# BACTERIOLOGICAL AND IMMUNOLOGICAL STUDY OF AGGRESSIVE PERIODONTITIS IN MOSUL


## ABSTRACT

**Aims of the study**: to isolate and identify microorganisms causing aggressive periodontitis, and to estimate the changes in the levels of IL-1β, TNF-α and CRP in serum as well as to determine peroxidase activity in saliva of patients with aggressive periodontitis. **Materials and Methods:** This study was carried out on total number of 40 (35 patients with aggressive periodontitis, 18 females and 17 males aged between 16-35 years and 5 control group between 20-30 years old). Samples were taken from the lesion for bacteriological study. Serum and saliva were collected and ELISA test was performed. **Results:** The bacteriological results showed that *A. actinomycetemcomitans* was the most prevalent bacteria in aggressive periodontitis, followed by facultative anaerobic. The serological and biochemical studies showed that interleukin-1β was significantly elevated in the study group while tumor necrosis factor α was not, whereas peroxidase enzyme activity and C-reactive protein were also highly significant elevated in the study group. **Conclusion:** *Actinobacillus actinomycetemcomitans* was the major etiologic bacteria of this disease as well as significantly high levels of, CRP, peroxidase activity and IL-1β could be regarded as strong markers for more precise understanding the immunological aspect of this disease.



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**Key Words:** aggressive periodontitis, bacteriology, immunology.


## INTRODUCTION

Periodontitis is defined as "an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession, or both. Aggressive periodontitis (formerly known as early onset periodontitis) is periodontal destruction that is become clinically significant around adolescence or early adult hood. The disease has been classified as localized and generalized. Other terms were used to describe the aggressive forms of periodontitis which include juvenile, localized juvenile, generalized juvenile, rapidly progressive, severe, and pre pubertal periodontitis [(1)]. The prevalence of aggressive periodontitis is higher in African-American than in whites 4.63% versus 0.91%. Whether or not the prevalence of aggressive periodontitis differs by gender is unclear although Loe and Brown [(2)] showed equal distribution of the disease equal between the genders.

Different microorganisms have been implicated in the development of aggressive periodontitis but the predominant was *A. actinomycetemcomitans* [(3)]. Some immune defects have been

implicated in the pathogenesis of aggressive periodontitis. Several investigators have shown that patients with aggressive periodontitis display functional defects of polymorphonuclear leukocytes, monocytes, or both [4]. Environmental factors may affect the clinical expression of aggressive periodontitis.

The current study was conducted to isolate and identify microorganisms causing aggressive periodontitis as well as estimate the levels of different immunological and biochemical markers.

## MATERIALS AND METHODS

### Subject Groups

This study was carried out on total number of 40 subjects in College of Dentistry/Mosul University. The study group consisted of 35 individuals; aged between 16-35 years old, (17 males and 18 females) referred to Periodontology Unit in the College of Dentistry/Mosul University. Patients were diagnosed and selected according to the recommended criteria [5,6]. The control group consisted of 5 healthy individuals (officials and dentists) working in the College of Dentistry of Mosul University. The age of this group ranged between 20-30 years old. This group had no signs of any systemic disease, gingivitis or any type of periodontitis.

### Collection of Samples

The supra gingival plaque was removed with sterile cotton pellets before taking the pocket sample. One pooled sample per patient was obtained by sampling the four deepest periodontal pockets more than 5mm- (one in each guardant) using paper points and the paper points kept there for 10 seconds [7]. The paper points then transferred to 5ml thioglycolate broth and incubated at 37˚C for 2 hours and sample was streaked on Dentaid-1 and blood agars. The blood agar was incubated at 37˚C for 1-2 days while the Dentaid-1 agar was incubated at 37˚C for 2-3 days [8].

About 4-5 ml of unstimulated saliva was collected, centrifuged at 3500 rpm for 10 minutes, and then the clear supernatant separated and placed in sterile plastic Eppendorff tubes and stored at -20˚C until the time of analysis [9].

