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The Relationship between Periodontal Disease and Predisposing Factors

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Key words
Periodntics, oral microbiology, periodontitis.

Abstract

Periodontitis is an inflammatory lesion mediated by host-bacterial interactions which results in a non-resolving inflammation that leads to local connective tissue attachment loss from the tooth surface, loss of alveolar bone and ultimately tooth loss Indeed, periodontal disease is the leading cause of tooth loss in the western world and developing countries. The aims of this study was to estimate the relationships between periodontal disease and predisposing factors. One hundred eighty samples (paper point inserted into periodontal pocket) were examined in the present study . Patients were of both sexes(140 males and 40 females).Their ages ranged from 16-69 years old. Clinical measurements of periodontal parameters used included dental plaque index, gingival index, bleeding on probing, probing pocket depth and clinical attachment loss . The results showed that periodontal disease was the most common in age group 20-29 years old and only a case reported with periodontal disease in 65 years old patients. According to sex distribution of patients, periodontal disease was mostly found in males than females. The periodontitis was more common in non-educated,treated ,smoking patients they living in rural area than educated,non treated ,non-smoking patients . they living in urban area.The most common anaerobic periodontal bacteria isolated from patients were peptostreptococcus prevotii which represented 15(8.3%) isolates, while prevotella intermedia, prevotella melani, prevotella disiens, Bifidobacterium sp., Fusibacterium mortiferum peptostreptococcus tetradius ,and Wolinella sp. represented only 1 (0.5%) of anaerobic isolates .Also another anaerobic subgingival bacteria isolated from inflamed sites in patients were fusibacterium varium, vellionella sp., campylobacter gracilis, capnocytophaga sp., peptostreptococcus magnus, peptostreptococcus micros, peptostreptococcus niger,peptostreptococcus anaerobius, staphylococcus saccharolyticus, streptococcus consellatus, and gemella morbillorum.

Introduction

Periodontal disease could be defined as a disorder of supporting structures of teeth, including the gingiva, periodontal ligament and alveolar bone. Periodontal disease develops from a pre-existing gingivitis. However, not every case of gingivitis develops into a periodontal disease. The inflammation of gingiva alone is termed gingivitis, and the severe

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inflammation of the periodontal ligament with destruction of alveolar bone is called periodontal disease[1] . The current concept concerning the etiology of periodontal disease considers three groups of factors which determine whether active periodontal disease will occur: A susceptible host, the presence of pathogenic species, and the absence of so-called "beneficial bacteria" [2].

It is generally accepted that the oral biofilm in association with anaerobic bacteria is the main etiological factor in periodontal disease [3,4].The oral biofilm consists mainly of microbes and host proteins that adhere to teeth within minutes of a dental oral hygiene procedure. Healthy gingival sulcus has a flora dominated by equal proportions of gram positive cocci, especially *Streptococcus spp,* and *Actinomuces sp.* Later, plaque "matures" resulting in a flora consisting from facultative anaerobic microorganisms, spirochaetes and motile rods. The proportions of strict anaerobic, Gram negative organisms increase significantly in accordance with increasing severity of disease. Disease activity in periodontal disease may range from slow, chronic, progressive destruction to brief and acute episodic bursts with varying intensity and duration[5] .The composition of the subgingival microbial flora and the level of pathogenic species differ from subject to subject as well as from site to site. The search for the pathogens of periodontal diseases has been underway for more than 100 years, and continues up today[5] .More than 300 species of bacteria colonize subgingival area and their cell wall components can trigger immune activation[6].

