irrigation of the root canal with sterile normal saline. Sterile paper points are inserted into the root canal and kept for 60 seconds.

The paper points are transferred to a bottle containing Thioglycolate broth media and transferred to the microbiology laboratory. Samples are incubated at 37^0 C for 24 hours.

Then, they are inoculated into the culture media (Blood agar and Mac Conkey agar plates) and incubated again at 37^0 C for 24 hours. Followed by this microbial colony count, biochemical tests are performed. The microorganisms associated with root canal treatment failure are identified in each case.

Figure 3

Result

The aerobic microorganisms identified from twenty samples obtained are given below:

Sample Number	Tooth Number	Microorganism Identified(Colony Count 10^5 CFU)	Type of Bacteria	Gram Positive / Gram Negative
1	21	Candida Albicans, Diphtheroids	Bacillus	Gram Positive
	22	Enterococcus Faecalis	Coccus	Gram Positive
3	11	Enterococcus Faecalis	Coccus	Gram Positive
4	31	Citrobacter Freundii	Bacillus	Gram Negative
5	47	Klebsiella Pneumoniae	Bacillus	Gram Negative
6	36	Klebsiellapneumoniae	Bacillus	Gram Negative
7	46	Klebsiellaoxytoca	Bacillus	Gram Negative
8	13	Enterococcus Faecalis	Coccus	Gram Positive
9	11	Enterococcusfaecalis	Coccus	Gram Positive
10	12	Klebsiella Pneumoniae	Bacillus	Gram Negative

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11	11	Klebsiellapneumoniae	Bacillus	Gram Negative
12	44	Candida Albicans	Bacillus	Gram Positive
13	36	Enterococcus Faecalis	Coccus	Gram Positive
14	11	Enterococcus Faecalis	Coccus	Gram Positive
15	45	Enterococcus Faecalis	Coccus	Gram Positive
16	36	Citrobacter Freundii	Bacillus	Gram Negative
17	37	Klebsiella Pneumoniae	Bacillus	Gram Negative
18	11	Klebsiella Pneumoniae	Bacillus	Gram Negative
19	11	Enterococcus Faecalis	Coccus	Gram Positive
20	45	Enterococcus Faecalis	Coccus	Gram Positive

- In the present study aerobic microorganisms were isolated that was associated with root canal treatment failure.
- Enterococcus faecalis is isolated in maximum number of cases(9).
- 42.8% of samples showed the presence of Enterococcus faecalis(9).
- KlebsiellaPneumoniae was isolated from 28.5% samples(6).
- Other than that Candida Albicans 9.5% (2), CitrobacterFreundii 9.5% (2), Diphtheroids 4.7% (1), KlebsiellaOxytoca 4.7%(1) were also isolated.

Discussion

For microorganisms to endure treatment and be detected in post-treatment samples, they must resist intracanal disinfection procedures and adapt to the drastically changed environment. They can adapt by creating strongly attached biofilms which colonize in distant inaccessible areas from the main canals (apical deltas, isthmuses, lateral canals), be protected by tissue residues, dentin, serum and dead cells, deeper penetration into dentinal tubules. They are likely to escape the effects of instruments (because of physical limitations) and irrigants (because of time constraints) used during chemo mechanical procedures[6, 8].

The microbiota associated with failed cases differs markedly from that reported in untreated teeth (primary root canal infection). Whereas the latter is typically a mixed infection, in which gram-negative anaerobic rods are dominant, the former is usually composed of one or a few bacterial species, generally gram-positive bacteria, with no apparent predominance of facultative or anaerobes[1].Möller (1966), after examining failed cases, reported a mean of 1.6 bacterial species (E. faecalis-29%, Anaerobic bacteria- 51%) per root canal. Significant association was also found between history of pain, tenderness to percussion, sinus, Coronally unsealed teeth and anaerobic bacteria / Candida Species[7].José F Siqueira Jr et al (2004) found that Enterococcus faecalis was detected in 77% of the root canal treatment failure cases. Pseudorami bacterial actolyticus (52%), Propioni bacterium propionic cum(52%), Dialisterpneumosintes(48%), Filifactoralocis (48%) and Candida albicans (9%) of the samples[11].In another study by R Vidana et al(2011) showed that Enterococcus faecalis infection are derived from an exogenous source and not from patient's own microflora determined by using pulsed field gel electrophoresis[9]. Kishore Kumar Singh et al(2020) conducted a DNA extraction and analysis by PCRstudy of E. faecalis and Red complex

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bacteria (P. gingivalis, T. denticola, T. forsythia) which showed an association between tenderness on percussion and secondary infection (E. faecalis -60%; Red complex bacteria-86%)[10].Hengameh Ashraf et al (2007) studied the association of periapical lesions in cases with re infection of root canals by culturing samples on Mac Conkey and blood agar culture media and found presence of Candida Albicans in cases with periapical lesions[12].

In this study, we have identified the aerobic group of microorganisms associated with root canal treatment failure since the culture of anaerobic microorganisms has not been possible due to difficulty in culturing techniques. Cases with tenderness on percussion showed the presence of Citrobacter Freundii and Klebsiella Oxytoca whereas those with pain showed the presence of Enterococcus Faecalis. In cases of short obturation/ Coronally unsealed teeth & periapical lesionthe presence of Klebsiella Pneumoniae, Candida Albicans and Diphtheroids was found.

