Bacterial Etiology And Risk Factors Of Periodontal Diseases In Enugu Metropolis, South East Nigeria

U C Maduakor, N F Onyemelukwe, S N Maduakor, N C Azubuike, A O Onyemelukwe, E B Nnedu

Citation

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Abstract

Background: Periodontal disease is plaque induced inflammation of surrounding tooth structures. It is a major factor in adult tooth loss. There is paucity of information on microbial etiology of periodontal diseases in Enugu.

Aim: The study focused on the isolation and identification of bacteria in periodontal diseases and associated risk factors among patients in Enugu.

Materials and methods: This was a case controlled study that was conducted from August 2017 to September 2018. A total of 433 samples were randomly collected comprising of 140 patients with periodontitis, 173 with gingivitis and 125 healthy subjects. Sterile paper points were used for the sample collection. Standard culture and biochemical techniques were used for the isolation and identification. Structured questionnaires were used to record demographic variables and other risk factors. Analysis was done with Graph pad prism Version 6. Categorical variables were reported using descriptive statistics.

Results: A total of 1,044 microorganisms were isolated. Gingivitis accounted for 430 isolates, periodontitis 352, and healthy subjects 262 isolates. Anaerobic microorganisms predominated in gingivitis with Actinomyces spp preponderant and Capnocytophaga spp. trailing. Periodontitis had predominant Fusobacterium nucleatum followed by Aggregatibacter actinomycetecomitans then Actinomyces odontolyticus. Aerobes dominated in healthy subjects. Females presented more significantly in gingivitis.

Conclusion: This study is novel in Enugu. Mixed bacteria growth was evident in the study. Age, level of education, family history, and bleeding gums were significant risk factors. Knowledge of the microbial etiology of periodontal diseases and the risk factors is the key for a successful periodontal therapy.

INTRODUCTION

There is a high prevalence of periodontal diseases globally and identifying the etiology is the fundamental key to its control. Periodontal disease is a chronic bacterial infection characterized by continuous inflammation, breakdown of connective tissue and destruction of alveolar bone [1]. Periodontal disease is broadly grouped into gingivitis and periodontitis. Gingivitis is inflammation of the gingiva caused by the accumulation of dental plaque and it is reversible. It may be common in children as young as 5 years [2]. It is a consequence of inadequate oral hygiene practices (such as tooth brushing and use of interdental cleaning aids [3]. Periodontitis is the chronic inflammatory disease started by built up of dental plaque biofilm and sustained by a deregulated immune response and usually preceded by gingivitis resulting in irreversible destruction of the supporting tissues surrounding the tooth including the alveolar bone [4,2,1]. Periodontal diseases are polymicrobial, multifactorial, diseases with many host factors involved in determining the individual susceptibility to disease [5]. It has been reported severally that the initiation and progression of the disease is akin not only to the presence of bacterial strains pathogenic for the periodontium but also due to the absence or minimal proportions of the beneficial commensals in the susceptible

host [5,6,7]. Limited numbers of periodontal pathogens in the complex biofilm have been reported to initiate periodontal diseases. Evidences have confirmed that certain bacterial strains in the periodontal environment can induce gingival tissue inflammation and bone destruction. These bacterial strains are referred to as periodontal pathogens [8,9]. These periodontal pathogens when present even in very small quantity possess the ability to damage the periodontal structure [5]. Most periodontal pathogens are anaerobes but the biofilm may also habour facultative aerobes, capnophiles and microaerophiles whose number is dependent on the environment in developed biofilm and periodontal pocket [5]. It was reported that of 800-1,000 species that colonize the oral cavity 50 species were identified to be strongly related to periodontal disease [10]. These Socrasky and Haffeajee classified into 5 complexes, namely yellow complex or early colonizers, green complex or secondary colonizers, then the orange, purple and red complexes. Red complex are the secondary colonizers that are the main pathogens associated with bleeding on probing [11]. There is a strong microbial succession in the infection of oral cavity that may be influenced by age, diet or location of the infection [12]. The metabolic action of the primary colonizers in the gingival crevice changes the environment and enhances colonization by secondary microorganisms. These secondary colonizers are more pathogenic and when they exceed threshold, disease can occur, although the presence of periodontopathogens itself does not necessarily result in disease [11]. The union of various bacterial virulence factors, the activity and composition of the commensal microorganisms, and host immune factors, are needed for the initiation of the disease process [13].