Five ml of blood sample was taken from each subject, centrifuged at 4500 rpm for 10 minutes and the serum separated and placed in a sterile plastic Eppendorff tubes and stored at -20˚C) until the time of analysis [10].

### Cultivation and Identifications of Samples

Bacteria were identified on the basis of morphological, cultural characteristics and biochemical tests.

### Immunological and Biochemical Assay

Boster's human IL-1β and TNF-α ELISA kits were used (Boster-China) in which human IL-1β specific monoclonal antibodies (clone No. ILB1-H67) and human TNF-α specific monoclonal antibodies (clone No.28401.111) were precoated on to 96-well plates.

Peroxidase enzyme activity was measured by colometric method [11]. The method of CRP test was based on the principle of the latex agglutination assay described by [12].

### Statistical Analysis

Student t-test, Pearson Chi-Square test, and Mann-Whitney test were used for the statistical analysis using 11.5 SPSS program.

## RESULLTS

This study showed that 88.57% of aggressive periodontitis patients were in the age between 16-30 years old, while only 11.43% of them were older than 30 years old. Females were more than males affected by aggressive periodontitis and the percentage of females was 51.4%, while

the males were 48.6% (Figure 1). Localized form of aggressive periodontitis represented (57.1%), while the generalized form represented 42.9%.

Bacteriological analysis of the study group showed that *A. actinomycetemcomitans* isolated in 77.1%, while 22.9% showed facultative anaerobes (Figure2). The later included oral streptococci (54.54%), *Staphylococcus* species (36.36%), and *Actinomyces* species (9.1%) and the biochemical tests for them are illustrated in Table (1).

