Role Of Anaerobic Bacteria In Mild, Moderate And Severe Cases Of Chronic Periodontitis

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Citation

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Abstract

Purpose: Periodontitis is the important cause of tooth loss in individuals over the age of 45 years and along with dental caries, is the most frequent oral health problem in the world. The aim of this study was to isolate and identify strict anaerobic bacteria associated with mild, moderate and severe grades of chronic periodontitis and to study their antibiotic sensitivity pattern. Material and methods: Hundred consecutive clinically diagnosed patients with chronic periodontitis were included in the study. Sterile paper points were used for sample collection from the deepest pocket sites. Strict anaerobes were isolated by culture and their antibiotic sensitivity pattern was tested.Results: Anaerobes were isolated in 83% of periodontitis cases. Gram negative anaerobes were isolated in 75.6% cases while Gram positive isolates were seen in 24.3% cases. Peptostreptococus micros was the commonest isolate in mild, Porphyromonas gingivalis in moderate and Fusobacterium nucleatum in severe cases of periodontitis. Metronidazole showed good sensitivity against Gram negative anaerobes, while Tetracycline against the Gram positive ones.Conclusion: The study demonstrates the role played by anaerobic bacteria in the causation of chronic periodontitis and the importance of their antibiotic susceptibility testing in planning of the treatment strategies.

INTRODUCTION

Oral health is an important aspect of overall health status of an individual. Teeth and their supporting structures (peridontium) are of main importance to oral health [1]. Periodontitis (diseases of the peridontium) is the important cause of tooth loss in individuals over the age of 45 years and along with caries, is the most frequent oral health problem in the world [2]. At least 48 specific periodontitis categories are now recognized. By far the most common is chronic periodontitis [3]. The etiology of the disease is multifactorial and bacterial deposits play an essential role in its pathogenesis. The bacteria that are involved in periodontitis accumulate in the sub- gingival plaque that comprises predominantly of strict anaerobic Gram-negative rods [4]. The disease severity may be described as mild, moderate and severe. In chronic periodontitis, the amount of tissue destruction is consistent with the local etiologic factors of plaque and is associated with variable microbial pattern [5]. Hence knowledge of microflora in different stages of disease is essential.

Periodontitis is a lifelong disease characterized by periods of exacerbations and remissions. It is curable before bone resorption starts by antimicrobials and good oral hygiene. Elimination or adequate suppression of periodontal pathogens in the subgingival microflora is essential for periodontal healing to take place [6]. As periodontopathic microflora includes a variety of microorganisms with differing antimicrobial susceptibility, inappropriate antibiotic therapy can give rise to drug resistance. Moreover the misuse of broad spectrum antibiotics in the treatment of periodontitis may enhance the development of bacterial resistance, which will diminish their therapeutic potential and may cause problems in the treatment of serious infectious diseases [7]. Scarce information is available in scientific literature on microbial pathogens in chronic periodontitis, while we are unaware of reports regarding their antimicrobial sensitivity pattern in India.

Hence, in light of paucity of information, the present study was undertaken to isolate and identify strict anaerobes associated with mild, moderate and severe cases of periodontitis and study their antibiotic susceptibility pattern.

MATERIAL AND METHODS

The subjects for the present study were selected from the outpatient Department of Dentistry, B.J.Medical College, Pune, Maharashtra. The study was approved by the Ethics

Committee of our institute.

STUDY POPULATION

The study population included 100 consecutive chronic periodontitis patients who were referred for diagnosis or treatment of periodontitis.

For each patient a set of full mouth dental radiographs was done. Subsequently all teeth were radiologically examined on the mesial and distal aspects. The patients were further grouped into those having mild, moderate and severe disease [5] by the examining dentist.

48 patients had mild, 38 moderate and 14 severe degrees of periodontitis. Informed consent of all subjects was taken.

SAMPLE COLLECTION

Samples were collected with complete aseptic precautions with the assistance of dentists. Initially the site of sample collection was isolated with cotton rolls, carefully cleaned with sterile cotton pellets, and air-dried. For single sites, two sterile paper points were inserted to the bottom of the pocket for a 20-s period and then transferred to Robertson's cooked meat medium. For pooled samples, at least one paper point per site from upto four sites was collected [8] .