However, besides periodontopathogens, genetic and environmental factors predispose to the development of the disease. The risk of periodontal disease is elaborated by several factors including any health condition that triggers bacterial defense mechanisms such as human immunodeficiency virus (HIV), diabetes and neutrophil disorders2. Obesity, tobacco smoking, poor nutrition, and sedentary lifestyle are also associated with increased risk of periodontitis [14].

Periodontitis is the major cause of tooth loss in adult population globally and these people are at risk of multiple tooth loss, edentulism and masticatory dysfunction as a result of which there is negative effect on nutrition, quality of life, self-esteem thereby imposing great socio-economic effect and healthcare cost [3,15,16].

Most information on periodontopathogens have emerged from studies done in Europe and USA, only recently has this been done in under developed world and the results showed differences both in quality and quantity when compared [17].

MATERIALS AND METHODS

POPULATION SAMPLED

Four hundred and thirty eight samples comprising of 125 “apparently” healthy subjects, gingivitis 173 and periodontitis 140 were included in the study.

The study was carried out in Microbiology Department of University of Nigeria Teaching Hospital Ituku-Ozalla, Enugu. Control subjects were drawn from ‘apparently’ healthy individuals who escorted patients to the clinics and periodontal health was defined as absence of clinically obvious oro-dental infection and manifestation of systemic disease.

Test samples were drawn from patients attending the outpatient department of dental clinics of University of Nigeria Teaching Hospital, Ituku Ozalla, Federal School of Dental Technology and Therapy and some private dental clinics all within Enugu metropolis. Gingivitis was defined as the inflammation of the gingiva which is plaque-induced in at least one site. Periodontitis was defined as the presence of pocket > 2 mm on more than one tooth.

ETHICAL GUIDELINES

The work was conducted in accordance with the Declaration of Helsinki (1964). Ethics Committee of the University of Nigeria approved the study. The purpose of the study was explained to subjects and those who agreed to participate were given consent form to sign.

Exclusion criteria

None of the patients had received antibiotic treatment three weeks prior to sample collection.

SAMPLE COLLECTION

Subjects were given pretested questionnaires to fill directly or were helped to fill. Information on general, personal and other relevant data were contained in the questionnaire.

Supragingival plaque was removed from the sampling site using sterile cotton swab. For the control group, gingivitis and periodontitis cases, samples were collected with the help

of sterile, absorbent paper point from subgingival plaque, gingival sulcus and the base of the pocket respectively.

INOCULATION

Samples were inoculated into Robertson's cook meat (RCM) (Himedia) and carried to the laboratory and incubated at 37oC for 48 hours [18].

Subcultures were made from the Robertson's cook meat media into different media using standard wire loop.

For aerobic cultures, subcultures were made from the top of Robertson's medium into sheep blood agar (Columbia agar, Oxoid) and MacConkey agar and incubated at 37oC for 18-24hrs.

For facultative anaerobes, subcultures were made onto blood agar, chocolate, Mitis Salivarius and brain heart infusion agar from the middle of the Robertson's medium and incubated at 5-10oC carbon dioxide at 37oC for 24-48 hrs using improvisation with candle jar.

