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Presence and location of bacteria in human periapical pathosis: A histopathological study

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ABSTRACT

Periapical granulomas are sterile or infected have been controversial since long. Various studies mostly used culture methods to evaluate the microbial flora of the periapical lesions, but still landed up with variable and sometimes false positive results. Present study used Modified Brown and Brenn stain to study the presence and location of bacteria in human periapical lesions. Thirty three periapical lesions attached to non-endodontically treated extracted teeth were fixed in 10% buffered formalin, decalcified in 10% HNO_3 and were processed for paraffin embedding. Serial sectioning was done and the slides were stained with H&E stain, and Modified Brown and Brenn stain. Of 33 lesions, bacteria were found in 28 lesions (17 granulomas and 11 cysts). Five granulomas were found to be sterile. Majority of the bacteria that were seen were gram-positive cocci, followed by gram-positive bacilli. Gram-negative bacilli were rarely seen. Yeast like structures were also observed in 12 lesions. Knowledge in this field is essential to the clinicians to effectively carryout the endodontic treatment.

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Periapical lesions are viewed as a dynamic encounter between microbial factors and host defenses at the interface between infected radicular pulp and periodontal ligament that results in local inflammation, resorption of hard tissues, and eventual formation of various histopathological categories of apical periodontitis, commonly referred to as periapical lesions.[1] Microorganisms attribute virtually to all pathoses of these lesions. Therefore to treat effectively the endodontic infections, clinicians must be familiar with the nature of infection of the root canal system and periradicular tissue.[2] But it has been difficult for the researchers to cultivate and identify all organisms because of the polymicrobial interaction and nutritional requirements of the microorganisms.[3] And also whether asymptomatic periapical granulomas are sterile has been controversial. It is generally believed that bacteria usually stayed confined to the root canal system of an infected tooth, except when associated with an abscess or cellulitis. It is believed that "a granuloma is not an area in which bacteria live, but is an area in which they are destroyed".[4] In the past the researchers used both the staining and culture methods to study the presence and location of bacteria in all periapical lesions but had landed up with variation and conflict, concluding that staining method gives less false positive results.[5] Hence this study was conducted with the aim to evaluate the presence and location of bacteria in human periapical lesions.

1. Materials and method

The study material consisted of 33 human teeth with attached periapical lesions, collected from the Department of Oral and Maxillofacial Surgery, Modern Dental College & Research Centre, Indore. The extracted teeth were either not restorable or the patient did not agree to save the teeth.

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1.1. *Inclusion criteria*

1. The teeth had to be non-endodontically treated as true undisturbed microflora can actually be studied if the tooth is untreated. It is known that the procedure and chemicals used during the endodontic treatment can modify the periapical flora.[6]
2. The lesions had to be attached to the apices of the extracted teeth, to avoid contamination of the lesion.[7–10]

After extraction the specimens were immediately immersed in 10% neutral buffered formalin[11] for fixation for at least 24 hours in sterile containers. After fixation the samples were decalcified in 10% nitric acid. The end point of decalcification was checked manually. The compressibility of the tooth and the ability of hypodermic needle to penetrate into the thickest portion of the tooth without any resistance were considered to be the end point of decalcification.[12]

After the end point of decalcification was achieved, the teeth were rinsed under running water for 24 h for complete removal of the residual acid, which if retained could affect the quality of staining. The rinsed samples were then put in automatic tissue processor for dehydrating, clearing and paraffin embedding. The paraffin blocks of processed samples were prepared using Leuckhart's 'L' molds. All samples were oriented parallel to the long axis of the main root.

The glass slides of dimensions 76 × 25 (1″ × 3″) and thickness 1.35 mm were coated with Mayer's albumin. The identification number of the sample and number of the serial section were written on the slides. Serial sections were taken with the microtome on the Mayer's albumin[11] coated slides at 5 μm until the whole specimen was consumed.

Every 5th, 10th, 15th…, sections were randomly selected to be stained with hematoxylin and eosin stain (H&E)[13] for classifying the lesion. Every 4th, 9th, 14th… sections i.e. every section prior to the H&E stained section were stained with Modified Brown and Brenn stain[11] to evaluate the presence and location of bacteria.