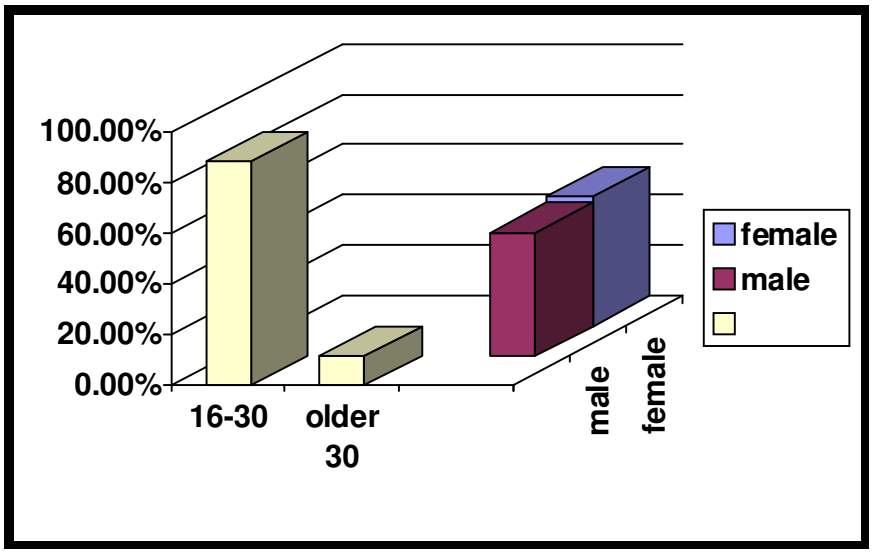


Figure (1): Percentages of gender and age in the study and control

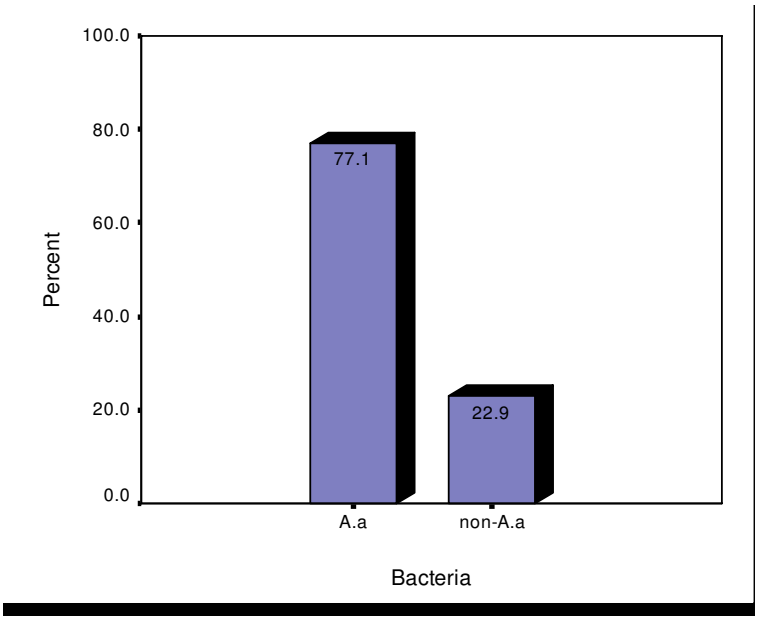


Figure (2): Percent of isolation of *A. a* and non- *A. a* in the study group.
A.a= *Actinobacillus actinomycetemcomitans*

Table (1): Biochemical tests for facultative anaerobes.

| Bacteria / Tests | Oral streptococci | *Staphylococcus aureus* | Non-coagulase positive staphylococci | *Actinomyces* spp. |
|---|---|---|---|---|
| **Number*** | **6** | **2** | **2** | **1** |
| **Catalase** | _ | **+** | **+** | _ |
| **Oxidase** | **/** | **/** | **/** | _ |
| **Coagulase** | **/** | **+** | _ | **/** |
| **Blood hemolysis** | **α** | **/** | **/** | **/** |
| **Mannitol Salt agar fermentation** | **/** | **+(yellow)**** | **_(red)***** | **/** |
| **MacConkey's agar growth** | _ | **/** | **/** | _ |
| **Neomycin** | **/** | **/** | **/** | **S** |
| **Metronidazole** | / | / | / | R |
| **Optochin** | R | / | / | / |

*Number: mean the number of isolates from samples; **yellow discoloration of the Mannitol Salt agar i.e. ferment mannitol. ; ***red discoloration of the Mannitol Salt agar i.e. don't ferment mannitol; (/): not done, (S): sensitive, (R): resistance, (+): positive, (-): negative.

**Comparison between Study and Control Groups in Relation to Interleukin-1β, Tumor Necrosis Factor-α, Peroxidase Enzyme Activity, and C-Reactive Protein**

The mean value of IL-1β in the study group was (97.82) pg/ml, which was higher than the control group (63.62) pg/ml, and the difference was significant (P<0.05), while the mean value of TNF-α in the study group was (56.1) pg/ml which was higher than in the control group (38.8) pg/ml with no significant difference (P>0.05). The mean value of peroxidase enzyme activity in the study group was (0.073) U/ml, higher than in the control group (0.028) U/ml with no significant difference (P<0.01) (Table 2).

Using Pearson Chi-Square test, 55.9% of the study group showed CRP value of 6mg/L, while 100% of the control group was negative for CRP test. These results indicated a relation between CRP and aggressive periodontitis which was highly significant (P<0.01) (Table 3).

Table (2): Comparison of IL-1β, TNF-α, and peroxidase activity between study and control groups

| Groups / Parameters | Study group | | | Control group | | | t-value | p-value |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | SE | Mean | SD | SE | | |
| **IL-1β (pg/ml)** | 97.82 | 35.3 | 6.24 | 63.62 | 23.34 | 10.43 | 2.083 | 0.045 |
| **TNF-α (pg/ml)** | 56.1 | 27.48 | 4.94 | 38.8 | 6.4 | 2.91 | 1.385 | 0.175 |
| **Peroxidase activity (U/ml)** | 0.073 | 0.081 | 0.014 | 0.028 | 0.004 | 0.002 | 3.195 | 0.003 |

Table (3): Comparison of CRP between study and control groups.