SAMPLE PROCESSING

The samples were processed within 24 hours. For isolation of strict anaerobes, the samples were plated on non-selective blood agar plates (5%) supplemented with hemin and menadione. Kanamycin-Vancomycin blood agar plates were used for selective recovery of obligate anaerobic Gram-negative rods. The plates were incubated in Lapiz anaerobic jar at 37ºC under anaerobic conditions for 7 days. After 7 days of incubation, colonies with differing characteristics were subjected to various tests. Identification was based on cell morphology, Gram stain reaction, biochemical and enzymatic tests including catalase, oxidase, indole hydrolysis, esculin hydrolysis, gelatin hydrolysis, urea hydrolysis and fermentation of sugars. All strict anaerobes were identified according to flow charts by Baron and Citron MD [9] and Koneman et al [10] .

Antibiotic sensitivity testing was done by the standardized antimicrobial disc susceptibility testing of anaerobic bacteria as described by Sutter et al [11] . The antibiotics tested were Penicillin (10units), Erythromycin (15 ug), Clindamycin (10ug), Metronidazole (5ug) and Tetracycline (10ug). The results were interpreted as sensitive (S), intermediate (I) or resistant as per the estimates of susceptibility by inhibition zone diameter measurements given by Sutter et al11,12 . Bacteroides fragilis ATCC 25285 was used as the control.

RESULTS

Anaerobes were isolated in 83% cases of periodontitis.

Polymicrobial pattern of microbial flora was seen in 72.25% cases.

Table I shows the spectrum of anaerobes isolated in mild, moderate and severe grades of periodontitis. Peptostreptococus micros was the commonest isolate in mild, Porphyromonas gingivalis in moderate and Fusobacterium nucleatum in severe cases of periodontitis.

Tables II and III show the in vitro susceptibility pattern of Gram-negative and Gram-positive organisms respectively.

Figure 1

Table I. Spectrum of anaerobes in mild, moderate and severe grades of chronic periodontitis

Organisms	**Mild** N= 48 Anaerobes isolated (70.38%)	**Moderate** N = 38 Anaerobes isolated (92.10%)	**Severe** N=14 Anaerobes isolated (100%)	**Total** N=100 Anaerobes isolated (83%)
Gram negative organisms (75.6%)				
Porphyromonas gingivalis	14	23	11	48
Prevotella melanogenica	03	08	03	14
Prevotella intermedia	03	07	02	12
Fusobacterium nucleatum	03	08	13	24
Bacteroides spp.	00	02	03	05
Veillonella spp.	08	01	00	09
Gram positive organisms (24.3%)				
Peptostreptococcus micros	16	06	00	22
Peptostreptococcus productus	03	01	00	04
Streptococcus intermedius	04	01	00	05
Proprionibacterium acnes	00	01	00	01
Actinomyces viscosus	02	00	00	02
Eubacterium lentum	00	02	00	02

Figure 2

Table II. In vitro susceptibility pattern of Gram negative anaerobes

Organism	**P** N (%)	**E** N (%)	**Cd** N (%)	**T** N (%)	**M** N (%)
Porphyromonas gingivitis	40 (83.3)	44 (91.6)	48 (100)	48 (100)	48 (100)
Prevotella melaninogenica	11 (78.5)	12 (85.7)	14 (100)	13 (92.8)	14 (100)
Prevotella intermedia	06 (50.0)	10 (83.3)	11 (91.6)	09 (75.0)	12 (100)
Fusobacterium nucleatum	13 (54.1)	11 (45.8)	24 (100)	23 (95.8)	24 (100)
Bacteroides spp.	03 (60.0)	04 (80.0)	05 (100)	04 (80.0)	04 (80.0)
Veillonella spp.	07 (77.7)	08 (88.8)	09 (100)	08 (88.8)	09 (100)
Total	**80 (71.4)**	**89 (79.4)**	**111 (99.1)**	**105 (93.7)**	**112 (99.10)**

P – Penicillin, E – Erythromycin, Cd – Clindamycin, T –Tetracycline, M – Metronidazole

Figure 3

Table III. In vitro susceptibility pattern of Gram positive anaerobes

Organism	P N (%)	E N (%)	Cd N (%)	T N (%)	M N (%)
Peptostreptococcus micros	17 (77.2)	13 (59.0)	17 (77.2)	21 (95.4)	20 (90.9)
Peptostreptococcus productus	03 (75.0)	02 (50.0)	03 (75.0)	03 (75.0)	04 (100)
Streptococcus intermedius	03 (60.0)	03 (60.0)	04 (80.0)	04 (80.0)	05 (100)
Proprionibacterium acnes	00	01 (100)	01 (100)	01 (100)	00
Actinomyces viscosus	01 (50.0)	02 (100)	02 (100)	02 (100)	01 (50.0)
Eubacterium lentum	01 (50.0)	02 (100)	02 (100)	02 (100)	01 (50.0)
Total	**25 (69.4)**	**23 (63.8)**	**29 (80.5)**	**33 (91.6)**	**31 (86.1)**