In order to differentiate between artifacts and bacteria, definitive identification criteria were followed as,[14]

- The bacteria must be in the same plane as the surrounding tissue;
- The morphology of neighboring organisms must be uniform;
- The staining response of the microorganisms must be consistent;
- Inflammatory cell response of the tissues must be present in the same plane as that of bacteria, as well as adjacent to the microorganisms.

1.1.1. *Modified Brown–Brenn stain results*

- Gram-positive bacteria – blue
- Gram-negative bacteria – dark pink
- Nuclei – red
- Background – yellow.

The slides were thus ready to be evaluated for reporting the bacteria in the periapical lesions and correlating it with the type of lesion. Every stained section of each sample was observed and results were tabulated. Reagents used for Modified Brown–Brenn stain were, iodine, basic fuchsin, picric acid, acetone and potassium iodide.

2. Results

All the serial sections of thirty three periapical lesions attached to the teeth were examined. Out of 33 specimens 22 (67%) were diagnosed as periapical granulomas (Fig. 1) and 11 were diagnosed as cysts with help of H&E stained sections (Figs. 2 and 3).

All the selected sections for bacteriological examination were stained with Modified Brown and Brenn staining method and observations were tabulated (Table 1). Twenty eight lesions (85%) were positive for bacteria of which 17 were granulomas and 11 were cysts. Of the 22 periapical granulomas, 17 (77%) were infected and 5 (23%) were sterile. All the reported 11 cysts were infected (Fig. 4).

In all the instances, bacteria were dispersed uniformly throughout the lesion in slight to moderate concentration (Fig. 5). Moreover the cysts had bacteria even within the lumen. Of the 28 lesions showing microorganisms, 15 lesions showed intracellular microorganisms (Fig. 6). Gram-positive cocci (Fig. 5) were seen in all 28 lesions whereas gram-positive bacilli were seen in 5 lesions (Fig. 7). Only 2 lesions (7.14%) showed gram-negative bacilli (Fig. 8).

Yeast like round gram-positive structures of variable size were also seen in Modified Brown and Brenn stain in 12 (36%) lesions of which 4 (12%) lesions showed budding spores like pattern and 2 (6%) showed pseudohyphae like pattern (Fig. 9).

3. Discussion

All endodontic diseases are either directly or indirectly related to the presence of microorganisms. The fundamental

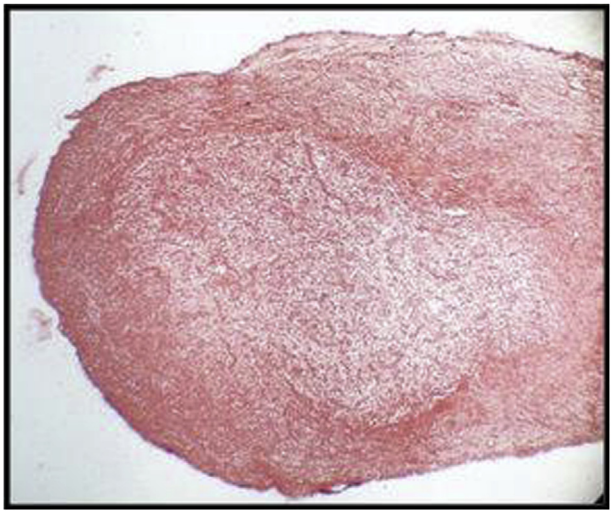


Fig. 1 – Periapical granuloma showing dense connective tissue capsule at the periphery and in the center it shows foamy macrophages (H&E stain 40×).

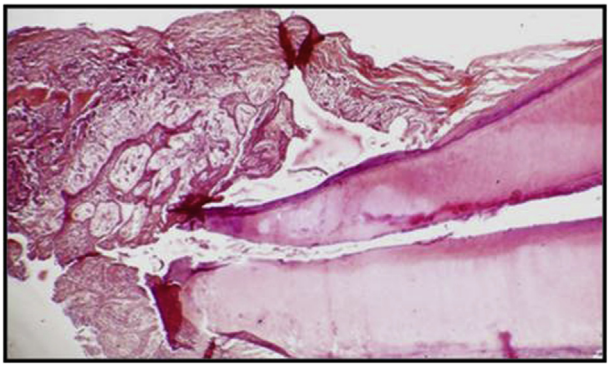


Fig. 2 – Periapical cyst attached to the tooth apex. Cystic lumen is continuous with the root canal and is lined by proliferative epithelium in arcading pattern (H&E stain 40×).

relationship between microorganisms and endodontic diseases has been established since a century and has variable findings.

