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Microbiological Study of Periodontitis in the West of Algeria

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Abstract: In pathology, oral flora responsible for periodontal disease is polymorphic. The periodontal infection results either from the penetration of pathogenic microorganisms in the tissues, or even the activation of already existing germs, but not pathogenic under normal conditions. Of a population of 500 patients consulting in Periodontology, 149 were diagnosed as suffering from periodontitis. More than Twenty bacteria had been isolated and identified from subgingival plaques of patients affected with aggressive (28%) and chronic periodontitis (18.4%),. In general, the bacterial flora of aggressive periodontitis had shown dominant gram-negative and motile morphotypes, among them, straight rods, curved, spirochetes and fusiform Gram negative. For chronic periodontitis the bacterial flora is characterized by the dominance of rods and motile to the rare gram-negative morphotypes. Certain bacteria known as periodontopathogens were identified in aggressive periodontitis as *Aggregatibacterium actinomycetemcomitans* (7.3%), Prevotella intermedia (4.2%), *Eikenella corrodens* (6.3%), *Bacteroides fragilis* (4.6%) and *Capnocytophaga* sp (4.7%).

Key words: Periodontitis disease · *Aggregatibacterium actinomycetemcomitans* · *Prevotella intermedia* · *Eikenella corrodens* · *Bacteroides fragilis* and *Capnocytophaga* sp.

INTRODUCTION

The periodontal infection results either from the penetration of pathogenic microorganisms in the tissues, or even the activation of already existing germs, but not pathogenic under normal conditions. This responsible flora is polymorphic, Gram-negative and microaerophilic or strictly anaerobic. Only ten or twenty species, regarded as pathogens, play a role in the pathogenesis of periodontal destruction [1]. Due to the fact that periodontitis is caused by bacteria, the most important causal periodontal treatment is the elimination of these bacteria [2]. Even on an individual tooth, periodontal disease does not progress uniformly. This can make two Hypotheses: either all tooth surfaces are not vulnerable to the same point, or pathogens are not distributed uniformly over the dental arches [2].

Each type of periodontal disease has a subgingival flora consists of a combination of micro-organisms of its own. The concept of bacterial specificity has been demonstrated that through advances in techniques for anaerobic cultures and the development of new selective culture media. Most microorganisms involved in these disease are gram-negative bacilli, anaerobes (Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum, Campylobacter rectus) or capnophiles (*Aggregatibacterium actinomycetemcomitans, Eikenella corrodens, Capnocytophaga ochracea*. ..) [3].

The juvenile periodontitis were divided into two clinical entities: localized juvenile periodontitis and generalized juvenile periodontitis, each with a different microbiology. The microbiology of generalized juvenile periodontitis is more complex and association of Porphyromonas gingivalis (10 to 15%) and other gram-negative bacilli (*Eikenella corrodens, Capnocytophaga* sp. *Aggregatibacterium actinomycetemcomitans* [3].

The rapidly progressive periodontitis is an aggressive form of periodontitis. The subgingival flora is typically composed of significant proportions of *Porphyromonas gingivalis.* Prevotella intermedia and other bacteria of the genus Bacteroides but Porphyromonas gingivalis appears to be one of the essential causative microorganisms in rapidly progressing periodontitis [3].

In recent years researchers in bacteriology trying to identify the causative bacteria.

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The aim of our work is to isolated identify the bacterial flora characteristic of aggressive and chronic periodontitis.

MATERIAL AND METHODS

232 patients aged 14 -35 years with periodontitis are presented in the department of Periodontology (Oran, Algeria) and were divided as aggressive or classification of Armitage [4]

Bacteriological Study

This Study Includes Several Steps

Selection of Site: The sampled sites were either molars and incisors, because these areas are most frequently affected in localized juvenile periodontitis.

Sampling: After removal of supragingival plaque by means of sterile cotton balls, subgingival plaque was collected on Gracey curette inserted to the depth of the periodontal (Figure 1). After sampling, samples of subgingival plaque were collected and deposited on tubes containing sterile saline or otherwise sterile distilled water [5]

Culture: the sample plate and collected shall be deposited in a tube containing 2 ml of saline (0.9% NaCl). The samples were mixed using a vortex for one minute to allow the dispersal of bacteria. Samples of subgingival plaque were shaken vigorously for 30 seconds using the mixer before being prepared for analysis.

