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SIGNIFICANCE OF OBLIGATE ANAEROBIC GRAM NEGATIVE BACTERIA IN ORAL ABSCESS: A STUDY OF WESTERN UTTAR PRADESH

Surgery

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ABSTRACT

Introduction: Periodontal oral abscesses represent approximately 8% of all dental emergencies in the world. The proportions of strict anaerobic, Gram negative organisms increase significantly in Oral Abscess. **Aim & objectives:**The purpose of this study was to look at the frequency of strict anaerobic bacteria in patients with oral abscess.Lack of data regarding the role of anaerobic bacteria in oral abscess from this geographical area prompted us to carry out this study. **Material and Methods:** Study comprises of 40 patients. 20 apparently healthy subjects without clinical signs and symptoms of Oral abscess constituted the control group and 20 patients with Oral abscess comprise of study group Sub gingival plaque Samples were collected into sterile test tubes containing 2 ml of transport media that is Robertson cooked meat (RCM) broth Samples were processed from RCM Broth, for both aerobes and anaerobes bacteria. **Results:** Anaerobe isolation was 81.67% in Oral abscess and 48.33% in healthy subjects, which shows increased rate of isolation of anaerobe has strong association with oral abscess.
In our study most common isolate was Prevotella spp (Oral Abscess=70%,Healthy=30%) followed by Fusobacterium sp. (Oral Abscess= 60%, Healthy=15%), Porphyromonas sp. (Oral Abscess = 50%, Healthy=10%)and Bacteroides sp. (Oral Abscess= 30 %, Healthy=10%) .p- Value for Prevotella sp., Fusobacterium sp., Porphyromonas sp. & Bacteroides sp. were 0.011, 0.003, 0.005, and 0.114 for Oral Abscess which shows strong association of these anaerobic GNB with Oral Abscess. **Conclusion:** In our study, statistically significant decrease in aerobic –anaerobic ratio suggests that anaerobes play a major role in oral abscess.Cultural methods are still economical and gold standard. Diversity of anaerobic bacteria in oral abscess should be considered in the treatment strategy of the patients. Considering the scarce data on microbial flora in the Indian population, further studies for assessment of microbial profile in various forms of oral abscess should be carried out.

KEYWORDS

INTRODUCTION

A Periodontal Oral Abscess (also termed "Lateral Abscess", or "Parietal Abscess"), is a localized collection of pus (i.e. an abscess) within the tissues of the Periodontium.[1]

It occurs alongside a tooth, and is different from the more common Periapical Abscess, which represents the spread of infection from a dead tooth (i.e.which has undergone pulpal necrosis). [2][3]

Among all emergency dental conditions, periodontal abscesses represent approximately 8% of all dental emergencies in the world, and upto 14% in the USA. [4]

The signs and symptoms of the acute dental abscess are pain, fever, swelling, trismus and erythema usually localized to the affected tooth, although the suppuration can frequently spread to the nearby tissues causing fatal complications. Deep neck and descending necrotizing mediastinal abscesses are a rare complication of the dental abscess and spread of odontogenic infections accounts for a large number of deep neck abscesses. [5]

Death usually occurs due to sepsis and multi-organ failure although airway occlusion is also a significant complication and requires early management by tracheostomy. [6]

The microorganisms that colonize the periodontal abscesses are primarily Gram negative anaerobic rods. The most commonly isolated genera include anaerobic *Streptococci*, *Fusobacterium species* and the black-pigmented anaerobes such as *Prevotella* and *Porphyromonas species.* [7]

Prevotella species have been reported as the most frequent isolates in numerous studies, found in 10-87% of dentoalveolar abscesses.[8][9][10][11] The proportions of strict anaerobic, Gram negative organisms increase significantly in oral abscess.

Clinical interests in these organisms are linked to the therapeutic problems usually encountered in treating mixed orodental infections. The relevant medical/dental history and examination is mandatory for the proper diagnosis of such cases.

Data on anaerobic periodontal microflora in the Indian population is very scarce.

The purpose of this study was to look at the frequency of strict anaerobic bacteria in patients with oral abscess. Lack of data regarding the role of anaerobic bacteria in oral abscess from this geographical area prompted us to carry out this study.