The samples were collected after complete removal of the gutta percha from the root canals of the retreated teeth by using sterile paper points and transferred to a bottle containing Thioglycolate broth media and then cultured in Blood agar and Mac Conkey agar plates. Samples are incubated at 37^0 C for 24 hours. The cultured strains were then subjected to gram staining test and microscopic examination to identify the microorganisms followed by some biochemical tests if required for further confirmation.

Another study in an Iranian population by Maryam Pourhajibagher et al (2017) investigated the microorganisms associated with primary and secondary endodontic infections via culture methods, biochemical tests and molecular approaches where the transport media used was VMGA III followed by plating on the BHI agar[13]. In a study by MW Egan et al (2002) Sabourand Dextrose Agar was used for culturing yeast & fungi which showed C. Albicans and R.Mucilagenosa as the most prevalent isolates[14].

The results of this studyrevealed that Enterococcus Faecalis-42.8% (9) and KlebsiellaPneumoniae-28.5% (6) were isolated from maximum number of samples.Other than that Candida Albicans- 9.5% (2), CitrobacterFreundii- 9.5% (2), Diphtheroids- 4.7% (1), Klebsiella Oxytoca 4.7% (1) were also isolated.

A bacteriological investigation by R. Vidana et al(2011) had been made in two cases of persistent periapical infections where Enterobacter cloacae was only isolate in case 1; Klebsiellapneumoniae and Enterococci were found in case 2. Obligately anaerobic bacteria were not found[9].

Another study was done to combine multiple displacement amplification and checkerboard DNA-DNA hybridization to qualitatively and quantitatively evaluate the microbiota present in infections refractory to endodontic treatment which showed the highest mean proportions of Corynebacteriumdiphtheriae (8.03 ± 0.98), Porphyrom onasgingivalis(5.42 ± 2.09), Streptococcus sobrinus (5.33 ± 0.69), and Stenotrophomon as Maltophilia (4.72 ± 1.73)[11]. Therefore, acquiring an adequate knowledge regarding the micro biota associated with failures we can understand that drugs that can specifically target destruction of these microorganisms can be used to give a proper treatment.

- 2% chlorhexidine in combination with sodium hypochlorite as root canal irrigants can combat E.Faecalis[16].
- It seems that the combinations of $Ca(OH)_2$ with camphorated paramonochlorophenol or chlorhexidine can be used as effective intracanal medicaments for fungal infection cases[17].
- Carbapenum is effective against klebsiellapneumoniae[20].

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- A wide range of beta lactum, aminoglycosides, quinolones, cephalosporins are used to combat klebsiella infections [20].
- Ceftriaxone, a third generation cephalosporin is effective against Citrobacterfreundii infections [21].
- Diphtheroidsare treated by using doxycycline or vancomycin [22].
- Percentage reduction with CH has been found to be 82% for facultative anaerobes respectively and 61% for Candida spp.Its mechanism of action is based on the lethal effects of hydroxyl ions on bacterial cells that disrupts the established nutritional relationships [23].
- Septomixine forte contains two antibiotics ie, neomycin and polymixine B sulphate. This can be effective against gram positive and gram negetive bacteria [24].
- Agents with high intrinsic activity against K pneumoniae include third-generation cephalosporins (eg, cefotaxime, ceftriaxone), carbapenems (eg, imipenem/cilastatin), aminoglycosides (eg, gentamicin, amikacin), and quinolones. These agents may be used as monotherapy or combination therapy.
- Combined spectrum of antimicrobial activity and synergistic or additive actions of antibiotics Ciprofloxacin, Metronidazole, and Minocycline found in TA paste [18].
- Individually, ciprofloxacin has broad spectrum activity and acts against both Gram-positive and Gram-negative bacteria by inactivating enzymes and inhibiting cell division [19].
- Metronidazole is effective against obligate anaerobes, which are common in the deep dentin of infected root canals and acts by disrupting bacterial DNA [19].
- Minocycline is a broad-spectrum tetracycline antibiotic and acts by inhibiting protein synthesis and inhibiting matrix metalloproteinase enzyme[19].
- Combination of these three antibiotics overcomes bacterial resistance and achieves higher antimicrobial action[20].

It can be recommended to take samples from root canal failure cases and conduct a microbiological culture to find out the specific organism for treatment failure. However, this method can be used to culture the aerobic and facultative microorganisms since the reports can be obtained within a short time of 3 days otherwise for anaerobic culture it would take 2 weeks.

Hence, it is preferable to use a combination of broad-spectrum antibiotics, Chlorhexidine, Sodium hypochlorite and calcium hydroxide to combat the microorganisms and render the root canal space free from further invasion.

Conclusion

So, from this study we can conclude that:

- The streptococcus species are responsible for causing root canal re infection in maximum number of cases.
- Adequate care should be exhibited not to leave any portal of entry for the microbes to re grow in the canals again and cause infection.
- If preventive measures are not effective then retreatment should be done with great skill.
- A good biomechanical preparation, use of effective irrigants and medicaments, establishment of a fluid tight seal can provide a good prognosis and restore the tooth back to its healthy state.

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