For anaerobic cultures, subcultures were made from near meat particles onto Rogosa agar and anaerobic basal agar (Oxoid CM0972) supplemented with 5% horse blood. The medium was inoculated by surface plating to obtain single colonies. These were incubated in anaerobic jar (gas pack) at 37oC for a minimum of 72 hrs. Anaerobic condition was achieved using the Oxoid AnaroGen Atmosphere Generation System (AN0025). Anaerobiosis was monitored with the help of a biological indicator (Pseudomonas aeruginosa) and a chemical indicator (methylene blue)

IDENTIFICATION OF ISOLATES

Colony characteristics were examined in case of any growth. Identification of micro-organisms was done using colonial morphology, Gram stain and other biochemical tests. Identification up to species level was done using Rapid identification system (Oxoid). Rapid ID 32 strep was used for Streptococcus species and Rapid ANA II for anaerobes. Other conventional biochemical tests were also used such as coagulase, catalase, oxidase, indole, citrate etc

STATISTICAL ANALYSIS

All statistical analyses were performed using GraphPad prism version 6.0 (GraphPad, San Diego, CA, USA). Categorical variables were described using descriptive statistics (frequencies and percentages). Chi-square (x2) test and Fisher's exact test (at 95% confidence intervals) were used to test for significant differences in the proportions/prevalence of Gingivitis and periodontitis in comparison to healthy controls, with respect to the socio-demographic characteristics and relevant predisposing risk indicators. P-value ≤ 0.05 was considered statistically significant. Multivariate/binary logistic regression analysis was used to measure the strength of the association between one predictor variable and the disease outcomes (in the context of other predictor variables).

RESULTS

Table 1 shows that overall, 39.5% of the study sample had gingivitis, 31.9% had periodontitis and 28.5 % had apparently healthy periodontium. Most participants were 16-31 years old. Table 1, shows that periodontitis was not common among subjects aged <15 years (4.8%). Above this age, the prevalence increased with increasing age 15.9% in 16-31, 27.5% in 32-47, 68.4% in 48-63 and 85.7% in 64+. In gingivitis, highest prevalence was found in <15 years with 53.4% and this decreased with increasing age.

Table 1

Age and Frequency distribution of Patients sampled and healthy subjects

Age (Years	Healthy Subjects(%)	Gingivitis(%)	Periodontisis (%)	Total (%)
<15	9(42.9)	11(53.4)	1(4.8)	21(4.8)
16-31	69(33.2)	106(50.9)	33(15.9)	208(47.5)
32-47	35(34.3)	39(38.2)	28(27.5)	102(23.3)
48-63	11(13.9)	14(17.7)	54(68.4)	79(18.0)
64+	1(3.6)	3(10.7)	24(85.7)	28(6.4)
Total	**125(28.5)**	**173(39.5)**	**140(31.9)**	**438**

Table 2 shows that all samples yielded microbes. Of 438 samples 9.4% were monomicrobial while 90.6 were polymicrobial. In periodontitis, 5.7% were monomicrobial, 12.7% in ginigivitis, 8.8% in apparently healthy control while others were polymicrobial.

Table 2

Number and frequency based on number of isolates per sample in gingivitis, periodiontitis and healthy subjects

No of Isolates	Healthy Subjects(%)	Gingivitis(%)	Periodontis	Total (%)
Mono	11(8.8)	22(12.7)	8(5.7)	41(9.4)
Two Isolates	53(42.4)	65(37.6)	52(37.1)	170(38.9)
Three isolates	50(40.0)	64(36.9)	50(35.7)	164(37.5)
Four Isolates	10(8.0)	20(11.6)	26(18.6)	56(12.8)
Five isolates	1(0.8)	2(1.2)	4(2.9)	7 (1.6)
Total	**125(28.6)**	**173(39.6)**	**140(32.0)**	**438**

Table 3 shows the aerobic and facultative anaerobes isolated,

of 405 isolates 153 (37.8%) were recovered from healthy subjects, 133 (32.8%) were recovered from periodontitis while 119 (29.4%) were recovered in gingivitis. Of the 153 recovered from healthy subjects, Streptococcus sanguis ranked highest 41 (32.8%) and the least was aerobic Lactobacilli. In gingivitis, of the 119 isolates, Staphylococcus aureus ranked highest 25 (14.5%) and the least was Pseudomonas aeruginosa 1 (0.6%). Of the 133 isolates in periodontitis, A. actinomycetecomitans ranked highest.