MATERIAL AND METHODS

The present study was carried out in Post Graduate Department of Microbiology, Subharti Medical College in collaboration with the Department of Periodontics, Subharti Dental College, Meerut for a period of one year.

Adult patients of eithersex, attending the OPD of Periodontology department and diagnosed clinically as Oral abscess were included in the study.

Patients who have taken any antibiotic, anti-inflammatory medications in past 6 months, or on any dental treatment or with any systemic illnesses were excluded from study.

The study has been approved by the research and ethical committee of Subharti Medical College. There is no conflict of interest.

Present study comprises of 40 patients. 20 apparently healthy subjects without clinical signs and symptoms of oral abscess constituted the control group and 20 patients with oral abscess comprise of study group

Informed and written consent of all the 40 subjects was taken for the study.

The culture media, reagents and chemicals used in the study were procured from Himedia Laboratories Private Limited, Mumbai, India. Sub gingival plaque Samples were collected into sterile test tubes containing 2 ml of transport media that is Robertson cooked meat(RCM) broth.(9)(HIMEDIA REF 149) with complete aseptic precautions.

Samples were processed from RCM Broth, for both aerobes and anaerobes bacteria:

For isolation of aerobes, samples were inoculated on Blood Agar, Chocolate Agar and Mac-Conkey agar culture plates.

For microaerophilic bacteria, plates were incubated under 5-10% carbon di oxide at 37 degree C for 24-48 hours. Aerobic isolates were identified on the basis of their gram staining, culture characteristic and biochemical reactions as per standard protocol. [8,12,13,14]

For isolation of anaerobes, sample was inoculated on Brain heart infusion Blood(BHIB) agar supplemented with vitamin K. Metronidazole disc(5 microgram) was placed on the well of sample inoculation to look for anaerobic growth away from it.Inoculated plates were placed in McIntosh Filde's jar with gas pack (Himedia Anaerogas pack) and incubated at 37°C for 5 days. Anaerobic isolates so obtained were identified on the basis of their gram staining, culture characteristics, aerotolerance test and biochemical reactions as per standard protocol. (8,12,13,14,15,16,17) For identification of gram negative anaerobes special potency discs test was done . Antibiotic discs Vancomycin (5 microgram), Kanamycin (1000 microgram) and Colistin (10 microgram) were placed on Brain heart infusion Blood agar (supplemented with vitamin K) plate with lawn culture of anaerobic isolates. [16,17]

Identification of both aerobic and anaerobic bacteria were also confirmed by the Automated Vitek 2 Compact System using GP card, GN card and ANC card (Bio Merieux, Marcy I' Etiole,France).

Interpretation and analysis of the obtained results were carried out using standard statistical tests of significance.

p Value of <0.05 was considered a statistically significant.

RESULTS AND DISCUSSION

In our study, more number of aerobic flora was isolated from healthy individuals that is 88.33% as compared to Oral abscess that is 58.33 %.(table 1)

Similarly Gomes *etal* [7] , William *etal* [16] isolated 53.3%,21.9% of aerobes in Oral Abscess.

Table1) Aerobic/facultative anaerobes isolated were:

AEROBIC FLORA	HEALTHY GROUP	ORAL ABSCESS
Samples processed(n) =60	20	20
Gram Positive Cocci	5(25%)	6(30%)
• ***Streptococcus viridans***	6(30%)	-
• ***Subspecies mutans***	2(10%)	4(20%)
• ***Subspecies salivarius***	2(10%)	4(20%)
• ***Subspecies mitior***	6(30%)	7(35%)
• ***Subspecies sanguis***	-	2(10%)
• ***Subspecies milleri***	14(70%)	2(10%)
• ***Staphylococcus aureus***		
• ***Staphylococcus epidermidis***	-	1(5%)
• ***Strepococcus pyogenes***		
• **Gram Negative Cocci**	13(65%)	4(20%)
• ***Moraxella catarrhalis***		
• **Gram Negative Bacilli**	2(10%)	1(5%)
• ***Escherichia coli***	2(10%)	-
• ***Klebsilla pneumoniae***	1(5%)	4(20%)
• ***Pseudomonas aeruginosa***		
TOTAL ISOLATES = 127	**53**	**35**
PERCENTAGE TOTAL	**88.33%**	**58.33%**