Human is associated with a widely diverse and complex subgingival microbiota encompassing both Gram positive and Gram-negative bacteria, facultative and anaerobic organisms, and possibly yeasts[7] Whilst numerous studies have investigated the composition of plaque and defined 5 bacterial complexes ranked by the strength of the groups relationship with clinical measures of periodontitis.The normal subgingival flora consists of mainly facultative (both aerobic or anaerobic capabilities) anaerobic Gram positive bacteria, and only 5% spirochaetes and motile rods. Generally ,periodontal disease progresses the proportion of anaerobic, Gram negative rod, and other bacteria increases. In chronic periodontitis approximately 75% of cells are Gram negative of which 90% are strict anaerobes[8]. This shift in microbial composition is represented by a change from mainly purple complex bacteria through to a large proportion of red complex bacteria in sites of active disease[9]. The currently recognized key Gram negative periodontopathogens include:

*Porphyromonas gingivalis, Prevotella intermedia, Bacteroides forsythus ,Aggregatibacter actinomycetemcomitans , Fusobacterium spesies ,Capnocytophaga species , Campylobacter sp.,wollenilla sp.,*and *Bifidobacterium*[6,10,11,12,13] . Also, the following bacteria could be isolated: *Eubacterium spp, Peptostreptococcus species Streptococcus consellatus, and Staphylococcus saccharolyticus* [5].

The relationship between periodontal disease socioeconomic status can be viewed globally, where there was a wide variation in the socioeconomic status among different peoples compared from developing countries which suggested that periodontal disease associated with nutritional deficiencies. Nibras *et al* found that increased sugar consumption was associated with education , living in low socio-economic area and brushing at least oral hygiene practices were higher for girls [14].

Aims of study

Many studies investigated the relationship between predisposing factors just like age,sex distribution, smoking, education, residence and treatment ..etc In general there is a conflict results provided by this studies ranging the probable relationship between periodontal disease and these factors.So,this study aims to disclose the possible suggested relationship between periodontal disease and predisposing factors**.**

Materials And Methods

One hundred eighty periodontal patients were examined and enrolled in the

present study ..They were referred to the Laboratory of Microbiology, Department of Microbiology, College of Medicine ,Tikrit University. Patients were of both sexes(140 males and 40 females).Their ages ranged from 16-69 years old. The selection was done randomly among patients in Tikrit University/college of dentistry/dental teaching hospital . The diagnosis of periodontitis was made by the clinical examination including taking dental, medical and family history from the subjects involved in this study, so patients fulfilling the criteria to be diagnosed as periodontitis. Clinical measurements of periodontal parameters used included dental plaque index, gingival index, bleeding on probing, probing pocket depth and clinical attachment loss using (graduated William's periodontal probe). Clinical diagnosis in each case was according to the dentist . The interviews were performed for each patient. The Questionnaire Form included general information about the patient e.g.: name, age, sex, smoking, education, residence and treatment if the patients treated or not treated by antimicrobials before sampling.

Sampling

Samples were obtained from periodontal pockets after supragingival plaque was removed from the teeth to be sampled[15]. The supragingival dental plaque was removed with sterile cotton, and the tooth surface was dried with compressed air to prevent contamination with saliva. The exclusion of moisture in the mouth with sterile cotton rolls and subgingival plaque was collected from the most inflamed sites by inserting a sterile paperpoint into the periodontal pocket for 10 seconds , when the pocket depth was from3-7mm.The sample was mixed with 1 ml thioglycollate broth (transport medium) , sealed tightly to avoid contamination and kept at 4 c^0. Samples were processed within 2 days of collection[16].

Cultural Technique

A plaque sample(loopful from thioglycollate broth containing subgingival paperpoint which inserted into periodontal pocket) was inoculated onto Brucella blood agar supplement with kanamycin 100 mg/l,vancomycin 7.5 mg/l Brucella blood agar, Enriched blood agar ,brain heart infusion agar,and trypticas soy agar with haemin and vitamin K1.The inoculated media were immediately incubated in an anaerobic environment generated with the anaerogens gas pack CO2 system Chemical, for 3 -7 days[16,17].

Isolation and Identification of Bacteria

Isolates were identified using cultural characteristics , gram -stain and biochemichal tests (convenentional methods) which includes : indole spot test, catalase test , oxidase test , arginine hydrolysis, lipase test, nitrate reduction , motility test ,growth in bile , urease test and growth on kanamycin(1mg),vancomycin(5µg), colistin(10µg) on Brucella blood agar [18,19] . Also anaerobic periodontal bacteria were identified by the API RapID ANA II system [remel,USA], API 20A [Biomereiux,France] and VITEK 2 ANC System [Biomereiux,France] [15,16,20] .