P – Penicillin, E – Erythromycin Cd – Clindamycin T –Tetracycline M – Metronidazole

DISCUSSION

In the present study anaerobes were isolated in 83% cases. Various studies in India and other countries have showed an isolation rate of anaerobes in periodontitis ranging from 42 to 100 % [4813] . The varying recovery rates of isolation can be due to varying criteria of patient selection, culture method employed, geographical differences and the molecular techniques used for identification [13] . Polymicrobial pattern of infection is routinely encountered in periodontitis. In the present study polymicrobes were found in 72.28% cases. Saini et al [13] and Salari et al [8] too have brought out the polymicrobial nature of periodontal flora in their studies.

In the present study Gram negative anaerobes (75.7%) were predominantly isolated than the Gram positive ones (24.3%). Porphyromonas gingivalis (48%), Fusobacterium nucleatum (24%), Peptostreptococcus micros (23%) and Prevotella spp. (26%) were the commonest organisms isolated. Other workers have isolated similar anaerobes in chronic periodontitis but in varying proportions [4813] . The varying bacterial proportions can be attributed to geographical variations.

The prevalence of anaerobes increases with the severity of disease and probing depth due to decreased oxygen tension in untreated human periodontal pockets, which is conducive to proliferation of anaerobes. In our study 48 patients had mild grade of periodontitis and anaerobes were isolated in 70.83% of them. Peptostreptococcus micros was the commonest isolate in these cases followed by Porphyromonas gingivalis and Veillonella. Anaerobes were isolated in 92.10% cases with moderate and 100% cases with severe periodontitis. Porphyromonas gingivalis, Fusobacterium nucleatum and Prevotella melanogenica were the commonest organisms isolated as the severity of disease increases. This can be explained on the basis of high production of butyric acid by these organisms that causes initiation of inflammatory changes and severe loss of attachment of connective tissue. Thus it can be said that Gram positive anaerobes are more prevalent in milder forms of disease, while Gram negative ones are seen in severe cases.

We are unaware of any studies regarding the antibiotic sensitivity pattern of periodontopathic microbiota in India. For the ease and sake of convenience in our setting we have used the disc diffusion method described by Sutter et al [11] . Disc diffusion method could give relatively accurate preliminary information [12] about the susceptibility pattern of the anaerobes isolated. In our study Gram negative isolates showed overall more sensitivity to antibiotics compared to Gram positive ones. The present study shows increased resistance of organisms to conventional antibiotics like Penicillin and Erythromycin. This is in confirmation with the statement of Wood R [14] who had reviewed the antibiotic sensitivity pattern of pathogenic organisms over a span of 20 years (1966-86). He stated that there is a continuous decline in the sensitivities of bacteria isolated to most of the antibiotics used in dental practice. Slowly and persistently resistant strains of all type of microorganisms encountered in dental practice are emerging. Penicillin, Amoxicillin, Erythromycin having been prescribed very frequently in dental practice have resulted in emergence of resistant strains as depicted in the study [14] . Metronidazoles, Clindamycin have been recommended for anaerobic infections. Both Gram-positive as well as Gram -negative organisms have fairly good sensitivity to these drugs in India. Interestingly Tetracycline that has been sparingly used in the past decade due to resistance and emergence of newer broad-spectrum antibiotics is showing high sensitivity. This can be attributed to reduction in the number of resistant strains due to restricted use.

In the study carried out by Van Winkellhoff et al [7] the anaerobic periodontal bacteria displayed higher level of resistance towards a number of antibiotics like Penicillin, Amoxicillin, Erythromycin Metronidazoles, Clindamycin and Tetracyclines. Other studies on subgingival microflora in periodontitis have indicated that bacterial resistance towards penicillins exists [1516] . Microbial resistance rates are not equally distributed around the world, thus even further studies on antimicrobial susceptibility patterns of periodontal pathogens in India are necessary which can help

in planning treatment strategies.

Thus from the present study it can be concluded that Gram - negative anaerobic rods play an important role in the causation of chronic periodontitis. Peptostreptococus micros was the commonest isolate in mild, Porphyromonas gingivalis in moderate and Fusobacterium nucleatum in severe cases of periodontitis. Metronidazole can be the choice of drug in case Gram negative anaerobes while Tetracycline holds good promise against the Gram positive ones. Considering the scarce data on periodontopathic microbiota in the Indian population more studies of similar nature should be considered for identifying the microbial profile in different forms of periodontitis and their antibiotic susceptibility pattern.

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