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"EVALUATION OF BACTERIAL PROFILE AND ITS ANTIBIOTIC SENSITIVITY IN ENDODONTIC-PERIODONTAL LESIONS NAMELY PRIMARY PERIODONTIC , PRIMARY ENDODONTIC AND TRUE COMBINED LESIONS"-A RANDOMIZED CONTROLLED TRIAL

Periodontology

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ABSTRACT

Endo –perio lesions are the lesions affecting both the pulp and the periodontium individually or combined in the same tooth. They are interrelated by various pathways like apical foramen, lateral canals, dentinal tubules which can result into the spread of disease from one tissue to another and have differing pathogenesis, making it necessary for the clinicians to have a complete understanding about the disease process for the successful diagnosis and treatment of the involved lesions. The aim was to evaluate the bacterial profile in endodontic- periodontal lesions and to assess antibiotic susceptibility to the four antibiotics namely Augmentin, Doxycycline, Ofloxacin+ornidazole, Cefotaxime.

KEYWORDS

Endo-perio lesions, microbial culture, Antibiotic susceptibility.

INTRODUCTION

The tooth with its pulp and the supporting periodontal tissues should be observed as one biologic unit. The tissues from which the tooth is formed includes ectoderm and ectomesenchyme. The proliferation of these ectomesenchymal cells results in the formation of dental papilla and dental follicle which are the precursors of periodontium and the pulp respectively.

There is a close interrelationship between the pulp and the periodontium embryonically, functionally and anatomically which influences each other during health, function and disease. The anatomic connections are established as a result of embryonic development which exists for the whole of life. Thus, the disease affecting one tissue may cause involvement of the other through its communication via the apical foramen, accessory/lateral canals and dentinal tubules.

Although the relationship between the pulp and the periodontium is studied extensively over time, it is still a topic of debate and discussion, accounting for more than 50% of the tooth mortality.

Various causes for the disease involvement of the endodontic and the periodontal tissues include etiologic factors like bacteria, viruses, fungi, as well as the contributing factors like trauma, perforation, root resorption and dental malformation.

Microbiology of the endo-perio lesions is a diverse one. Bacterial association between the endo-perio lesions is still under debate, despite of the established anatomic interrelationship, thus making it important for us to have an adequate knowledge about the diverse population of bacteria involved in the endodontic periodontal lesions.

In the present study microorganisms were detected by utilizing two culture media. Depending upon the various etiologic factors and communicating pathways between endodontium and the periodontium, the nature of the lesions may vary and treatment plan may involve root canal treatment, scaling and root planning, regenerative therapy etc. Though mechanical therapy remains the main treatment modality, it may not be a success in all clinical scenarios, leading to the need for systemic antimicrobial therapy. Systemic antimicrobial agents may have major impact in the areas beyond the reach of instrumentation and they also help in preventing recolonization and reorganization of the dental biofilm.

Hence the present study aims at evaluating the bacterial profile involved in endodontic periodontal lesions along with its susceptibility to commonly used antibiotics in dental practice.

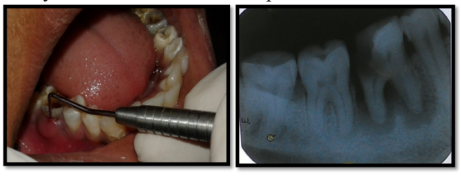

Figure 1: clinical and radiographic picture of endoperio lesion

AIM OF THE STUDY

To evaluate the bacterial profile in endodontic- periodontal lesions.

OBJECTIVES OF THE STUDY

- To evaluate the presence of *Porphyromonas gingivalis, Bacteroides forsythus, Porphyromonas endodontalis & Prevotella intermedia* in primary periodontal lesions, primary endodontic lesions and true combined lesions.
- To evaluate & isolate unknown species in the above groups.
- To evaluate antibiotic sensitivity to the combination of amoxicillin+clavulanic acid , Ofloxacin+Ornidazole, Doxycycline & Cefotaxime.

MATERIALS AND METHODS:

SAMPLE SIZE AND METHOD OF DATA COLLECTION:

The sample size included 75 subjects, with the age group between 25-45 years, and were divided into three and both plaque and root canal samples were collected from a single involved tooth of the same subject. Each group consisted of 25 subjects.

Group 1 : Primary periodontal group (n= 25)
Group 2 : Primary endodontic group (n=25)
Group 3a : True combined periodontal group } Group 3 (n=25)
Group 3b : True combined endodontic group }

The subjects were clinically examined for the periodontal & endodontic conditions and the data was recorded. Prior to starting the study, a written informed consent was obtained from all the study participants.

INCLUSION AND THE EXCLUSION CRITERIA FOR THE STUDY GROUPS INCLUDED:

GROUP 1: Primary Periodontal group

Inclusion criteria

A minimum compliment of 20 teeth, Bleeding index score of ≥ 2, Pocket probing depth of ≥ 6mm, Clinical attachment level of ≥ 4mm

Exclusion creiteria

No periodontal treatment in last six months& should not be on any systemic antibiotic therapy over last six months, smokers and patient consuming tobacco in any other forms, history of any systemic diseases, pregnant & lactating mothers.