Table 4 shows the profile of anaerobic microorganisms recovered, of 650 isolates, 109 (16.8%) were from healthy subjects, 230 (35.4%) from periodontitis and 311 (47.8%) from gingivitis. The most prevalent organisms in healthy subjects were Veillonella spp., in gingivitis, Actinomyces spp 99 (31.8%) and in periodontitis, Fusobacterium nucleatum ranked highest 42 (18.3%).

Table 3

The Number and frequency of Aerobic and facultative anaerobes in healthy subjects and periodontal disease in Enugu

Organism	Health subjects (%)	Gingivitis (%)	Periodontis (%)	Total(%)
Streptococcus mutans	21(16.8)	22(12.7)	10(7.1)	53(13.1)
Streptococcus sanguis	41(32.8)	20(11.6)	3(2.1)	64(15.8
Streptococus mitis	0(0.0	14(8.1)	7(5.1)	21(5.2)
other strept	4(3.2)	0(0.0)	0(0.0)	4(0.9)
Staphylococcus aureus	0(0.0)	25(14.5)	23(16.9)	48(11.9)
coag Negative Staph	15(12.0)	0(0.0)	1(0.7)	16(3.9)
Aerobic Lactobacilli	1(0.8)	4(2.3)	1(0.7)	6(1.5)
Candida albicans	4.8)	1(0.6)	5((3.6)	12(2.9)
Other yeast	0(0.0)	1(0.6)	5(3.6)	6(1.5)
Aggregatibacter actinomycetecomitans	25(20.0)	23(13.3)	39(27.9)	87(21.5)
Klebsiella spp	33(26.4)	8(4.6)	18(12.9)	59(14.6
Pseudomonas aeroginosa	7(5.6)	1(0.6)	8(5.7)	16(3.9)
Escherichia coli	0(0.0)	0(0.0)	13(9.3)	13(3.2)
Total	**153(37.8)**	**119(29.4)**	**122(32.8)**	**405**

Table 4

The number and frequency of anaerobes in Periodontal disease and healthy subjects

Organism	Healthy Subjects (%)	Gingivigtis(%)	Periodontitis(%)	total (%)
Fusobacterium nucleatum	13(11.9)	45(14.5)	42(18.3)	100(15.4)
Anaerobi Lactobacilli	0(0.0)	46(14.8)	15(6.5)	61(9.4)
Actinomyces viscosus	8(7.3)	51(16.4)	25(10.9)	84(12.9)
actinomyces odontolyticus	0(0.0)	14(4.5)	1(0.43)	15(2.3)
Actinomyces naeslundii	15(13.8)	25(8.0)	15(6.5)	55(8.5)
Actinomyces israelii	0(0.0)	9(2.9)	5(2.2)	14(2.2)
Peptostreptococcus anaerobius	17(15.6)	15(4.8)	13(5.7)	45(6.9)
Peptostreptococcus micros	25(22.9)	24(7.7)	19(8.3)	68(10.5)
Prevotella intermedia	2(1.8)	16(5.1)	26(11.3)	44(6.8)
Prevotella melaninogenica	0(0.0)	17(5.5)	14(6.1)	31(4.8)
Porphyromonas gingivalis	1(0.9)	10(3.2)	27(11.7)	38(5.8)
Bacteroides fragilis	0(0.0)	13(4.2)	0(0.0)	13(2.0)
Capnocytophaga spp	0(0.0)	4(1.3)	13(5.7)	17(2.6)
Veillonella spp	28(25.6)	22(7.1)	15(6.5)	65(10.0)

Table 5

Risk indicators of Gingivitis and Periodontitis in comparison to the control group.