Two other tests will be made for microscopic examination (one for fresh and one for Gram stain) [6].

The streaking were made on different selective agar media "trypticase soy agar with serum horse + bacitracin and vancomycin, TSBV) [7], Bacteroides bile esculin agar [8] and nonselective aerobic and anaerobic (Brucella agar and blood agar) and identify the bacteria found in these different clinical entities [9].

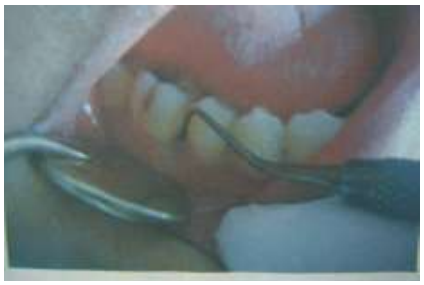

Fig. 1: subgingival sampling, CHU Oran.

After the bacteria are inoculated on solid media, Petri dishes were incubated under different atmospheres (ambient, microaerophilic and anaerobic) at 37°C for 2 to 7 days depending on the used media [6].

RESULTS

The study is based on results from a series of samples of subgingival plaque collected and treated with oral bacteriology laboratory, department of periodontology, Oran Algeria. 149 patients aged 14 - 35 years were included in this study.

Direct examination represented by fresh and Gram stain revealed in patients with aggressive periodontitis, a predominance of flora especially curved, fusiform motile rods and motile spirochetes and Gram negative bacilli and coccobacilli Gram negative and Gram-positive filaments (Figure2).

For chronic periodontitis, the flora is characterized by both motile and non motile morphology Gram negative and positive. The morphology of the most evocative are cocci and straight rods (Figure 3). The rate of coccobacilli is very negligible in this entity.

bacteria were isolated, some are identified as periodontal pathogens and others whose etiologic role in periodontal disease is not yet clarified. These bacteria are *Aggregatibacterium actinomycetemcomitans, Eikenella corrodens P. intermedia, Capnocytophaga,Campylobacter, H. pylori, Actinomyces*, Eubacteriu and Peptostreptococcus (Table 1).

Regarding aggressive periodontitis, bacteria that play a role in periodontal disease are *A. actinomycetemcomitans, A. actinomycetemcomitans, E. corrodens, P. intermdia, Capnocytophaga* sp. and *Bacteroides frgilis.*

Other bacteria have been identified including their involvement in periodontal disease is unclear such as: Haemophilus aphrophilus, Campylobacter species, Eubacterium *and* Actinomyces.

The enterobacteria were also identified in our study, such as E.coli, *Pseudomonas aeruginosa and Klebsiella pneumoniae* For chronic periodontitis, the absence of Bacteroides fragilis, Capnocytophaga sp as important bacteria in periodontal disease is noted in this entity (Table 1).

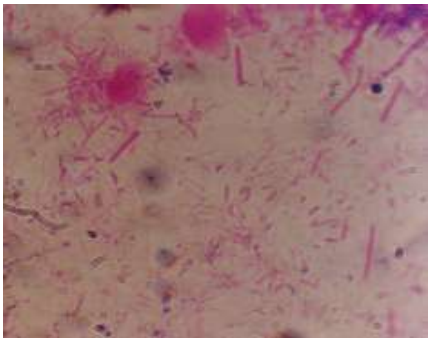

Fig. 2: Flora of aggressive periodontitis X1000.

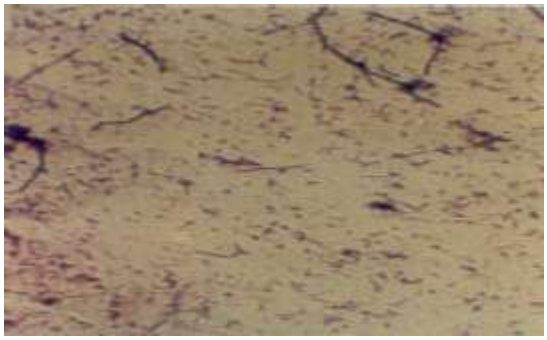

Fig. 3: Flora of chronic periodontitis X1000.