Discussion:

The present study showed that the highest incidence of periodontal disease was found among males when 180 patients were examined as showed in Table 1.It was noticed difference between males and females . The results were almost similar to that reported by Loe and Brown who found that periodontitis was higher among males than females[21] . In addition , Moise et al found that 50% of males suffered from periodontal disease compared with females (10%) . It was possible that females did not reach a threshold of inflammation that might have otherwise been associated with severe periodontal infections[22]. Shvayogi et al found that periodontal disease was more among males (68%) as compared to females (32%) [23] . Furthermore, these results were almost similar to those of Saxby who found that there was a highly significant differences among males and females in periodontal disease [24]. Gina et al found severe periodontal disease was 3-fold higher among men (12.5%) than women (4.2%) and approximately 2-fold higher among non-Hispanic black men (19.3%) and Mexican-American men (18.8%) than among non-Hispanic white men (9.4%) [25] . Batool et al found that

males are more predominance than females[26]. Shiau et al found that men are at greater risk for distructive periodontal disease than women[27].Moreover,the present study showed different results compared to Hayder conclusion who found that prevalence of periodontitis was higher in females than males[28]. Also, these results were different from those of Al-Barhawe and AbdulRahman who found that severe gingivitis and periodontitis were recorded in females more than males[15, 29]. However, Ramjford et al found that periodontal condition of young men in India who exhibited the clinical symptoms of general malnutrition was not different from the periodontal condition of well-nourished individual [30].The highest incidence in males may be due to dental care utilization rates are lower among men than women , and ignorance of oral hygiene and negligance or wrong tooth brushing[22, 15] .The gender differences reported here might be attributable to treatment bias, practice differences, or socioeconomic determinants. Smoking patterns for example , were different across genders. However, the fact that the present findings were similar in the subgroup of never being smokers makes this possibility less likely[23]. Assessment of the possible role of females hormones in destructive periodontal disease may help in the definitive increase in periodontal disease seen in men [31]. Plaque is the causative agent in periodontal disease affecting adult individuals, but the balance between bacterial challenge and host response was important .Gingivitis can progress to incipient adult type (chronic) periodontitis in a significant proportion of adolescent[23] . The present results showed that the age of patients studied group ranged from 16-69 years old .The high percentage of periodontal disease was in age groups 20-29 and 30-39 years old that represented 60.5% and 15% respectively as showed in Table 2. Table 3 shows that the majority of periodontal pathogens were among age groups 20-29 years , which represented 51(69.86%) of cases , while age group 60-69 represented only 1(1.37%) of cases studied .The results were almost similar to those of Hayder who found that severity of periodontitis was most common in age group 20-29 years old [28] . Gina et al found that the prevalence of periodontitis and periodontal pathogens increased among all adults [25]. On the other hand , the results presented here were different from those of Batool et al who found that the high percentage of periodontitis was at age 33-57 years old [26] . Shivayogi et al found that gingivitis and periodontitis were more prevalent in 15 years old and children (73.30%) [23] . This age was also a period of life when the psychological readiness to increase plaque control activities was often low [32].The studies of periodontal disease prevalent ,or extent and severity from epidemiologic studies more prevalence in adult as compared to children[31]. The present results were in consistent with other reports which showed that an association poverty , lower income and education, with higher levels of periodontal disease among adults [15]. The present study demonstrated a different microbiota in periodontal pockets. The finding presented here that Vellionella sp (7.7) Fusibcterium varium (5.5%) , Campylobacter gracilis (1.6) ,Capnocytophaga sp.(1.1) ,Prevotella intermedia(0.5%), Prevotella melani (0.5%) , Prevotella disiens (0.5%) ,Bifidobacterium sp. (0.5%) ,Fusibacterium nucleatum(0.5%),and Wolinella sp.(0.5%) were more frequent as isolated from periodontal pocket as showed in Table 4 .It was ,however, interesting to note that gram positive anaerobic bacteria, especially Peptostreptococcus spp. were isolated in high rates in periodontal pocket which includes Peptostreptococcus prevotii (8.3%), Peptostreptococcus magnus(1.1%), Peptostreptococcus micros (1.6%), Peptostreptococcus tetadius(0.5%), Peptostreptococcus niger (2.2%) , and Peptostreptococcus anaerobius (2.7%) .Moreover , Staphylococcus saccharolyticus , Streptococcus consellatus, and Gemella morbillorum were isolated in the present study in percentages of 2.7%,2.2%,and 1.6% respectively. These results were almost similar to those of Daniluk et al who found that the most common periodontal pathogens were Veillonella

