Microbiological Status of Periodontal Diseases in Lagos, Nigeria

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ABSTRACT

Objectives: *The dynamic nature of oro-facial infections made it imperative to study the epidemiology of gingivitis and periodontitis which are significant clinical conditions in the Nigerian environment.*
Methods: *The clinical and microbiological features of 162 patients with periodontal diseases (gingivitis 68, periodontitis 94) were analysed. The advantage of routine antibiotic susceptibility testing for oral pathogens in patients' management was investigated.*
Results: *The incidence of periodontal diseases was more in females than males with a ratio of 0.53 though the difference was not significant. A high incidence of gingivitis (55.9%) occurred within the first 29 years with a cluster of cases (48.6%) between 10 and 29 years of age with incidence tending to decline with advancing age. Though the incidence of periodontitis was highest amongst adults over 40 years (42.6%), the incidence of 8.5% in children below 10 years of age was high. Polybacterial aetiology was characteristic; aerobes were the predominant flora in gingivitis with a preponderance of Streptococcus spp, while anaerobes predominated in periodontitis with such species as Porphyromonas, Prevotella, Fusobacterium and Actinobacillus. Significant reduction in duration of treatment was obtained when patients were treated based on susceptibility results as opposed to empirical knowledge* ($p < 0.05$).
Conclusion: *The diversity of microbial aetiology of periodontal infections may put much demand on routine laboratory investigations for patient management, but it may be necessary to weigh the benefit of additional cost against the cost of treatment failure associated with antibiotic resistance in bacteria.*

Estatus Microbiológico de las Enfermedades Periodontales en Lagos, Nigeria

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RESUMEN

Objetivos: *La naturaleza dinámica de las infecciones orofaciales hizo imperativo el estudio de la epidemiología de la gingivitis y la periodontitis – condiciones clínicas significativas en el medio ambiente nigeriano.*
Métodos: *Las características clínicas y microbiológicas de 162 pacientes con enfermedades periodontales (gingivitis 68, periodontitis 94) fueron analizadas. Se investigó la ventaja de la prueba de rutina de la susceptibilidad antibiótica para los patógenos orales en el tratamiento de los pacientes.*
Resultados: *La incidencia de las enfermedades periodontales fue mayor en las mujeres que en los hombres con una proporción de 0.53, aunque la diferencia no fue significativa. Una alta incidencia de gingivitis (55.9%) ocurrió dentro de los primeros 29 años de edad con un grupo de casos (48.6%) entre 10 y 29 años de edad, tendiendo la tendencia a disminuir con el progreso de la edad. Aunque la incidencia de la periodontitis fue más alta entre adultos mayores de 40 años (42.6%), la incidencia de 8.5% en los niños de 10 años de edad fue alta. La etiología fue característica. Los aerobios fueron la flora predominante en la gingivitis, con preponderancia de Streptococcus spp, mientras que los anaerobios predominaron en la periodontitis con especies tales como* Porphyromonas, Prevotella, Fusobacterium *y* Actinobacillus. *Se obtuvo una reducción significativa de la duración del tratamiento cuando los pacientes fueron tratados a partir de los resultados de la susceptibilidad, en vez del conocimiento empírico (*$p < 0.05$).

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Conclusión: *La diversidad de la etiología microbiana puede causar una demanda excesiva de las investigaciones de rutina de los laboratorios para el tratamiento de los pacientes, pero puede ser necesario sopesar el beneficio del costo adicional frente al costo del fracaso del tratamiento asociado con la resistencia antibiótica de las bacterias.*

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INTRODUCTION

The oral cavity and its associated tissues house over 300 cultivable bacterial species many of which have been ascribed periodontopathogenic status (1, 2). *Streptococcus mutans* is the only organism consistently isolated from all decayed tissues and found in greater numbers in carious teeth (3, 4). In the presence of gingivitis, the prevalent subgingival floras are anaerobic Gram-negative rods with *Prevotella intermedia* as the most commonly isolated (5, 6). In rapidly progressive periodontitis, *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* lead the pack (7, 8) while *A actinomycetemcomitans* and *Capnocytophaga* spp predominate in juvenile periodontitis (1, 9). In suppurative odontogenic infections, *Fusobacterium, Porphyromonas*, *Prevotella*, *Peptostreptococcus*, *Actinomyces* and *Streptococcus* predominate (1, 10). Thus, the microbiology of infections in the oral cavity and tissues reveals a dynamic microbial succession whose composition may be influenced by age, diet or location of infection.