The present study was carried out on 33 non-endodontically treated human periapical lesions attached to the extracted teeth with the objective to evaluate the presence and location of bacteria in periapical lesions. The teeth included in the study were non-endodontically treated. This criterion was followed because true undisturbed microflora of periapical lesions could be studied if the tooth is untreated, as the procedure and chemicals used during the endodontic treatment can modify the periapical flora.[6] The other inclusion criteria, of selecting periapical lesions attached to the roots were followed in this study to avoid the contamination of the lesion, as recommended by Winkler TF, Mitchell DF, Healy HJ (1972),[7] Block RM, Bushell A, Rodrigues H, Langeland KA (1976),[8] Simon James (1980)[9] and Riccuci D, Pascon EA, PittFord TR, Langeland K (2006).[10] This strict inclusion criteria was the reason for the small sample size.

The serial sectioning method was adopted in our study (until the whole specimen was consumed) so that no findings related to the periapical lesion are missed. Histopathological study was preferred over the culture method for studying bacteria. The reason was that culture method grows both periapical pathogens as well as contaminants, which are not always differentiable. This could lead to false positive results, which is evident by the study carried out by Wayman Blake (1992).[5] He had detected bacteria histopathologically as well as by culturing. Histopathologically 14% lesions were infected, however culture method showed bacteria in 88% lesions. The routine culture method involves sending half specimen for microbiological examination and the other half is used for histopathology. This procedure not only causes contamination,

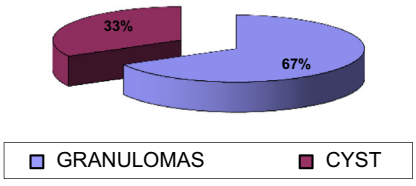


Fig. 3 – Percentage of granulomas and cyst.

Table 1 – Bacteria in the periapical lesions of all 33 samples.

Samples	Bacteria	Yeast like structures	Diagnosis
1	–	–	PG
2	+C, +B	Seen	PG
3	–	–	PG
4	+C	–	PG
5	+C	Seen	PG
6	+C	Seen	PG
7	+C, +B	Seen	PG
8	–	–	PG
9	+C	–	PG
10	+C	Seen	PG
11	+C	–	PG
12	+C	–	PG
13	–	–	PG
14	–	–	PG
15	+C	Seen	PG
16	+C	–	PG
17	+C	–	PG
18	+C	Seen	PG
19	+C	–	PG
20	+C	–	PG
21	+C	Seen	PG
22	+C, +B	–	PG
23	+C	–	PC
24	+C	–	PC
25	+C	–	PC
26	+C	Seen	PC
27	+C, +B, −B	Seen	PC
28	+C	–	PC
29	+C	–	PC
30	+C, −B	–	PC
31	+C, +B	Seen	PC
32	+C	Seen	PC
33	+C	–	PC

(+C = gram-positive cocci, +B = gram-positive bacilli, −B = gram-negative, PG = periapical granulomas, PC = periapical cyst).

but also the sample received for diagnosis may not be representative of the lesion. To eliminate this error, entire periapical lesion along with the tooth was used to diagnose the lesion. To evaluate the bacteria histopathological technique/Modified Brown and Brenn stain was used.[10] H&E stain was also used to diagnose the lesions and to correlate the presence of bacteria with the type of the lesion.

In the present study the Modified Brown and Brenn technique used for demonstration of bacteria is the modification of the gram staining technique. This stain was used in previous studies by Engback K, Johansen KS, Jensen ME (1979),[15]

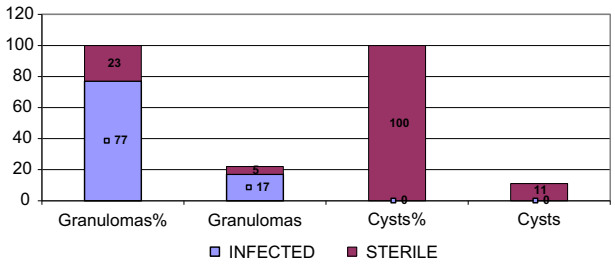


Fig. 4 – Percentage of sterile and infected periapical lesions.