| CRP (mg/L) | <6 (mg/L) | 6 (mg/L) | 12 (mg/L) | 24 (mg/L) | 48 (mg/L) | $\chi^2$-value | p-value |
|---|---|---|---|---|---|---|---|
| **Study** | 17.6% | 55.9% | 11.8% | 11.8% | 2.9% | | |
| **Control** | 100% | 0% | 0% | 0% | 0% | 14.599 | 0.006 |

## DISCUSSION

In the study group, 88.57% of patients with aggressive periodontitis were in the age between 16-30 years old which is compatible with others [13-15] who showed that aggressive periodontitis affected at age of 30 years, but older and younger patients also may be affected.

Males to females percentage in the present study concerning aggressive periodontitis was 48.6/51.4. This finding is acceptable since many studies showed that the prevalence of aggressive periodontitis was nearly equally distributed in males and in females [15-17]. Few studies found significant relation between gender and aggressive periodontitis, in which the percentage of males to females was 28/72 [18], and this may be due to that more females seeking or attending dental clinic than males, the and the hormonal changes observed more in females [19].

In this study, the prevalence of LAP was higher than GAP, (57.1% compared to 42.9%) which are consistent with other studies [14-20].

In the present study, the prevalence of *A. actinomycetemcomitans* was high (77.1%) in patients with aggressive periodontitis. Similar data were reported by others [21-23]. The linkage or strong relation between aggressive periodontitis and *A. actinomycetemcomitans* is due to the major virulence factor leukotoxin, which selectively kills human leukocytes, and also induces apoptosis in T lymphocytes and PMNs [24]. Also, this bacterium produces a protease which degrades the native type I collagen, and fibronectin leading to destroy the periodontal connective tissues [25]. The isolation of facultative anaerobes was very small in numbers; however *Staphylococcus* species, oral streptococci and Actinomycetes were the most commonly isolated. Similar findings were also reported by others [26-28].

### Comparison between Study and Control Groups

In the present study, the level of IL-1β was significantly higher in aggressive periodontitis patients than in controls. Armitage (2004) also found an elevated level of IL-1β in patients with aggressive periodontitis due to hyper responsive macrophages. Other study showed elevated levels of IL-1, IL-6, TNF-α and IFN-γ in aggressive periodontitis at different stages of disease

suggesting that the intensity of immune response correlated with the increased mediators that represented evolution and monitoring markers of the periodontitis progression [29].

The present study showed elevated level of TNF-α in the study group compared to control group but with no significant difference. Similar finding was reported by other [30]. Although, the effect of TNF-α as pro inflammatory markers in aggressive periodontitis is questionable despite of its high level in aggressive periodontitis compared to control groups. This level remained higher after periodontal therapy in generalized aggressive periodontitis compared to healthy periodontium subjects [31].This emphasized a weak or no association between aggressive periodontitis and TNF-α which is explained by others [19,32].

The increase in the activity of peroxidase enzyme in saliva of patients with aggressive periodontitis in the present study was highly significant compared to individuals with healthy periodontium. Other studies demonstrated an elevated level of myloperoxidase (MPO) in aggressive periodontitis, suggesting that MPO may serve as an inflammatory marker for periodontitis [33-36]. Another study observed decreased in salivary peroxidase and myloperoxidase enzyme activity in juvenile periodontitis suggesting that the suppressed peroxidase-mediated host defense mechanisms could be characteristics of juvenile periodontitis [37].

The elevated level of CRP in the study group of the current study revealed a highly significant relation of CRP with aggressive periodontitis which is comparable with others [9-38]. This result supports the hypothesis that biochemical analysis of saliva might be an important non-invasive way of study disease process in periodontitis. The elevated level of serum CRP may be due to elevated level of IL-6[39]. The elevated level of inflammatory markers like TNF-α, IL-1, and IL-6 which all stimulated hepatocytes to produce CRP, other acute-phase proteins and procoagulant mediators [40-41].

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