Besides caries, gingivitis and periodontitis are the most frequently diagnosed oral diseases in hospitals. Various reports showed a prevalence of 21– 25% while other conditions including pulpitis, dentoalveolar abscess and pericoronitis each accounts for less than 5% (11, 12). Age seems to play an important role in the pathology of oral diseases. Akinlaja *et al* (12) reported that the risk of suffering from oral diseases increases with age from 14.5% at 10–19 years to 100% at 50 years and above. The literature is equivocal on the predisposing role of gender to dental diseases and where there is a male to female preponderance, no physiological explanation has been adduced (13, 14).

Management of oro-facial infections requires a better understanding of factors that predispose to infection, aetiology and pathogenesis of each clinical condition in the oral cavity. It was in this respect that i) the role played by age and gender in the pathogenesis of periodontal infections and ii) the significance of microbiological investigation in the choice of antibiotics in the management of periodontal diseases were investigated.

SUBJECTS AND METHODS

Patients

The clinical and microbiological data of one hundred and sixty-two patients were analysed in this study (gingivitis 68 and periodontitis 94). The patients were seen over a period of two years (September 2001 to August 2003) and were drawn from the Departments of Preventive Dentistry and Oral and Maxillofacial Surgery of the Lagos University Teaching Hospital (LUTH) and College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria. Patients were admitted into this study only if they had not taken antibiotics three months prior to enrolment and on signing an informed consent form. Patients' biodata were obtained and then examined for the presence of one or two co-existing clinical entities.

Clinical examinations consisted of measurement of attachment loss made on all teeth with a Marquis probe on four sites per tooth (mesio-buccal, disto-buccal, mid-buccal and mid-lingual), probing depth, gingival index and plaque index (15), and assessments of alveolar bone loss by radiography. Patients were diagnosed for periodontitis when there were evidences of periodontal tissue destruction in at least two areas where pockets could be probed to 8mm and where 50% of the marginal alveolar bone had been lost or had at least four sites with pockets of over 5 mm probing depth and showed distinct evidence of alveolar bone loss by radiographs (16). Cases of gingivitis were established in the presence of at least two pockets with depths greater than 3 mm, bled on gentle probing and showed obvious changes in colour and texture of the gingiva.

Sampling procedures

Subgingival pocket plaque was sampled from the deepest portion of the periodontal pocket. After removing the supragingival plaque as completely as possible, three sterilized paper points (size 40; Piece, Tokyo, Japan) were separately inserted into the periodontal pockets and left there for 60 seconds. Two of the samples were transferred separately to 1 ml of reduced thioglycollate broth, dispersed by Vortex mixer for 30 seconds, and then diluted serially 10-fold (17). The third paper sample was placed in 1ml phosphate buffered saline (PBS, pH 7.2) and treated as above.

Microbiological monitoring

A set of specimens in reduced thioglycollate broth was inoculated in 0.1 ml aliquot onto the following media: blood agar plates supplemented with vitamin K_1 (1 μg/ml) and L-cysteine hydrochloride, neomycin blood agar (100 μg/ml neomycin), Wilkins-Chalgren agar and trypticase soy agar plates all supplemented with 10% defribinated sheep blood, 5 μg/ml haemin and 0.5 μg/ml menadione. Also, trypticase soy agar plus bacitracin, vancomycin and horse serum (TSBV) plates were inoculated for the isolation of *A actinomycetemcomitans*. The plates were incubated anaero-

bically in 10% CO_2 and 10% H_2 using the GasPak anaerobic jar system (Oxoid) at 37°C for 7 – 10 days. The second set of specimens in thioglycollate broth was inoculated onto blood agar plates, enriched trypticase soy agar (ETSA) and TSBV and incubated in air + 5% CO_2 at 37°C for 5 days. Specimens in PBS were inoculated onto blood agar, MacConkey agar and mannitol salt agar plates and incubated aerobically at 37°C for 24 – 72 hours. Colonies on the anaerobic and aerobic culture plates were counted where necessary and then identified.