Isolation of Anaerobic flora was higher in Oral abscess as compared to healthy group. Anaerobe isolation was 81.67% in Oral abscess and 48.33% in healthy subjects, which shows increased rate of isolation of anaerobe has strong association with oral abscess. (Table 2)

Table 2) Obligate anaerobes isolated were:

ANAEROBIC FLORA	HEALTHY GROUP	ORAL ABSCESS
Samples processed(n) = 60	20	20
Gram Positive Cocci	-	1(5%)
• ***Peptoniphilus assachrolyticus***	1(5%)	4(20%)
• ***Peptostreptococcus anaerobius***	-	1(5%)
• ***Parvimonas micra***	-	-
• ***Anaerococcus prevoti***		
• **Gram Negative Cocci**	5(25%)	1 (5%)
• ***Veillonella sp.***		
• **Gram Negative Bacilli**	6(30%)	14(70%)
• ***Prevotella species***	2(10%)	10(50%)
• ***Porphyromonas species***	2(10%)	6(30%)
• ***Bacteroides species***	3 (15%)	12(60%)
• ***Fusobacterium species***		
• **Gram Positive Bacilli**	1(5%)	-
• ***Bifidobacterium sp***		
• ***Actinomycetes***	5(25%)	-
• ***subsp.naesulundi***	1(5%)	-
• ***subsp.odontolyticus***	1(5%)	-
• ***Clostridium sp.***		
***TOTAL ISOLATES* = 125**	**29**	**49**
PERCENTAGE TOTAL	**48.33%**	**81.67%**

In the present study obligate Gram negative anaerobes (85.71%) were predominantly isolated than the gram positive (12.24%) in patients suffering from Oral Abscess

In our study most common isolate was *Prevotella spp* (Oral Abscess=70%,Healthy=30%) followed by *Fusobacterium sp.* (Oral Abscess= 60%, Healthy=15%), *Porphyromonas sp.* (Oral Abscess = 50%, Healthy=10%)and *Bacteroides sp.* (Oral Abscess= 30 %, Healthy=10%) .

p- Value for *Prevotella sp., Fusobacterium sp., Porphyromonas sp. & Bacteroides sp. were* 0.011, 0.003, 0.005, and 0.114 for oral Abscess which shows strong association of these anaerobic GNB with Oral Abscess. (Table 3 & 4)

Increased isolation rate of *Prevotella*, *Fusobacterium sp.* and *Porphyromonas sp.* showed statistically significant results with Oral Abscess with significant p value of 0.011, 0.003 and 0.005 respectively proving *Prevotella, Fusobacterium* and *Porphyromonas sp.* as a causative agent for Oral Abscess.

Similar findings were recorded in other studies like Gomes *et al,* 2006[7] , Kuriyama *et al*., 2005[11] ; Baumgartner *et al.,* 2004[18] ; Fazakerley *et al.,* 1993[19] ; Kolokotronis, 1999 [20] ; Kulekci *et al.,* 1996[21] ; Lewis *et al.,* 1993[22] ; Riggio *et al*., 2006 [23] .Roche & Yoshimori, 1997[24] ; Siqueira *et al.,* 2001b, d[25][26] Wade *et al.,* 1994 [27] for Oral Abscess.

Table 3 & 4) Comparative spectrum of aerobe/facultative anaerobes & obligates anaerobes in patients with Oral Abscess & healthy control group subjects & and their statistical analysis.

AEROBIC FLORA	HEALTHY GROUP	ORAL ABSCESS	p value*	
Streptococcus viridans subsp.mutans	25%	30%	**0.723**	**NOT SIGNIFICANT**
Streptococcus viridans subsp. salivarius	30%	0%	**0.008**	**SIGNIFICANT**
Streptococcus viridans subsp. mitior	10%	20%	**0.375**	**NOT SIGNIFICANT**
Streptococcus viridans subsp. sanguis	10%	30%	**0.113**	**NOT SIGNIFICANT**
Streptococcus viridans subsp.milleri	30%	35%	**0.735**	**NOT SIGNIFICANT**
Staphylococcus aureus	0%	10%	**0.146**	**NOT SIGNIFICANT**
Staphylococcus epidermidis	70%	10%	**0.001**	**SIGNIFICANT**
Streptococcus pyogenes	0%	5%	**0.311**	**NOT SIGNIFICANT**
Moraxella catarrhalis	65%	20%	**0.004**	**SIGNIFICANT**
Escherichia coli	10%	5%	**0.548**	**NOT SIGNIFICANT**
Klebsilla pneumoniae	10%	0%	**0.146**	**NOT SIGNIFICANT**
Pseudomonas aeruginosa	5%	20%	**0.151**	**NOT SIGNIFICANT**