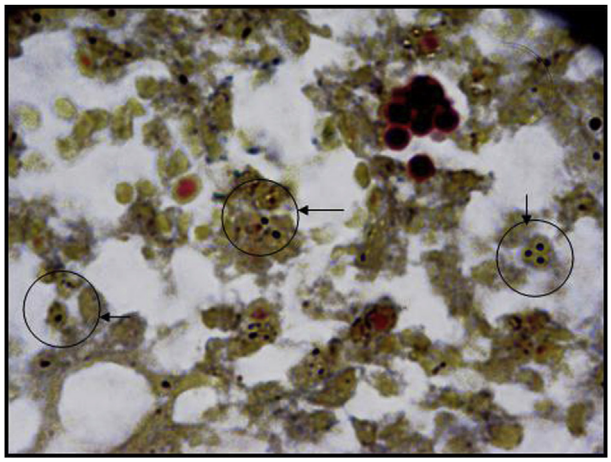


Fig. 5 – Gram-positive cocci are seen scattered throughout the periapical lesions (Modified Brown and Brenn stain 1000×).

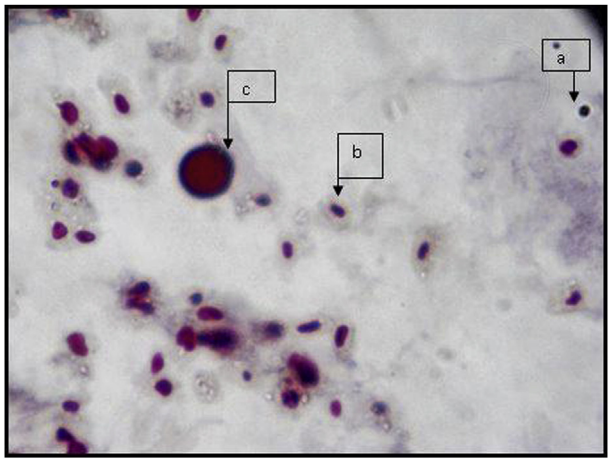


Fig. 7 – (a) Gram-positive cocci, (b) gram-positive bacilli, and (c) yeast like structure. In the periapical lesion (Modified Brown and Brenn stain 1000×).

Block RM, Bushell A, Rodrigues H, Langeland KA (1976),[8] Egan MW et al (2002),[16] Riccuci D, Pascon EA, PittFord TR, Langeland K (2006).[10] The reported frequency of the bacterial cells in the periapical lesions does not exclude the presence of bacterial components such as cell walls or metabolic products that are not identified by staining technique. In the present study the bacteria were reported in 28 (85%) lesions by following the identification criteria.[7,17] Out of these 28 infected lesions 17 were granulomas and 11 were cysts. This shows that all the cysts were infected. Of the 22 granulomas 17 (77%) were infected and 5 (23%) were sterile. However the findings of previous studies are variable. According to Hardnt (1926)[8] and Riccuci D, Pascon EA, PittFord TR, Langeland K (2006)[10] all granulomas are sterile. Whereas according to Bullied (1934), Mela (1934), almost all the granulomas are infected.[8] They reported 100% and 98.2% of the periapical granulomas to be infected respectively. Whereas studies of Chris Iwu (1990), Burkett (1938), Melville (1967), and Grossman (1959) on granulomas found them to be infected in 88%, 49%, 35%, and 15% respectively.[8,18] In the present study of all the organisms identified in the lesions, gram-positive cocci were the most prominent, followed by gram-positive bacilli. However the absence of gram-negative cocci and rarity of gram-negative bacilli in the periapical lesions was evident. These findings coincide with those quoted by Winkler et al (1959)[19] and (1972).[7] In the lesions, which were negative for bacteria, the bacteria were stained within the dentinal tubules, which show the efficacy of the staining method used. The absence of bacteria in the periapical tissue of the same section can therefore not be false negative. No culture was made of the specimen. Therefore in the absence of culture, identification of the species of organisms was not possible. But the gram reactivity of the bacteria is of considerable importance as the gram-positive bacteria differ not merely in staining characteristics and in structure but also in several other properties such as growth requirements, susceptibility to antibiotics and pathogenecity.[15,20] Forcing of the bacteria into the periapical lesion from the inevitable rocking of the tooth during the extraction or the oral contamination of the central periapical

