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Anaerobic Organisms and Its Antibiotic Sensitivity in Diabetic Dentoalveolar Abscess – A Cross Sectional, Descriptive Study

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ABSTRACT

Background: The presentation of bacterial infection of dental origin is constantly changing and is a measurable reflection of modern evolution of oral flora. The bacteriological studies have shown variations in the microbiology of acute dental infections however, the bacterial species encountered are usually regarded as members of the commensal oral flora and because of this it is unclear which of their potential pathogenicity factors cause abscess formation. The prevalence of diabetes is increasing worldwide with diabetic individuals usually having higher predisposition to infections the self-medication and frequent use of broad spectrum antibiotics in the treatment of oral infections and other body infections may enhance the development of bacterial resistance. **Methods**: This descriptive, cross sectional study was conducted on 60 Diabetic patients with Dentoalveolar abscess. Purposive sampling was done. The pus sample collected; cultured (aerobically and anaerobically). Morphological, biochemical and antibiotic sensitivity tests were done to study the isolates. **Results**: A total of 116 isolates obtained, 31 of them were Peptostreptococcus, 26 of them were Peptococccus, 29 of them were Fusobacterium, 19 of them were Prevotella, 11 of them were Bacteroides. Among the entire anaerobically cultured bacteria, Metronidazole was the most sensitive drug (100%) followed by Amoxicillin/clavulanic acid (95%), Clindamycin (83%), Cefotaxime (72%) and Ciprofloxacin (60%). The least effective drug was amoxicillin. **Conclusion**: Anaerobic bacteria are prevalent in Diabetic patients. Due to emergence of antibiotic resistance pathogens, culture specimens and antibiotic sensitivity testing are essential for correct management of infections and the selection of appropriate antibiotics.

Keywords: Diabetes Mellitus, Abscess, Bacterial Infections, Metronidazole.

INTRODUCTION

Dental or Dentoalveolar abscess is a denomination used to describe localized collection of pus in the alveolar bone at the root apex of the tooth. It usually occurs secondary to dental caries, trauma, deep fillings or failed root canal treatment. Once the intact pulp chamber is breached, colonization of the root canals occurs with a diverse mix of bacteriological agents. After entering the periapical tissues via the apical foramen, these bacteria are capable of inducing acute inflammation leading to pus formation. The presentation of bacterial infection of dental origin is constantly changing and is a measurable reflection of modern evolution of oral flora.[1,2] the bacteriological studies have shown variations in the microbiology of acute dental infections however, the bacterial species encountered are usually regarded as members of the commensal oral flora and because of this it is unclear which of their potential pathogenicity factors cause abscess formation.

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Spreading odontogenic infections (SOIs) are the most common type of serious orofacial infection encountered by oral and maxillofacial surgeons, SOIs represent the transformation from a localized Dentoalveolar infection, usually a periradicular abscess, to a destructive infection that can spread rapidly through tissue planes, resulting in a significant incidence of mortality.[3] Infections caused by anaerobic bacteria are common, and may be serious and life-threatening. Anaerobes predominate in the bacterial flora of normal human skin and mucous membranes, and are a common cause of bacterial infections of endogenous origin. Infections due to anaerobes can evolve all body systems and sites.[4]

Condition predisposing to anaerobic infections include: exposure of a sterile body location to a high inoculum of indigenous bacteria of mucous membrane flora origin, inadequate blood supply and tissue necrosis which lower the oxidation and reduction potential, host defense mechanisms, functional or anatomical abnormalities of the host, and virulence of the infecting microorganism. It is not only the host defense that determines the outcome of infection, but the timing and appropriateness of antimicrobial treatment as well.[5] The prevalence of diabetes is increasing worldwide with diabetic individuals usually having higher predisposition to infections. Infections

represent a frequent and severe systemic complication of diabetes mellitus and are said to be associated with sustained hyperglycemia.[6] As bacterial pathogens and antibiotic resistance patterns may change over time and based on location, in diabetic patients, the self-medication and frequent use of broad spectrum antibiotics in the treatment of oral infections and other body infections may enhance the development of bacterial resistance.[7,8]

In view of the preceding scenario, our study was designed with an objective to comprehend the role of anaerobic bacteria in Dentoalveolar abscess in Diabetic patients by isolating and identify anaerobic organisms and testing their antibiotic sensitivity in order to provide guidelines for effective treatment.