species, Fusobacterium, Staphylococcus saccharolyticus ,Streptococcus consellatus, Gemella morbillorum , Peptostreptococcus spp. and Prevotella intermedia[33]. Moreover, these results were similar to those of Spartt , Winkelhoff et al , Piovano , Haffajee and Socransky who found that Veillonella species, Fusobacterium, Staphylococcus saccharolyticus ,Streptococcus consellatus, Gemella morbillorum, Peptostreptococcus spp. Prevotella intermedia, Campylobacter gracilis and Bifidobacterium sp. [32,34,35,36] . Furthermore , Mohammad et al found that Actinobacillus actinomycetemcomitans (26.8%), Porphyromonas gingivalis (21.9%), Capnocytophaga sputigena (16.7%), Eikenella corrodens (13.2%), Prevotella intermedia (10.5%), Prevotella disiens (3.1%), Capnocytophaga gingivalis (2.2%), Peptostreptococcus micros (2.9%), Prevotella corporis (1.8%), Peptostreptococcus magnus (1.3%) , and Fusobacterium nucleatum (0.4%)were most common isolates[37]. Spratt et al was found that Capnocytophga sp. involvement in some forms of periodontitis[38]. According to Gürsoy et al Prevotella intermedia is associated with periodontal disease [39]. Mayorga-Fayad et al revealed that the frequency increases Prevotella intermedia in patients with periodontitis [40] . The present results were different from those of Yacoubi et al who found that Aggregatibacterium actinomycetemcomitans , Eikenella corrodens are primary causative agent in periodontitis[41]. Moreover , Cisar et al and Ximenez-Fyvie et al found that actinomyces were the most common component of periodontal disease[42,43]. The data from the investigation suggest that there was heterogeneity in the subgingival periodontopathogenic bacteria among subjects. However, as many bacteria in the oral cavity cannot be cultured, it was likely that these still uncharacterized bacteria could play a role in the initiation and progression of periodontal disease. Table 5 shows that the majority of periodontal pathogens were among males than females which represented 50 (68.5%). Table 6 shows that the periodontal pathogens was the most common pathogen isolated from non- educated patients .Furthermore, the present study showed that the majority of periodontal pathogens were among non treated than treated patients which represented 60 (82.2%) as shown in Table 7. The relationship between periodontal disease socioeconomic status can be viewed globally, where there was a wide variation in the socioeconomic status among different peoples compared from developing countries which suggested that periodontal disease associated with nutritional deficiencies. Nibras et al found that increased sugar consumption was associated with education , living in low socio-economic area and brushing at least oral hygiene practices were higher for girls [44]. Al-Barhawe found that the highest ratio of severe gingivitis was in uneducated group ,this is due to their unhealthy deal of oral hygiene , not using tooth brush and having bad nutrient habit [15] . Polander found that the education level influences the oral diseases and in planning appropriate preventive measures [45]. The distribution of pathogens among patients shows that the periodontal pathogens was the most common pathogen isolated from patients who living in urban area as shown in Table 9. Abdul-Rahman found similar results that urbans have gingivitis more than rurals[29]. The finding suggests that there are factors other than the oral hygiene , such as sugar intake , self perception of oral health , and socioeconomic status to which adequate attention has not paid. Table 8 shows that the majority of periodontal pathogens were among smoking patients than non smoking patients which represented 35 (70%) . Moreover , Ziyad found that there was significant differences between smoker and non smoker males who having gingivitis and periodontitis with more accumulation of plaque in smokers than non smokers[45]. Furthermore ,these results were almost similar to those of Tonetti Muller and Erdemir who found that periodontitis was more common in smoker than non smoker males[46,47,48]. On other hand ,the results presented here were different from those of Danielson et al and Giannopoulou et al who found that there was no differences between smoker and