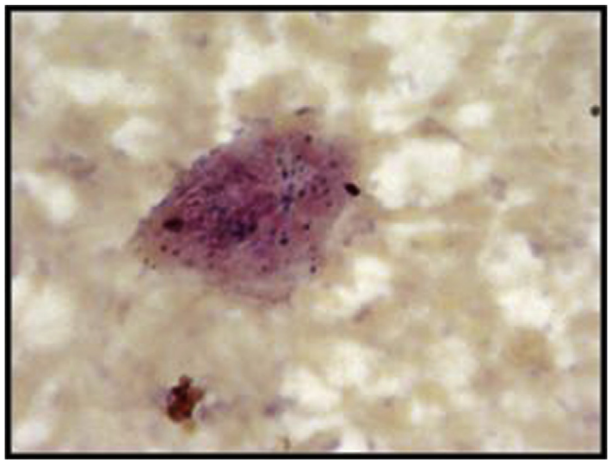


Fig. 6 – Intracellular microorganisms (Modified Brown and Brenn stain 1000×).

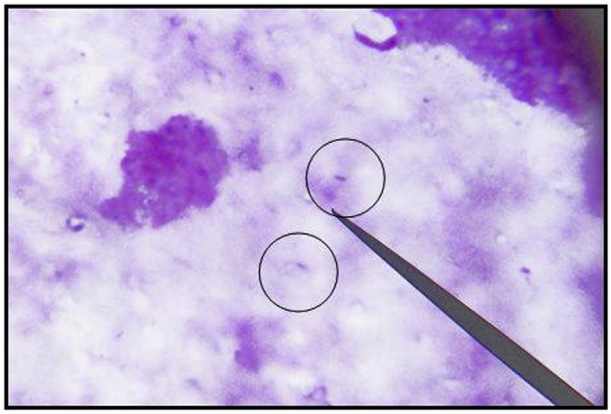


Fig. 8 – Gram-negative bacilli are seen scattered throughout the periapical lesion (Modified Brown and Brenn stain 1000×).

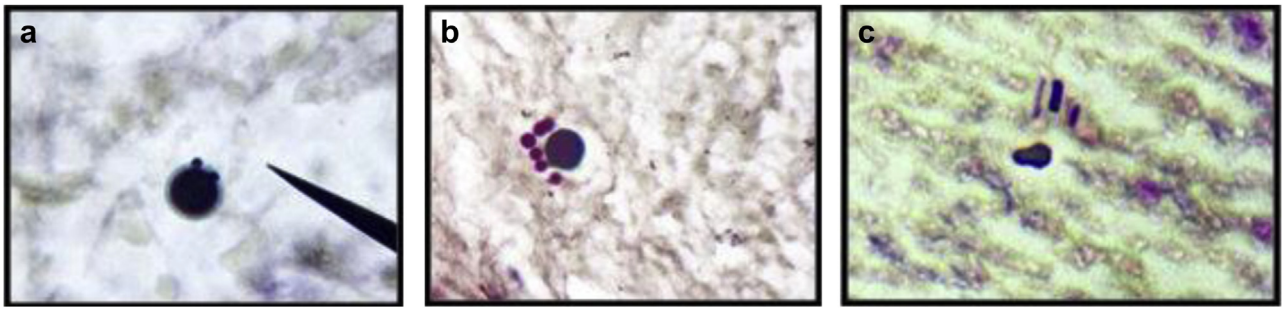


Fig. 9 – Grams-positive yeast like structure having, a – budding pattern, b – colony like pattern, c – pseudohyphae like pattern (Modified Brown and Brenn stain 1000×).

tissue appears to have been unlikely as histopathologically, the inflammatory cells often surrounded and engulfed the bacteria. Yeast like structure in the form of budding spore, pseudohyphae and colonies were also seen in the Brown Brenn stained sections. These structures could be yeast or Russell bodies as both are gram-positive structures.[13] It is difficult to differentiate them morphologically by Modified Brown Brenn stain. Both require special diagnostic procedures. For example, Gomori's methenamine silver stain[21] can confirm yeast whereas Russell bodies require immunohistochemistry. The Russell bodies are CD138 and CD79a positive.[22] The findings of this study revealed that most of the periapical lesions are infected. Further studies with larger sample size and more specific inclusion criteria are required. It may be emphasized from this study that all periapical lesions should be treated as infected lesions to increase the success rate of endodontic therapy. Further to find out the role of fungi in the periapical lesions as reported by previous reporters,[16,23–25] special stains can be used in further studies.

Conflicts of interest

All authors have none to declare.

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