MATERIALS AND METHODS

This cross-sectional, descriptive study was conducted from January 2019 to December 2019, after obtaining institutional ethical clearance; we included 60 patients with fasting blood sugar levels greater than 130 mg/dL or with a known history of diabetes mellitus who visited the outpatient department of oral medicine and radiology.

Purposive sampling was done; we included patients aged between 40 to 65 years with dent alveolar abscess with intraoral or extra oral swelling, [Figure 1] excluded patients with previous endodontic therapy of the affected tooth, teeth with periapical sinus/fistula, antibiotic therapy with in previous two months. A brief case history along with Diabetic history taken and investigated for blood HbA1c levels, if clinical findings satisfied the inclusion criteria, the patient was informed about all the procedures to be performed during the study. Following that, if patient was ready to be a part of the study, the patient was asked to sign the consent form.

Procedure was explained to the patient. Patient was seated on a dental chair and draped with a patient drape. Patient was asked to rinse mouth with water. For each patient, the oral mucosa overlying the abscess was scrubbed with tincture of iodine; a sterile 18- gauge needle fitted to a 3-ml disposable syringe was passed through the alveolar mucosa into the abscess, from which the contents are withdrawn. The needle was sealed immediately by cork [Figure 2]. The samples are transported to microbiology laboratory held at room temperature and processed within 30 mins.

Sample processing was done in the following way: Direct microscopy for Bacterial morphology: A thin smear of the pus sample was made on a clean glass slide and allowed to air dry. Smear was gently heat fixed, stained by Gram staining technique and examined under oil immersion objective of the light microscope, for the presence of pus cells, Gram positive and Gram negative organisms. The size, shape and arrangement of bacteria were noted.

Anaerobic culture- The sample was inoculated onto Brucella blood agar containing vitamin K and Hemin in which a Metronidazole disc was placed at the junction of primary and secondary streaking to identify the anaerobes presumptively. A MacConkey agar plate streaked with Pseudomonas aeruginosa ATCC strain which is an obligate aerobe was used as a negative control for anaerobiosis. The plates were immediately incubated anaerobically for 48 hours at 37°C in an anaerobic jar [Figure 3] (Hi media Anaerobic System Mark II LE 002 3.5L) with Gaspak (BD GasPak EZ Gas Generating Container Systems with indicator).

After 48hrs plates were examined for growth and colony morphology of different colonies were recorded. [Figure 4] Transmitted light was used to look for hemolysis. Gram stained smears of the colonies were examined under oil immersion objective of the microscope. Catalase and Spot indole test were performed. Each colony were then subcultured onto brucella blood agar plates and based upon gram staining Nitrate disk, Bile esculin disk and Special potency disk were used for gram negative, Sodium polyanethol sulphonate (SPS) disk was used for gram positive and the plates were incubated in the anaerobic jar with Gaspak at 37°C for 48 hrs. Aerotolerance test was done to differentiate between obligate and facultative anaerobes. Zone of inhibition were measured after incubation and results were interpreted. Biochemical tests (catalase, Nitrate disk reduction test, coagulase, Bile test, Spot indole test, Sodium polyanethol sulphonate test, Special potency antibiotic disc susceptibility) were performed.

Antimicrobial susceptibility testing was performed anaerobic isolates, by disc diffusion method. Colonies of bacteria are spread over Mueller- Hinton agar medium. Discs impregnated with antibiotics are placed by the help of sterilized forceps. This plate was again incubated for 12–24 h at 37°C. Zone of inhibition is measured by the help of the WHO quality control chart to access the sensitivity of the antibiotics (Amoxicillin, Amoxicillin & clavulanic acid, Clindamycin, Cefotaxime, Metronidazole, Ciprofloxacin, antibiotics were selected for testing).

RESULTS

The study population comprised of 60 patients, there were 37 males and 23 females [Table 1].