Table 1: cultivable flora of periodontitis

Bacteria	Agressive periodontitis (%)	Chronic periodontitis (%)
A. actinomycetemcomitans	8.4	0.3
Eikenella corrodens	10.8	20.7
Prevotella intermedia	8.0	5.4
Bacteroides fragilis	5.6	0.0
Capnocytophaga sp	2.4	0.0
Campylobacter sp	7.1	7.5
Helicobacter pylori	4.5	6.3
Haemophilus aphrophilus	7.1	2.1
Eubacterium sp	8.0	6.3
Eubacterium nodatum	1.4	0.0
Actinomyces israelii	1.7	1.8
Actinomyces naeslundii	1.7	0.9
Peptostreptococcus anaerobius	1.4	0.0
Peptostreptococcus micros	3.5	0.3
Staphylococcus aureus	4.2	0.0
Streptococcus viridans	11.8	38.7
E. coli	3.1	5.4
Pseudomonas aeruginosa	3.5	0.0
Klebsiella pneumoniae	3.1	0.0
Corynebacterium matruchotii	2.4	1.2
Neisseria mucosa	0.3	3.0

DISCUSSION

Oral microbiology did in the second half of the twentieth century considerable progress. The microbiological examination is recognized as indispensable in certain situations or to detect and quantify the presence of certain pathogenic bacteria that are suspected.

The flora of periodontal pockets are characterized by a high proportion of motile anaerobic Gram-negative bacteria (30%) and spirochetes [10]. In our study we found also a dominance of Gram negative -flora, motile as well as the high density of spirochetes.

Some Gram-negative anaerobes are associated with the incidence and progression of periodontal disease [11-13].

The Spirochetes were observed in all cases and play an important role in periodontitis [14]. Oral spirochetes are classified as periodontal pathogens [15]. Also, Loesche and Grossman[16] support this hypothesis. Other studies suggest that the predominance of Treponema denticola and other gram-negative bacteria in high numbers in periodontal pockets may play an important role in progression of disease [17, 18].

According, Loesche and Grossman [16] the oral spirochetes are often dominant bacterial types observed in subgingival plaque taken from sites with periodontal disease [19]. For Richard *et al*. spirochetes comprise a significant proportion (20-50%) of the total microscopic count of bacteria in inflamed pockets [15]. Also, Baehni and Guggenheim [20] have found spirochetes in periodontal pockets of patients with periodontitis. Sela has shown a positive relationship

between Treponema denticola and aggressive periodontitis [17].

Our study revealed that chronic periodontitis flora is diverse and composed of both motile and non motile types and gram positive and negative (cocci, rod-rights) as well as its rarity in gram-negative bacilli and gram-positive filaments.

We have found Eikenella corrodens in chronic (20.7%) and in aggressive periodontitis (10.8%). For Noiri *et al,* Eikenella corrodens is associated with various types of periodontitis [21]. Similarly, Nonnenmacher *et al*. [22] and the Academy report [14] Eikenella corrodens were detected in both groups.

The ecological niche of Aggregatibacterium actinomycctemcomitans is the oral cavity. It is mainly found in the subgingival flora [23].

Aggregatibacterium actinomycetemcomitans is detected in our study more in aggressive periodontitis (8.4%) compared to chronic periodontitis (0.3%); *Aggregatibacterium actinomycetemcomitans* has been implicated as primary causative agent in localized juvenile periodontitis [10, 3, 24]. Nonnenmacher *et al*. [22] have identified *Aggregatibacterium actinomycetemcomitans* in cases of localized juvenile periodontitis and rapidly progressing. The Academy report [14] was associated *Aggregatibacterium actinomycetemcomitans* cases of localized juvenile periodontitis and generalized. According to Loesche and Grossman, [16] *Aggregatibacterium actinomycetemcomitans* is present in all clinical entities. Baehni and Guggenheim [20] have reported its presence in subgingival sites of patients with periodontitis.