non smoker patients with periodontitis and periodontal pathogens isolates[49,50]. Garbin et al showed that the group with a high risk of the occupation periodontal disease are those with poor oral hygiene those who ate sweets frequently , and those who were of low – socioeconomic class[51]. Al-Barhawe found a highest ratio of severe gingivitis was in pregnant and in patients who living in rural area due to ignorance of oral hygiene and type of diet and not using toothbrush[15] .Moreover, these results presented here were almost similar to those of Mois et al and Loe who found that periodontitis increased among non-educated ,non-treated patients ,patients who living in rural area and among smoking males[22,52]. Furthermore, another explanations to the results was that the inflammatory gingival response to plaque accumulation may suppressed under the influence of predisposing factors such as cigarette smoking [53],and nervously mediated vasoconstriction in the healthy human gingival. However , it was speculated that small repeated vasoconstrictive attacks due to smoking may in the long run contribute to gingival vascular dysfunction and periodontal disease and formation biofilm by periodontal pathogens[54].The general increase of periodontal pocket and periodontal disease in patients can be also explained from the fact that the imbalance in the host bacterial interactions and imbalance between bacterial challenge and host response may due to changes in the composition of sub gingival plaque with increase in numbers and /or virulence bathogenic periodontal organisms ;changes in the host response to the bacterial challenge , or combination of both[55]. In addition, Haffajee and Socransky showed that smokers may have a higher proportion of sites harboring periodontal pathogen, especially in the palatal aspect of the maxillary teeth and the upper and lower incisor region[56]. In conclusion, the results of this study shows that the most common age group affected by periodontal disease was 20- 29 years old. The prevalence of periodontitis were more common in males than females.
The periodontitis was more common in non-educated,treated ,smoking patients they living in rural area than educated,non treated ,non-smoking patients . they living in urban area. peptostreptococcus prevotii , prevotella intermedia, prevotella melani , prevotella disiens, Bifidobacterium sp., Fusibacterium mortiferum peptostreptococcus tetradius ,and Wolinella sp. fusibacterium varium ,vellionella sp., campylobacter gracilis, capnocytophaga sp. ,peptostreptococcus magnus ,peptostreptococcus micros ,peptostreptococcus niger,peptostreptococcus anaerobius , staphylococcus saccharolyticus , streptococcus consellatus , and gemella morbillorum were the most common anaerobic periodontal pathogens isolated from patients in the present study.

Results:

1. Gender:

The present study showed that 77.8% of studied patients were males ,while 22.2% were females. As show in tabil 1.

2. Age Distribution:

The ages of patients included in this study ranged between 16-69 years old .Table 2 shows that 60.5% of patients were among age group 20-29 years ; whereas only 1.1% of the patients were among age group 60-69years old .

Table 3 shows that the majority of periodontal pathogens were among age groups 20-29 years which represented 51(69.86%) of cases , while age group 60-69 represented only 1(1.37%) of cases studied.

3. Identification of Anaerobic Periodontal Isolates:

Periodontal pathogens identified by conventional methods, API RapID ANA system II , API 20A ,and Vitek2 ANC systems for identification of anaerobic bacteria.

Table 4 shows the types of anaerobic periodontal pathogens isolated. In the present study the most common anaerobic periodontal bacteria isolated were peptostreptococcus prevotii which represented 15(8.3%) of all isolates, while prevotella intermedia, prevotella melani , prevotella disiens, Bifidobacterium sp., Fusibacterium mortiferum peptostreptococcus tetradius ,and Wolinella sp. represented only 1 (0.5%) of all isolates.

4. Gender Distribution of Isolated Periodontal Pathogens:

Table 5 shows that the majority of periodontal pathogens were among males than females which represented 50 (68.5%).

5. Distribution of Periodontal Pathogens according to Education Level:

The distribution of pathogens among patients showed that the periodontal pathogens was the most common pathogen isolated from non- educated patients as shown in Table 6.