Variables		Control	Gingivitis	Periodontitis	p-value*	p-value#
		N (%)	N (%)	N (%)		
Gender	Male	54	55	49	0.05	0.2069
	Female	71	118	91		
Age (Years)	< 15	9	11	1	0.7510	<0.0001
	16-31	69	106	33		
	32-47	35	39	28		
	48-63	11	14	54		
	>63	1	3	24		
Level of education	Illiterate	0	3	17	0.034	0.0371
	Primary	4	14	22		
	Secondary	18	46	35		
	Tertiary	103	110	6		
Method of oral hygiene	Brush and paste	90	137	81	0.3224	0.0371
	Chewing stick	12	10	26		
	Both	23	27	33		
Frequency of oral hygiene	Once per daily	81	123	104	0.1934	0.1414
	Twice per day	41	42	31		
	After each meal	3	8	5		
Family History	No	116	37	25	<0.0001	<0.0001
	Yes	9	136	115		
At what age	≤20	6	88	26	<0.0001	0.0016
	>20	119	85	114		
Visit to dentist	No	116	51	42	<0.0001	<0.001
	Yes	9	122	98		
Does gum bleed	No	122	74	72	<0.0001	<0.0001
	Yes	3	99	68		

Gingivitis vs. healthy control p-value*, Periodontitis vs. healthy control p-value#.

Table 5 shows that the prevalence of gingivitis was significantly higher (p=0.05) in females than males. There were significant differences in the distributions of gingivitis

among study subjects according to their level of education, history of toothache, toothache history age, visit to dentist and gum bleeding, in comparison to healthy controls. Similarly there was a significant difference in the prevalence of periodontitis according to age, level of education, history of toothache, toothache history age, visit to dentist and gum bleeding between subjects with periodontitis and healthy control group. There was however no significant difference when both gingivitis and periodontitis were compared with healthy control group according to method and frequency of oral hygiene.

Table 6

Multivariate logistic regression analysis of risk factors with Gingivitis and Periodontitis

Variables (dichotomous/binary)	OR*	p-value*	OR#	p-value#
Gender		0.0513		0.2100
Male	1		1	
Female	2.15		1.86	
Age		<0.0001		<0.0001
≤31 yrs	1		1	
>31 yrs	5.17		2.28	
Education		<0.0001		<0.0001
≤6 yrs	28.77		3.58	
>6 yrs	1		1	
Frequency of tooth brushing		0.0330		0.0108
Once daily	1.57		1.25	
>once daily	1		1	
Family history		<0.0001		<0.0001
Yes	59.29		5.23	
No	1		1	
Toothache history age		<0.0001		<0.0001
≤20	1		1	
>20	6.85		1.19	
Gum bleeding		<0.0001		<0.0001
Yes	38.41		2.58	
No	1		1	

OR* - Odd ratio for the predictor variables (risk factors) of gingivitis

P-value* - p-value for risk factors associated with gingivitis

OR# - Odd ratio for the predictor variables (risk factors) of periodontitis

P-value# - p-value for the risk factors associated with periodontitis

Multivariate logistic regression analysis of the risk factors associated with gingivitis and periodontitis showed that the risk factors significantly associated with gingivitis and periodontitis are; Age, level of education, frequency of tooth brushing, family history, toothache history age, as well as gum bleeding. These risk factors of disease outcome were each categorized into binary indicators of gingivitis and periodontitis as shown in table 6.

DISCUSSION

Periodontal disease is a growing health problem in Nigeria. Presently no work has been done to determine the etiological agents of periodontal disease in Enugu, few works done here dealt on the prevalence of the disease. The present study has shown evidence of diversity of microorganisms associated with gingivitis and periodontitis. There was higher dominance of anaerobic Gram-negative bacteria in periodontis 61.6%, in gingivitis Gram positive anaerobic bacilli predominated and in healthy subjects' aerobic Gram-positive cocci dominated. The higher prevalence of anaerobes is due to low redox potential of the pockets [12]. Studies done in other countries showed that the isolation rate of anaerobes ranged from 42% to 100% in periodontitis and 38% to 80% in healthy subjects [18, 19, 20]. Our work is in consonance with these works, we reported 58.4% in healthy subjects as against 63.4% in periodontitis.