Among 60 patients constituting the study group, 57 cultures had positive results, and 3 cultures had no growth [Table 2], Total isolates obtained were 116, among that 3 cultures revealed no growth, 9 cultures revealed one isolate, 48 cultures revealed 2 isolates, among the 116 isolates obtained, 57 of them were Gram +ve, 59 were Gram –ve [Table 2].

Among the 116 isolates obtained, 31 of them were Peptostreptococcus, 26 of them were Peptococccus, 29 of them were Fusobacterium, 19 of them were Prevotella, 11 of them were Bacteroides. [Table 3]

Table 1: Demographic Data

Parameter	Group	N (%)
Number of patients		60
Age (Years)	<50	14 (23%)
	50-70	34(56.6)
	>70	12(20%)
Sex	(M: F)	37:23 (61.66%:38.33%)
Duration of Diabetes	<10 Years	27
	10-20 Years	19
	>20 Years	14
Mean HbA1c		7.75
Poor glycaemic control	HbA1c > 8%	32(54%)
Average isolates per patient		1.933

Table 2: Culture Results

Finding of culture	Number of specimens	Percentage (%)
Total positive cultures	57	95
Total negative cultures	3	05
1 isolate per culture	9	15.78
2 isolates per culture	48	84.21
Gram +ve	57	49.13
Gram -ve	59	50.86

Table 3: Incidence of Various Anaerobic Bacterial Isolates

Isolated	Number of isolates	Percentage (%)
Gram Positive		
Peptostreptococcus	31	26.72
Peptococccus	26	22.41
Gram Negative		
Fusobacterium	29	25.00
Prevotella	19	14.65
Bacteroides	11	9.48

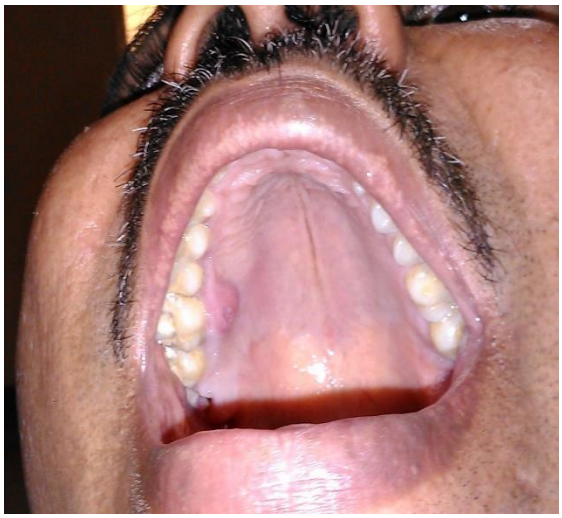

Figure 1: Dentoalveolar abscess in anterior palatal region

Figure 2: Sample in the syringe with widen cork

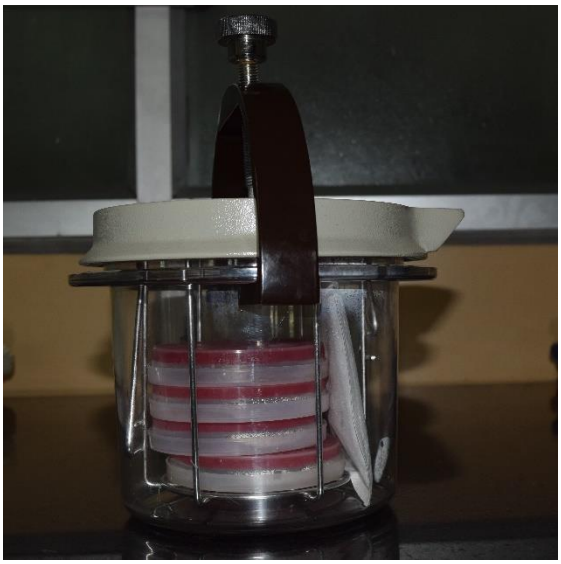

Figure 3: Anaerobic jar with plates and Gaspak

Figure 4: Growth of organisms

Peptostreptococcus spp (26.72 %) was isolated in 31 instances in which Metronidazole was the most sensitive drug (100%) followed by Amoxicillin/clavulanic acid (95%), Clindamycin (88%), Cefotaxime and Ciprofloxacin (70%) each. The most resistant drugs were amoxicillin (100%). Peptococccus (22.41 %) was isolated in 26 instances in which Metronidazole was the most

sensitive drug (100%) followed by Amoxicillin/clavulanic acid (92%), Clindamycin (80 %), Cefotaxime (75 %) and Ciprofloxacin (60%) The most resistant drugs were amoxicillin (100%).