Identification of isolates

Aerobic bacterial isolates were identified based on their colony and cellular morphology and conventional biochemical tests. The anaerobic isolates were identified using cultural characteristics, Gram staining reaction, motility, sugar fermentation test, indole production, dye tolerance test, susceptibility to antibiotics (the An-Ident test) and other biochemical characteristics using both the API 20A and the rapid API-ZYM enzyme systems (Montalieu, Vercieu, France). *Actinobacillus actinomycetemcomitans* was identified by colony morphology, sugar fermentation test, catalase and oxidase production.

Assessment of treatment efficacy

Patients were treated with antibiotics i) based on empirical knowledge of the disease and following routine treatment protocol (group A) or ii) based on laboratory investigations including antibiotic sensitivity test results (group B). Two groups with periodontitis comprising 18 patients each and two groups with gingivitis comprising 12 patients each were distributed between the two treatment groups following informed consent as follows: AP and AG comprising periodontitis and gingivitis patients respectively that received empirical therapy; BP and BG representing periodontitis and gingivitis patients respectively treated based on results of laboratory investigations.

For each treatment group, specimens were collected before the start of antibiotic therapy and one week after completion of treatment for bacteriological study. Also, the duration of treatment for complete resolution of symptoms was recorded for each patient under each group and treatment category. This corresponded with culture result post-therapy indicating 90% reduction in bacterial load. In practical terms, this represented not more than three colonies on primary isolation plates.

Statistical analysis

The differences in age and gender distribution among the clinical conditions were subjected to chi-square analysis at 5% confidence level while student's *t* test was used to determine if dependence on results of laboratory investigations will significantly reduce the duration of treatment.

RESULTS

Table 1 highlights the contributing roles of gender and age to the development of periodontal diseases. Females presented with a higher incidence of periodontal diseases with a male to female ratio of 0.53, though this difference was not significant ($p > 0.05$). On the contrary, there was a progressive increase of periodontal diseases with age (< 0.05) with patients over 40 years constituting the risk group. A high incidence of gingivitis (55.9%) occurred within the first 29 years with a cluster of cases (48.6%) between 10 and 29 years with incidence tending to decline with advancing age. Though the incidence of periodontitis was highest amongst adults over 40 years (42. 6%), the incidence of 8.5% in children < 10 years was high suggesting that juvenile periodontitis may be a problem in the study environment.

Initial bacterial mean counts pre-therapy in patients' samples with gingivitis were aerobes $10^{9.68}$ cfu/ml and anaerobes $10^{6.87}$ cfu/ml while for periodontitis it was $10^{8.75}$ cfu/ml for aerobes and $10^{8.99}$ cfu/ml for anaerobes. Table 2 presents the impact of laboratory investigations in the treatment of periodontal infections. Reduction of bacterial load by 90% post-therapy was achieved in 10 days and 7 days in gingivitis cases for empirical therapy and laboratory determined therapy (LDT) respectively. Similarly, in patients with periodontitis cure was effective with empirical therapy in 14 days and with LDT in 7 days. Significant differences occurred in reduction of treatment duration when choice of therapy was based on laboratory investigations ($p < 0.05$).

Tables 3 and 4 give the bacterial aetiologies of gingivitis and periodontitis. Infections were mostly polybacterial, with predominance of anaerobes in periodontitis (90.4%) and aerobes in gingivitis (85.3%). Mono-aetiological status was obtained for *Streptococcus pyogenes* and *Staphylococcus aureus* in two cases of gingivitis and α-haemolytic strep-

Table 1: Epidemiological data of periodontal diseases at LUTH, September 2001 to August 2003

Clinical conditions	No. of cases seen	Sex distribution		Age distribution (in years)				
		Female	Male	< 10	10–19	20–29	30–39	> 40
Gingivitis	68	44 (64.7)	24 (35.3)	5 (7.3)	15 (22.1)	18 (26.5)	18 (26.5)	12 (17.6)
Periodontitis	94	62 (66.0)	32 (34.0)	8 (8.5)	14 (14.9)	12 (12.7)	20 (21.3)	40 (42.6)

Percentage distribution is given in parentheses

Table 2: Effect of laboratory investigations on treatment efficacy of periodontal diseases

	Mean duration of treatment (days) for 90% bacteriological cure	Mean microbial count (log_{10} cfu/ml) post therapy	
		Aerobe	Anaerobe
Empirical therapy			
Gingivitis	10 ± 1.83*	0.40	0.26
Periodontitis	14 ± 2.47	0.15	0.11
LDT			
Gingivitis	7 ± 0.91	0.40	0.26
Periodontitis	7 ± 1.05	0.15	0.11