Polymicrobial pattern which is a characteristic of periodontal disease was evident in this study, 87.3% in gingivitis and 94.3% in periodontitis, this agrees with the works of many other researchers [12,18,19,20]. The presence of polymicrobial infection has important implications in management because it modifies the clinical course of disease, impacting on the selection of antimicrobial therapy and the anticipated response to treatment especially when it involves pathogens commonly exhibiting antimicrobial resistance [21].

Aggregatibacter actinomycetecomitans was the most prevalent facultatative anaerobe in periodontitis, 27.9% and in gingivitis 13.3 %. Of all the microorganisms in biofilm, three are said to be important in the initiation and progression of periodontal disease: A. actinomycetecomitans, Porphyromonas gingivalis and Tannerella forsythensis are named key pathogens or "red complex" bacteria [22,2]. Amel et al., detected A actinomycetecomitans in 8.4% in aggressive periodontitis and 0.3% in chronic periodontitis [23]. They are small short, straight or curved rods with rounded ends that is non motile and Gram negative and it is reported to be strongly associated with destructive periodontal lesions [5]. It possesses many virulence factors including leukotoxin, collagenase, protease, endotoxin, fibroblast inhibition factor inducing bone resorption [5].

In gingivitis, Actinomyces spp. were the most dominant microorganisms, this is in consonance with the work of Saini et al., but at variance with the work of Egwari et al. who reported a predominance of Porphyromonas [18,12]. Actinomyces are an important component of supra and subgingival plaques24. A. naeslundii in dental plaque may contribute to certain periodontal disease25. Johnson et al., isolated A israelii in periodontitis26. Actinomyces

odontolyticus belong to the purple complex with Veillonella parvula in current classification system of bacteria responsible for periodontal disease [11]. They serve as bridge between "Orange complex" and "Red complex".

Anaerobic microorganisms in order of ranks in this work were in periodontitis, Fusobacterium nucleatum 18.3%, Porphyromonas gingivalis 11.7%, Prevotella intermedia 11.3%, Peptostreptococcus micros 8.3% and many others, many researchers detected these organisms but in different proportions. The different recovery rates may be due to varying patient selection criteria, different culture methods, geographical differences and use of molecular techniques for identification18. Fusobacterium nucleatum is not a serious periodontopathogen but it is one of the oral species that is consistently associated with and in increased number at sites of periodontitis [27]. It belongs to the "Green complex in current Socransky and Haffagee's classifications. It elicits secretion of peptides which have effect on host cells and modulate immune response. It possesses coaggregation properties that enable it to transport periodontopathogenic bacteria [27].

Porphyromonas gingivalis, belongs to the "Red complex" group of periodontopathogens. It is a Gram negative, non-motile asacchorolytic rod-like obligate anaerobe. It possesses antiphagocytic structures such as capsules which protects against phagocytosis, presence of fimbriae for adhesion, and vesicles. It also produces virulence factors such as proteases (for destruction of immunoglobulin and the complement factors, and degradation of host cell collagenase inhibitors), collagenase, hemolysin, endotoxin, fatty acids, H2S, and NH4 [5].

Prevotella intermidia was 5.1% in gingivitis and 11.3% in periodontitis, Gursory et al., reported that P. intermidia is associated with periodontal disease. [28] It belongs to the "Green complex". It is a short round ended non motile Gram negative anaerobic pathogenic rod which is less virulent and less proteolytic than P. gingivalis [5].

Anaerobic Gram positive cocci were isolated, Peptostreptoccus micros, 7.7% in gingivitis and 8.3% in periodontitis and Peptostreptococcus anaerobius, 4.8% in gingivitis and 5.7% in periodontitis. P. micros belongs to the second major group complex according to Socransky. The role of these organisms in initiation and/ or progression of periodontitis are much less defined than that of the "Red complexes". Peptostreptococcus is associated with periodontal diseases [29].