ANAEROBIC FLORA	HEALTHY GROUP	ORAL ABSCESS	p –Value*	
Peptoniphilus assachrolyticus	0%	5%	**0.311**	**NOT SIGNIFICANT**
Peptostreptococcus anaerobius	5%	20%	**0.151**	**NOT SIGNIFICANT**
Parvimonas micra	0%	5%	**0.311**	**NOT SIGNIFICANT**
Anaerococcus prevoti	0%	0%		---
Veillonella sp.	25%	5%	**0.076**	**NOT SIGNIFICANT**
Prevotella sp.	30%	70%	**0.011**	**SIGNIFICANT**
Porphyromonas sp.	10%	50%	**0.005**	**SIGNIFICANT**
Bacteroides species	10%	30%	**0.114**	**NOT SIGNIFICANT**
Fusobacterium species	15%	60%	**0.003**	**SIGNIFICANT**
Bifidobacterium sp	5%	0%	**0.311**	**NOT SIGNIFICANT**
Actinomycetes subsp. naesulundi	25%	0%	**0.016**	**SIGNIFICANT**
Actinomycetes subsp.odontolyticus	5%	0%	**0.311**	**NOT SIGNIFICANT**
Clostridium sp	5%	0%	**0.311**	**NOT SIGNIFICANT**

Polymicrobial nature of the oral abscess has been brought out in this study. (table 5)

Table 5) Comparative evaluation of Aerobic/Facultative Anaerobic isolates in various groups showing Polymicrobial nature of oral microflora.

Aerobic	Healthy group (total=20)	Oral abscess group(total=20)
Positive culture	**18(90%)**	**20(100%)**
Negative culture	**2(10%)**	**0**
Monomicrobial	**0**	**2(10%)**
Polymicrobial	**18 (100%)**	**18(90%)**
2 isolates	**7(38.89%)**	**6(33.33%)**
3 isolates	**10(55.56%)**	**7(38.89%)**
4 isolates	**1(5%)**	**5(27.78%)**
5 isolates	**0**	**0**

The ratio of aerobes and facultative anaerobe to anaerobes was 1.828 & 0.714 in cases of Healthy group and Oral Abscess respectively. (table 6)

This depicted that aerobes and Facultative anaerobes were more in normal healthy gingiva while anaerobes predominated in oral abscess.

Table 6)Comparative evaluation of Aerobic-Anaerobic Ratio in various groups.

	HEALTHY GROUP	ORAL ABSCESS
Samples processed (n) =40	20	20
Aerobes	53	35
Anaerobes	29	49
AEROBIC ANAEROBIC RATIO	**1.828**	**0.714**

CONCLUSION

Anaerobic gram negative bacilli *Prevotella* sp, *Fusobacterium sp and* additional *Porphromonas sp.*were found as the causative agents of Oral Abscess. Aerobic / facultative anaerobe like *Staphylococcus aureus* , *Streptococcus pyogenes, Pseudomonas sp, Streptococcus viridians subspecies milleri, sanguis, mitior, mutans.,* and Anaerobes like*, Bacteroides sp, Peptonpihilus assachrolyticus, Peptostreptococcus anaerobius , Parvimonas micra* have increased isolation in this group and they might play role in Oral Abscess .

In our study, statistically significant decrease in aerobic –anaerobic ratio suggests that anaerobes play a major role in oral abscess.

Cultural methods are still economical and gold standard.

Diversity of anaerobic bacteria in oral abscess should be considered in the treatment strategy of the patients. Considering the scarce data on microbial flora in the Indian population, further studies for assessment of microbial profile in various forms of oral abscess should be carried out.

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