LDT, Laboratory determined therapy; *, Mean ± standard deviation

Table 3: Prevalent aerobic bacteria in periodontal diseases at LUTH, September 2001 – August 2003

Bacteria	Incidence (%) in Periodontitis	Gingivitis	MAS*
Streptococcus pyogenes	12 (12.8)	14 (20.6)	G2
α-haemolytic streptococci	19 (20.2)	20 (29.4)	G1
Enterococcus faecalis	5 (5.3)	2 (2.9)	
Staphylococcus aureus	10 (10.6)	8 (11.8)	G2
Staphylococcus epidermidis	3 (3.2)	4 (5.9)	
Pseudomonas aeruginosa	2 (2.1)	2 (2.9)	
Proteus spp	1 (1.1)	1 (1.5)	
Klebsiella aerogenes	8 (8.5)	3 (4.4)	
Escherichia coli	1 (1.1)	1 (1.5)	
Diphtheroids	3 (3.2)	3 (4.4)	
Total (%)	64 (68.1)	58 (85.3)	

*, G represents gingivitis, while G1 and G2 represent the number of gingivitis cases with mono-aetiology
MAS = mono-aetiological status

Table 4: Prevalent anaerobic bacteria in periodontal diseases at LUTH, September 2001 – August 2003

Bacteria	Incidence (%) in Periodontitis	Gingivitis	MAS*
Porphyromonas gingivalis	17 (18.1)	12 (17.7)	P2
Porphyromonas asaccharolytica	12 (12.8)	7 (10.3)	
Prevotella intermedia	9 (9.6)	4 (5.9)	
Prevotella melaninogenica	14 (14.9)	9 (13.2)	
Fusobacterium nucleatum	8 (8.5)	5 (7.4)	
Fusobacterium necrophorum	5 (5.3)	4 (5.9)	
Clostridium perfringens	3 (3.2)	0 (0.0)	
Actinobacillus actinomycetemcomitans	6 (6.4)	0 (0.0)	
Peptostreptococcus spp	11 (11.7)	6 (8.8)	
Total (%)	85 (90.4)	47 (69.1)	

P = periodontitis
MAS = mono-aetiological status

tococci in a case of gingivitis. *Porphyromonas gingivalis,* which was the predominant anaerobic isolate in both clinical conditions, was isolated in pure cultures from two cases of periodontitis. Gram positive aerobic bacteria, predominantly cocci, were more prevalent in the dental conditions than Gram negative bacilli. *Actinobacillus actinomycetemcomitans* was isolated from 6 (6.4%) cases of periodontitis but was not a component of the gingival flora. The peptostreptococci were isolated more from cases of periodontitis (11.7%) than from gingivitis (8.8%).

DISCUSSION

Though the data of this study did not reveal any statistical gender difference in incidence of periodontitis, many other studies seem to favour a female predisposition (10, 16, 17). This may not be unrelated to a higher preference for sugar based diet by women or changes in hormonal status. However, it may be necessary to investigate the exact role of these factors in the pathogenesis of periodontal diseases.

The data of the present study emphasize the importance of age in the pathogenesis of periodontal diseases (1, 18, 19). The data showed a progressive increase in incidence for both conditions in the first 39 years of life, thereafter incidence of gingivitis fell while periodontitis increased by two-fold. More importantly was the cluster of cases in early adult life (20 – 39 years). The epidemiological implication of this is that an increasing proportion of the population would have suffered from periodontal related diseases before middle age (40 – 50 years). A more predictive index to this is the seemly high incidence of juvenile periodontitis recorded in this study.

Antibiotic therapy is not considered in all cases of gingivitis and periodontitis. In quite a number of cases, the patients' management is limited to plaque removal by mechanical means, root planning, surgical debridement and/or irrigation or local delivery of antimicrobial agents. However, a number of studies has highlighted the importance of antibiotic therapy in periodontal health (20 – 22). In con-

templating systemic antibiotic therapy, Hancock and Newell suggested that clinicians must consider the following: what periodontal pathogens are present, susceptibility of the pathogens to drugs contemplated and at what level the drug is delivered into the periodontal pockets (23). This obviously is in consonance with studies that showed variations in antibiotic susceptibility of periodontal pathogens (24, 25).