We isolated Staphylococcus aureus in 14.5% in gingivtitis and 16.4% in periodontitis, many researchers have also reported this [23,30]. The use of antibiotic in periodontal diseases or other infections may predispose to the increase of the Staphylococcus spp in oral cavity. These microbes readily become resistant to antibiotics, and may culminate to superinfection. The ability to form biofilm has enabled Staphylococcus to survive in this environment [20,31]. Streptococcus spp were detected in high number by many researchers [12, 23]. Some Streptococci are beneficial to the host as their colonizing the pocket in large numbers could delay the process of periodontal disease [32].

The presence of Enterobacteriaceae was considered unusual in patients with periodontitis [33]. Several studies had associated enteric bacilli with periodontal diseases23. According to Botero et al., their role in periodontitis are not clear but are thought to indicate superinfection [34]. It is speculated that they are opportunists which thrive after periodontal treatment. The drug of choice for treating periodontal diseases includes amoxicillin, doxycycline, tetracycline and metronidazole. Enterobacteriaceae show resistance to these drugs and may therefore persist after administration. Futher studies are needful to explain their presence in the plaque biofilm and to explain their role in infection [34].

Candida albicans was isolated in our study and Daniluk et al reported it, Candida albicans could have a role in the infrastructure of periodontal microbial plaque and in its adherence to periodontal tissues [35,36].

Our study showed a statistical gender difference in gingivitis, but not in periodontitis, many other researchers seem to favor a female preponderance [37, 38]. However, our work is at variance with that of Ababneh et al., who reported a male predisposition and Susin and Albander that reported equal distribution [39, 40].

Multivariate logistic regression analysis of risk factors associated with gingivitis and periodontitis showed that age was significant. Many authors believed that age is not a determinant but the life time disease accumulation [39, 40, 41]. For subjects >31 years old, the odd of developing gingivitis increased by 5.17 times and that of periodontitis increased by 2.28 times. In our works, a low level of education was significantly associated with increased prevalence of periodontitis and the odds of having periodontitis increased by 28.77 times in patients' ≤ 6 years with gingivitis and 3.58 times in periodontitis. The higher

prevalence of periodontitis among subjects with low education has been reported in Jordan and in Thailand [39, 42]. In USA, subjects with < high school education were 3 times more likely to have periodontitis than subjects with a higher level of education [43] Periodontal disease has been reported to have a reciprocal relationship with educational level. Frequency of tooth brushing showed brushing once daily had an increased odd of 1.57 times of developing periodontitis. Patients with bleeding gum showed an increased odd of 38.41 times of having gingivitis and 2.58 times of having periodontitis than subjects who had no cases of gum bleeding. Gum bleeding has been reported to be one of the early signs of developing periodontal disease. The effect of maintaining good oral hygiene on the periodontium is well documented [44, 45]. Patients with previous family history are almost 60 times more probably going to develop gingivitis and about 5.23 times more probably going to have periodontitis than those without a previous family history. Positive family history of periodontal diseases has been reported by many researchers [39].

CONCLUSION

Our work has shown the diversity of periodontopathogens in gingivitis and periodontitis and all should be put into consideration in treatment strategy of patients. The factors that strongly associated with periodontitis were age, level of education, history of periodontal disease, and age at which odontalgia occurred including bleeding gum. Knowledge of the microbial etiology of periodontal diseases and some associated risk factors is the key for a successful periodontal therapy.

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Author Information

Uzoamaka C. Maduakor
Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, College of Medicine, University of Nigeria, Enugu Campus
Nigeria

Ngozi F. Onyemelukwe
Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, College of Medicine, University of Nigeria, Enugu Campus
Nigeria

Sam N. Maduakor
Department of Periodontology, Faculty of Dentistry, College of Medicine, University of Nigeria, Enugu Campus
Nigeria

Nkiruka C. Azubuike
Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, College of Medicine, University of Nigeria, Enugu Campus
Nigeria

Anulika O. Onyemelukwe
Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, College of Medicine, University of Nigeria, Enugu Campus
Nigeria

Ebuka B. Nnedu
Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, College of Medicine, University of Nigeria, Enugu Campus
Nigeria