GROUP 2: Primary endodontic group

Inclusion criteria: Irreversible pulpitis, apical Periodontitis, non vital teeth, periapical radiolucencies suggestive of apical abscess, periapical granulomas & cyst. Diameter of periapical lesion ≤ 1 cms.
Exclusion creiteria: Periapical radiolucency ≥ 1 cms, root fracture, root resorption (internal or external), periodontal pathology

GROUP 3 : True combined group

Inclusion criteria

Tooth should have an endodontic lesion as well as a periodontal lesion

initiating separately and then meeting together. The other criteria were the same as prescribed for group 1 & 2

Exclusion creiteria
Were the same as that for group 1 & 2

PROCEDURE:
Collection of saliva
All participants were refrained from eating and drinking 2 hrs before saliva collection. After rinsing the mouth thoroughly with water and to spit the saliva directly into the saliva collecting tube (50 ml of centrifuge tube). Saliva samples were immediately placed on ice bag for transport to the laboratory followed by centrifuging at 2,600g for 10 mins to spin down large debris & eukaryotic cells. The supernatant was mixed from all the samples which was referred to as pooled saliva and was stored at -80 degree Celsius which was used throughout the study.

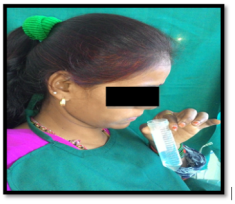

Figure 2. saliva collection

Collection of root canal microbial samples:

- Involved tooth was isolated with rubber dam & disinfected with 30% hydrogen peroxide & 5% iodine tincture for 60 seconds. After administering local anesthesia the pulp chamber was opened aseptically with sterile high speed carbide bur with sterile saline irrigation. A sterile K file was than introduced into the canal. The handle of K file was cut off with a diamond bur without water and the instrument was then transferred into the tube containing reduced transport fluid. Then two sterile paper points (size 20) were inserted into the same canal separately & retained in position for 1 min. The paper points were immediately placed in tube containing reduced transport fluid & immediately transported to the Basic Science Research laboratory.

Figure 3: Collection of endodontic sample

Collection of the periodontal plaque samples:
Supragingival plaque was removed & sterile cotton roll was inserted into the vestibule to prevent saliva from disturbing the sampling process. Subgingival plaque samples were taken at selected sites by inserting Gracey curette into the bottom of the deepest periodontal pocket. The curette was removed & transferred to the tube containing reduced transport fluid which was the transported to the laboratory immediately.

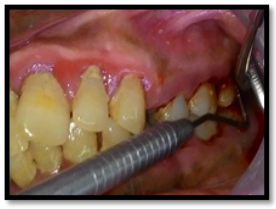

Figure 4: collection of periodontal sample

MICROBIOLOGICAL PROCEDURE:
After collecting the samples from the root canal and the periodontal pocket , vortexing was done , following which samples were cultured using:
1) Blood agar with hemin & vitamin K
2) SHI medium with saliva pooled & sterilized

- Both these culture media were incubated anaerobically for 5-7 days in anaerobic chamber with 5% CO2 at 37 degree Celsius.

After 5-7 days the bacteria grown on the culture were identified with their phenotypic characteristics. Carbohydrate utilization test was performed for identification of the microorganisms. Antibiotic sensitivity test was done for organisms isolated from the endo-perio lesions by microbroth dilution method for estimation of minimum inhibitory concentration.

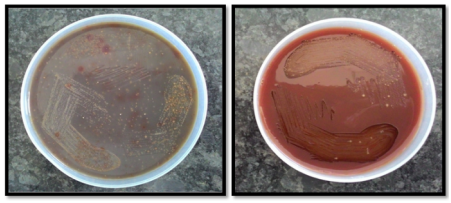

Figure 5: culture plates

RESULTS AND OBSERVATIONS
Table 1: SBI, PPD and CAL scores in four groups (1, 2, 3a, 3b)

Variables	Groups	Mean	Std. Deviation	Std. Error	95% CI for Mean	
					Lower Bound	Upper Bound
SBI	Group 1	3.88	0.52	0.10	3.67	4.10
	Group 2	1.67	0.53	0.11	1.46	1.89
	Group 3a	1.94	0.53	0.11	1.72	2.16
	Group 3b	1.95	0.55	0.11	1.72	2.17
PPD	Group 1	6.42	0.52	0.10	6.20	6.63
	Group 2	2.46	0.99	0.20	2.05	2.87
	Group 3a	2.89	0.60	0.12	2.64	3.14
	Group 3b	2.89	0.59	0.12	2.64	3.13
CAL	Group 1	6.23	0.47	0.09	6.04	6.42
	Group 2	0.65	1.23	0.25	0.15	1.16
	Group 3a	2.37	0.52	0.10	2.16	2.59
	Group 3b	2.42	0.46	0.09	2.24	2.61

Table 2: Comparison of four groups (Group 1, Group 2 , Group 3a, Group3b) with respect to organisms present