It is common practice by clinicians to give antibiotics empirically. Goene *et al* (20) emphasized the significance of microbiological investigation in the treatment of severe periodontitis. In their study *A actinomycetemcomitans* was eliminated from periodontal pockets of their patients following combination therapy of amoxicillin 375 mg and metronidazole 250 mg three times daily for 7 days based on microbiological data. The patients had received initially, tetracycline or minocycline which are mainstay for treatment of *A actinomycetemcomitans* in addition to mechanical management but patients' conditions were refractory. The results of this study give impetus to routine microbiological investigations as a guide to choice of antibiotics in periodontal disease. It is important to note that this practice will reduce the duration of treatment and thus reduce the overhead cost both to the patient and hospital. Empiric choice of therapy may encourage emergence of bacterial strains resistant to antibiotics especially when therapeutic failure is frequent necessitating constant change in antibiotic regimen. Though it is true that good *in vitro* susceptibility test result has not always yielded corresponding *in vivo* outcome, the clinician's experience and the laboratory findings should be placed side by side in deciding the most appropriate drug to give.

The present study showed the preponderance of anaerobic bacteria in periodontitis and aerobic bacteria in gingivitis; these findings reflect the oxygen tension in different periodontal pocket depths as earlier reported (26). In a separate study, the dominance of aerobic over anaerobic bacteria both in gingivitis and periodontitis was reported (11) and this is in variance with the results of the present study. This variation could be due to better sampling procedures in the present study which included the use of paper points of different sizes.

In reference to our previous report (11), the α-haemolytic streptococci remain the most significant aerobic bacteria in this study. The importance of *Streptococcus* spp in periodontitis has been demonstrated in experimental animal studies where their LPS induced significant bone loss (4, 26). Although with a few exceptions, Gram negative aerobic bacteria are not considered serious periodontal pathogens (11, 14). Sandros *et al* (28) demonstrated the ability of *P gingivalis* but not *E coli* to adhere to and invade human pocket epithelial cells *in vitro* while Kigure *et al* (29) showed that *P gingivalis* could be carried by *Treponema denticola* deeper into periodontal pockets. These two studies have helped to describe the pattern of colonization of microorganisms in periodontal pockets.

Porphyromonas gingivalis was the predominant anaerobic isolate with approximately 18.1% incidence in periodontitis and a marginal difference of 0.4% incidence in gingivitis. *A actinomycetemcomitans* occurred exclusively in patients 10–19 years old suggesting a prevalence in juvenile periodontitis (9, 20). The most frequent bacterial association observed in this study was those between *A actinomycetemcomitans* and *F nucleatum*, *Porphyromonas* spp and *Prevotella* spp and association between *Peptostreptococcus* spp, *Prevotella* and facultative anaerobic bacteria (data not shown). It is worth noting that *P gingivalis* and *A actinomycetemcomitans* association was not observed in the present study. This seems to confirm the observation of Page *et al* (10) that patients with rapidly progressive periodontitis seldom show elevated antibody titres to both *P gingivalis* and *A actinomycetemcomitans*. Johansson *et al* (30) was later to explain that antagonism between *P gingivalis* and *A actinomycetemcomitans* accounted for why both organisms do not co-inhabit the same periodontal pockets. In another study, the ability of *P bivia* to produce subcutaneous abscess in mice was enhanced in the presence of *E coli* and *Peptostreptococcus* spp (31). This may explain in part the association between *Peptostreptococcus*, *Prevotella* and facultative anaerobic bacteria as observed.

CONCLUSION

A comparison of the data in this study and others showed that age is a risk factor in the development of periodontal infections more so as it determines the pathophysiological nature of the disease. Incidence of periodontal infections was generally higher in the female population, though the difference in incidence in both genders was not significant. Investigations of the roles of factors such as pregnancy, hormonal changes and diet may create better understanding of the pathogenesis of periodontal diseases. Polymicrobial aetiology was the rule in the periodontal infections; a shift of aerobic dominance in gingivitis to anaerobic preponderance in periodontitis occurred. This ecological shift was related to different oxygen tensions at supragingival plaques, subgingival plaques and root canal. The diversity of microbial aetiology of periodontal infections may put much demand on routine laboratory investigation for patient management, but the benefit of such practice should be considered in the light of increase and proliferation of bacteria resistant to antibiotics. In locality with no established antibiotic data for oral pathogens, reliance on routine culture and antibiotic sensitivity test results will be a useful guide to therapy.

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