Among the entire anaerobically cultured bacteria, Metronidazole was the most sensitive drug (100%) followed by Amoxicillin/clavulanic acid (95%), Clindamycin (83%), Cefotaxime (72%) and Ciprofloxacin (60%). The least effective drug was amoxicillin (100%) [Figure 5].

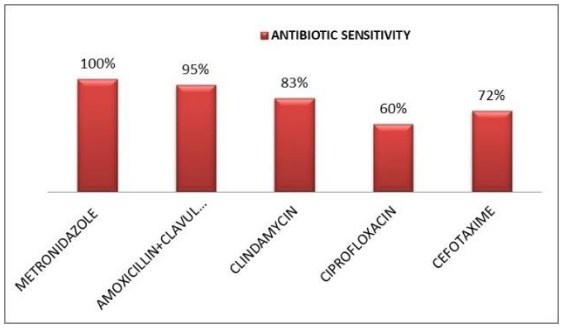

Figure 5: Antibiotic sensitivity pattern

DISCUSSION

Since Dent alveolar abscess are induced by microorganisms that are part of the normal oral microflora, it is therefore extremely important to disinfect the mucosal surface overlying the abscess, thus, a variety of sampling techniques have been used in past studies, previous studies have used iodine solution for surface disinfection.[9,10] Few used 70% ethyl alcohol solution.[11,12]

In our study for each patient, the oral mucosa overlying the abscess was scrubbed with tincture of iodine; a sterile 18- gauge needle fitted to a 3-ml disposable syringe was passed through the alveolar mucosa into the abscess, from which the contents are withdrawn, the needle was sealed immediately by cork. Specimen transport - Most studies do not describe the method used in transporting the specimen from the patient to the laboratory for culture, an ideal transport medium keeps the microbes alive and preserves their proportions in the sample. In our study after the aspiration the needle was sealed immediately using wooden cork, the specimens were transported in the same syringe to microbiology laboratory and processed within 30 mins, this method was employed in the previous studies.[13,14]

Culture techniques - The majority of early investigation on dental periapical abscesses actually failed to utilize adequate anaerobic culture techniques.[15,16] Later studies utilized different culture media for primary isolation which may well explain the variance in the previously published data. Blood agar plates were almost universally used, but their composition unfortunately varied widely.

Kuleki et al used anaerobic blood agar (trypticase soy agar with yeast extract, hemin, vitamin K, and defibrinated sheep blood) to obtain total bacterial counts and anaerobic blood agar with kanamycin to select for Prevotella and Porphyromonas species.[9] Mahalle A et al used nutrient broth for aerobic microflora and thioglycolate media for anaerobic microflora.[10] Shah A et al used culture media containing chocolate agar or Mac Conkey agar medium. Plates were incubated at 37°C for 12–24 h in an aerobic atmosphere.[17] In a study by Kityamuwesi R et al specimen was immediately inoculated in a transport medium, Soybean casein digest broth - BD BACTEC Plus + Anaerobic/F Medium.[12] Habib A et al inoculated swab immediately into a tube of thioglycollate broth, the specimens were incubated for 24 hours at 37°C. then sub cultured onto 2 solid agar plates, one blood agar plate for aerobic incubation for 24 hours and one brain heart infusion agar for anaerobic incubation for 48-72 hours.[18] The use of an anaerobic chamber is currently considered the best technique available for recovery of stringent anaerobes since specimens and cultures can be protected from oxygen at every stage of the procedure.