We identified Capnocytophaga only in aggressive periodontitis. According to Spratt *et al*. [27], *Capnocytophaga* sp. has been shown his involvement in some forms of periodontitis.

We found Prevotella intermedia and Haemophilus aphrophilus more in aggressive than in chronic periodontitis. According Gürsoy *et al*. [25], Prevotella intermedia is associated with periodontal disease. For Mayorga-Fayad *et al.* [26], the frequency increases Prevotella intermedia in patients with periodontitis. According Tempro and Slots [28] Haemophilus aphrophilus is present in low proportion in the subgingival plaques and play a role that are not clear in advanced periodontal disease. For the same researchers, Dental plaque is a primary ecological niche for Haemophilus aphrophilus.

In our study, Eubacterium is present in aggressive periodontitis (8%). Indeed, Poco and al. [29], have found up to 54% of the microflora of subgingival plaque from periodontal pockets. For Hill *et al*. [30], this bacterium was isolated between 35 and 42%.

According Ximenez-Fyvie *et al*. [31] Actinomyces are an important component of supra and subgingival plaques. Cisar *et al*. [32] have found Actinomyces naeslundii in dental plaque may contribute to certain diseases such as periodontitis. Johnson *et al.* [33] noted Actinomyces israelii in periodontitis cases. We isolated Actinomyces naeslundii (1.7%) and Actinomyces israelii (1.7%) in aggressive periodontitis, Actinomyces naeslundii (0.9%) and Actinomyces israelii (1.8%) in chronic periodontitis.

We isolated Staphylococcus aureus (4.2%) and Peptostreptococcus anaerobius (1.4%) from the periodontal pockets of patients with aggressive periodontitis. Other studies have reported the presence of Staphylococcus aureus in such infections [14]. Nonnenmacher *et al*. [22], Staphylococcus aureus were observed in cases of periodontitis. Kumar [34] *et al*. Have reported that Peptostreptococcus is associated with periodontitis and the percentage of this organism is very high in these entities. Similarly, Mouton [35] attributed a causative role in Peptostreptococcus (Peptostreptococcus anaerobius *and* Peptostreptococcus micros) in cases of periodontal disease.

We noted the presence of Campylobacter in both types of periodontitis. Macuchi and Tanner [36], have identified at least seven species of Campylobacter in subgingival sites. According to Lakshman [37], Campylobacter spp. is associated with different forms of periodontitis.

The Neisseria were isolated with very low levels in aggressive periodontitis (0.3%). For the expert group - INSERM-[23], Neisseria are primary colonizers of the film but gained no exogenous pathogenic role they have not been assigned.

Streptococci were observed with high percentage in chronic periodontitis (38.7%). For Baehni and Guggenheim, [20], Streptococci are considered beneficial for the host and colonizing the pocket in large numbers, can delay the process of periodontal disease.

Helicobacter pylori is grown in chronic periodontitis (6.3%) more than aggressive periodontitis (4.5%). For Stolzenberg-Solomon *et al.* [38] Helicobacter pylori was found in dental plaque and associated with periodontal disease. According Namiot *et al*. [39] Studies have shown that Helicobacter pylori colonizes not only the stomach but also the oral cavity and there are strains of Helicobacter pylori that were isolated from dental plaque.

Stolzenberg-Solomon *et al*. [38] have shown that periodontal disease and especially the deep periodontal pockets associated with Helicobacter pylori.

E. coli was grown in both entities. According Betancourth *et al*. [40] micro-organisms of the family Enterobacteriaceae (gram negative rods) were considered as microorganisms unusual in patients with periodontitis. Steffens *et al*. [41] have mentioned that there are several studies that have associated enteric bacilli to periodontal disease.

Corynebacterium matruchotii is found in the case of aggressive (2.4%) and chronic (1.2%) periodontitis. Barrett *et al*. [42] have reported the possible role of *Corynebacterium matruchotii* in dental plaque and the pathogenesis of periodontitis.

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