Organisms	Group 1	%	Group 2	%	Group 3a	%	Group 3b	%	Total
ND	3	12.0	0	0.0	6	24.0	6	24.0	15
B.forsythus	5	20.0	0	0.0	4	16.0	0	0.0	9
P.endodontalis	1	4.0	1	4.0	3	12.0	1	4.0	6
P.endodontalis & P.gingivalis	1	4.0	0	0.0	3	12.0	2	8.0	3
P.gingivalis	13	52.0	13	52.0	8	32.0	13	52.0	47
P.gingivalis & B.forsythia	1	4.0	0	0.0	1	4.0	0	0.0	2
P.intermedia	0	0.0	11	44.0	0	0.0	2	8.0	13
P.intermedia & B.forsythus	1	4.0	0	0.0	0	0.0	1	4.0	2
Total	25	100.0	25	100.0	25	100.0	25	100.0	100

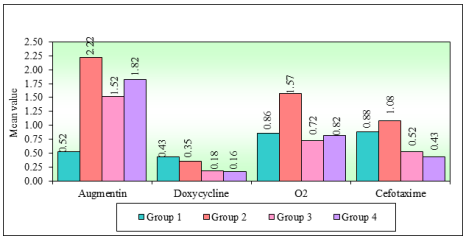

Figure 6- coamprison of four groups (1, 2, 3, 4) with respect to augmentin, Doxycycline, O2 and Cefotaxime (in 10^3 µglml) scores

1. SBI, PPD & CAL scores were predominantly higher in primary periodontal group and secondary endodontic lesions.
2. Periodontal pathogens namely *P. gingivalis* can be isolated from root canals and endodontic pathogens like *P.endodontalis* can be isolated from periodontal pockets.
3. All the bacteria assessed showed sensitivity to Augmentin Doxycycline, Ofloxacin+ornidazole and Cefotaxime. However a combination therapy would be the treatment of choice in the management of endo-perio lesions
4. In this study, on culturing the samples unknown species were not isolated.

DISCUSSION

Endodontic and periodontal problems perhaps account for a majority of complaints that patients report to a dental operatory with. Sometimes they may appear to occur concomitantly or at times be coexisting as separate entities. They often pose treatment dilemmas to the clinician as well as at times are refractory to the common treatment methodologies. Therefore we decided to explore the microbial profiles of these endo-perio lesions and also assess their antibiotic susceptibility to the commonly used antibiotics and antimicrobials in day to day dental practice.

SBI score was recorded in Group 1 and was statistically significant (p=0.0001) when compared to Groups 2,3a and 3b on an intergroup comparison basis. This can be correlated with the higher incidence of *P. gingivalis* (52%) and *B. forsythus* (20%) in this group which are predominantly the red complex bacteria. However the presence of 52% levels of *P. gingivalis* in the primary endodontic lesion group (Group 2) is a salient feature and indicates the bidirectional movement of the red complex bacteria in endo-perio lesions Therefore the endodontic and the periopathogenic microflora may be acting in synergy when it comes to the cumulative destruction that occurs in endo-perio lesions. This is also endorsed by the higher incidence of pocket probing depth PPD in Group 1 when both inter group and intra group comparisons were made. It can be therefore inferred that endodontic bacteria may also contribute to the destructive mechanisms in a pre-existent periodontal infection, however whether the converse happens is unclear.

When CAL were compared, the primary periodontal group showed the highest value which was statistically significant when compared to the other groups. This can be again defined by the predominant presence of red complex periodontal pathogens that are primarily proteolytic in nature and bring about connective tissue destruction. It is however surprising to note that Groups 3a and 3b showed greater CAL loss compared to Group 2. This endorses the fact that endodontic pathogens in them can induce and bring about greater connective tissue destruction when there is pre-existent periodontal disease. However the same does not hold true for Group 2 where the periodontal pathogens cannot cause increased CAL loss in the presence of a pre-existent endodontic lesions.

With respect to antibiotic sensitivity, Group 1 had a greater sensitivity towards Augmentin and Doxycycline when compared to Ofloxacin+ornidazole and Cefotaxime. However it was sensitive to the latter drugs as well. The lesions of Group 2 tended to be more sensitive to Ofloxacin+ornidazole combination and Cefotaxime when compared to the other groups. This can be attributed to the predominantly anaerobic gram negative flora that is seen to occur in primary endodontic lesions. However, since there is an interdispersion of flora between the endodontic and periodontal compartments in combined lesions one needs to take into consideration the preference for usage of an antibiotic combination rather than single drug therapy to avoid recurrence as well as drug resistance in endo –perio lesions.

Thus we can safely infer that endodontic flora does influence the severity of destruction in pre existent periodontal disease but the vice versa may not occur. There is a definitive migratory process between the two spaces when overall microbial profiles for the various endo-perio lesions are evaluated. Furthermore the possibility of causation of the lesions independently by different pathogens and newer niches of colonization needs to be explored in the future, as well as evaluation of their sensitivity to combination drug therapy should help the clinician in management of such cases.

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