Specimen processing and Identification- all the anaerobic and aerobic plates were examined. The colonies of bacteria were identified by their macroscopic and microscopic appearance. Gram smear and aerotolerance testing was done. If the colony grows on aerobic culture, it is unlikely to be an obligate anaerobe (except Clostridium septicum and Actinomycetes). If no growth occurs on aerobic culture, the organism is presumptively identified by means of Gram's reaction, colony characters, and available biochemical tests.[10] Anaerobic bacteria were further cultivated on Anaerobic agar with addition of 50 mg/ml kanamycin to selectively inhibit facultative anaerobes and aerobes while permitting growth of strict anaerobes.[12] Biochemical tests (catalase, oxidase, coagulase, indole test) were performed to identify the species. 18 In our study isolates were identified based on microscopic characteristics, aerotolerance test, colonial characteristic and biochemical tests comparable with previous studies.[19] Significant improvement in the routine diagnostic yield from acute dental abscesses has occurred with employment of meticulous specimen collection and processing on selective and nonselective agars under appropriate atmospheric conditions. However, despite the close attention to detail, it is apparent that many genera of bacteria have yet to be cultured.

The studies using swabs of purulent material have demonstrated poor recovery of strict anaerobes and low mean numbers of isolates per sample (range 1.0-1.1).[20] Major limitation of past cultural studies is that a large percentage of the oral microflora

does not grow on conventional artificial culture media in the laboratory. 21 Our study has revealed 95% positive cultures of anaerobic microorganisms (57/60 cases). A total of 116 isolates were recovered, accounting for 1.93 isolates per specimen. This finding is comparable with other workers.[18,19]

Our study has revealed Peptostreptococcus (26.72%), Peptococccus (22.41%), Fusobacterium (25%), Prevotella (14.65%), Bacteroides (9.48%), regarding aerobic isolations, the finding are comparable to other workers but the proportion of Gram negative (49.13%) isolates is much more than that reported by other workers. Thus, from the present study it can be concluded that Anaerobic Gram negative bacteria play an important role as causative agent in diabetic patients.

The prevalence of bacteria increases with the severity of diseases, immunity of patient and the effect of predisposing factors. Our study is similar to previous studies in which both gram positive and gram negative organisms are fairly involved in dental diseases in diabetic patients.[22,23] Prevotella species have been reported as the most frequent isolates in numerous studies, found in 10-87% of dentoalveolar abscesses.[24,25]

In this study diabetic (Type II) patients were selected because there is little knowledge available regarding pathogenesis, progression and healing of periradicular and pulpal diseases. Anaerobic infection is also increased by vascular problems associated with diabetes, which may be associated to shortened oxygen diffusion over the capillary wall. Because of neutrophil microbicidal suppression, synergism of aerobic and anaerobic bacteria, infection becomes more severe and it also lasts longer due to anoxia.[26,27] It is worth visualizing, that isolated microorganisms are not same in each case but differ from one individual to other, i.e., Bacteroides, Fusobacteri¬um, Peptostreptococcus, Peptococcus and Eubacterium and these bacteria have been found out to be the main causative agent of endodontic infection.[28]

Among the entire anaerobically cultured bacteria, Metronidazole was the most sensitive drug (100%) followed by Amoxicillin/clavulanic acid (95%), Clindamycin (83%), Cefotaxime (72%) and Ciprofloxacin (60%). The least effective drug was amoxicillin (100%). The highest rates of penicillin resistance have been observed with the members of the genus Bacteroides and Prevotella.[29-31]

The use of either metronidazole indicating 100% overall sensitivity of the pathogenic flora in the case of this association or amoxicillin in combination with clavulanic acid should be considered as alternatives. Clindamycin remains an alternative in individuals who are allergic to the penicillin group of antibiotics; other authors report high overall in vitro rates of sensitivity to cefazolin, cefotaxime and ciprofloxacin.[32,33]

CONCLUSION

The present study has highlighted the incidence of anaerobic bacteria is higher in Diabetic patients. The most common anaerobic bacteria are Peptostreptococcus species, Peptococccus, Fusobacterium species and Prevotela species. Due to emergence of antibiotic resistance pathogens, culture specimens and antibiotic sensitivity testing are essential for correct management of infections and the selection of appropriate antibiotics. Metronidazole indicating overall sensitivity of the pathogenic flora or amoxicillin in combination with clavulanic acid should be considered as alternatives. Clindamycin remains an alternative in individuals who are allergic to the penicillin group of antibiotics.

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