6. Distribution of Periodontal Bacteria according to Treatment with Antibiotics:

Table 7 shows that the majority of periodontal pathogens were among non treated patients compared to treated patients which represented 60 (82.2%).

7. Distribution of Periodontal Pathogens according to Smoking:

Table 8 shows that the majority of periodontal pathogens were among smoking patients compared non smoking patients which represented 35 (70%).

8. Distribution of Periodontal Pathogens according to Residence

The distribution of pathogens among patients showed that the periodontal pathogens was the most common pathogen isolated from patients who living in urban area as shown in Table 9.

Table 1: Gender Distribution among Patients with Periodontal Disease.

Gender	No. (%) of patients
Males	140 (77.8%)
Females	40 (22.2%)
Total	180 (100%)

Table 2: Distribution of Periodontal Patients according to Age .

Age group (years)	No. (%)Patients with periodontitis
10-19	27 (15%)
20-29	109 (60.5%)
30-39	24 (13.4%)
40-49	9 (5%)
50-59	9 (5%)
60-69	2 (1.1%)
Total	180 (100%)

Table 3: Age Group Distribution in Relation to Anaerobic Periodontal Pathogens Isolated from Pockets of Patients.

Age Group	Periodontal pathogens isolated from patients	Patients with negative cultures
	No.(%)	No.(%)
10_19	11 (15.07%)	16 (14.95%)
20-29	51 (69.86%)	58 (54.21%)
30-39	5 (6.85%)	19 (17.76%)
40-49	2 (2.74%)	7 (6.54%)
50-59	3 (4.11%)	6 (5.61%)
60-69	1 (1.37%)	1 (0.93%)
Total	73 (100%)	107 (100%)

X^2=7.153; df=5; p =0.2 not significant

Table 4: Types of anaerobic bacteria isolated from periodontal patients:

Types of isolates	**No. (100%) of isolates**
prevotella intermedia	1 (0.5%)
prevotella melani	1 (0.5%)
prevotella disiens	1 (0.5%)
Bifidobacterium sp	1 (0.5%)
Fusibacterium nucleatum	1 (0.5%)
Fusibacterium varium	10 (5.5%)
Vellionella sp.	14 (7.7%)
Campylobacter gracilis	3 (1.6%)
Wolinella sp.	1 (0.5%)
Capnocytophaga sp	2 (1.1%)
Peptostreptococcus prevotii	15 (8.3%)
Peptostreptococcus magnus	2 (1.1%)
Peptostreptococcus tetradius	1 (0.5%)
Peptostreptococcus micros	3 (1.6%)
Peptostreptococcus niger	4 (2.2%)
Peptostreptococcus anaerobius	2 (1.6%)
Staphylococcus saccharolyticus	5 (2.7%)
Streptococcus conselatus	4 (2.2%)
Gemella morbillorum	2 (1.6%)
Total	73 (40.7%)

Table 5: Gender distribution of Isolated Periodontal Pathogens.

Gender	**No. (%) of isolated periodontal pathogens**
Males	50 (68.5%)
Females	23 (31.5%)
Total	73 (100%)

Table 6: Distribution of Periodontal Pathogens according to Education Level.

Patients	**No. (%) of periodontal pathogens isolates**
Educated patients	16(21.9%)
Non-educated patients	57(78.1%)
Total	73(100%)

Table 7: Distribution of Periodontal Bacteria Isolated according to Treatment with Antibiotics.

Patients	No. (%) of periodontal pathogens
Treated patients	13 (17.8%)
Non-treated patients	60 (82.2%)
Total	73 (100%)

Table 8: Isolation of Periodontal Pathogens from Smoking Males.

Patients	No. (%) of periodontal pathogens isolates
Smoking	35 (70%)
Non smoking	15 (30%)
Total	50 (100)

Table 9: Distribution of Periodontal Pathogens according to Residence.

Residence	No. of periodontal pathogens isolated(%)
Urban area	42 (57.5%)
Rural area	31 (42.5%)
Total	73 (100%)

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