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# Pro-inflammatory cytokine concentration in gingival fluid in obese

## Abstract

**Introduction:** High levels of pro-inflammatory cytokines both in periodontal disease and in obese people are well documented in the literature. However, there is weak evidence of the relation between both pathologies and cytokines concentration both in serum as in gingival fluid (i.e. Interleukin 6 and Gamma Interferon). The objective of our exploratory study is to measure the concentration of pro-inflammatory cytokines such as Interleukin 6 and Gamma Interferon in the gingival fluid of the obese patients with different clinical periodontal status.

**Methods:** Twenty one adult patients with a body mass index >30Kg/m$^2$ were included. Baseline measurements, including clinical parameters and gingival fluid samples were taken to all subjects. Gingival fluid sample was taken in order to measure the Interleukin 6 and Gamma Interferon concentrations.

**Results:** Nineteen patients (90%) had periodontal disease. Among them, the disease was mild in 43%, moderate in 14% and severe in 33% of the patients. The average cytokine concentration in gingival fluid for Interleukin 6 was 2,06+/ - 0,96pg/ml and for Gamma Interferon 2,73 +/- 1,13 pg/ml. Interleukin 6 concentration in patients with concomitant diseases was 2,66 +/- 0,91pg/ml and in those without concomitant diseases was 1,63 +/- 0,77pg/ml (p=0.01); the Gamma Interferon concentration was 3,48 +/- 1,13 pg/ml and in the patients without concomitant diseases 2,19 +/- 0,79pg/ml (p=0.009).

**Conclusion:** Even though the strong association between obesity and periodontal disease has been proved, the estimation of the persistence of the inflammatory response through the high levels of pro-inflammatory cytokines seems to be more related to the concomitant diseases with inflammatory origin disorders than with the association itself.

**Keywords:** periodontal disease, obesity-IL6-gamma interferon, gingival fluid

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Olavegogeascoechea P,[1] Brusca MI,[1] Noier M,[1] Carballo B,[1] Casal M,[1] Palazzi J,[2] Grandinetti J[1]

[1]Department of Medicine and Health Sciences, Dentistry career, Inter-American Open University, Argentina

[2]International Integral Clinical Trial Labs, Argentina

**Correspondence:** Olavegogeascoechea P, Department of Medicine and Health Sciences, Dentistry career, Inter-American Open University, Av. San Juan 951, Autonomous City of Buenos Aires, Buenos Aires, Argentina, Email polavego@gmail.com

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**Abbreviations:** IL6, interleukin 6; INF-γ, gamma interferon; BMI, body mass index; CD, celiac disease; WHO, world health organization

## Introduction

The prevalence of obesity is increasing around the world. This trend has prompted the WHO to describe the increase as a global epidemic; therefore it would be considered a major problem in public health.[1] It is strongly associated with a higher risk for diseases such as diabetes, hypertension, some types of cancer, vascular disease[2] and periodontitis.[3,4] Periodontitis is a chronic inflammatory affection, related not only to risk factors for vascular disease but also related to cardiovascular and neurovascular events (i.e. acute myocardial infarction and ischemic stroke).[5] Its pathogenic process is complex[6] and would bear a part in similar paths to arteriosclerosis.[7] The mechanisms whereby the obese patients develop periodontitis most commonly are not well known, however, it is known that the adipose tissue is a major producer and reservoir of pro-inflammatory mediators.[8] The relation between both diseases,[9] could be mediated by the constant production of pro-inflammatory cytokines.[10–12] The presence of high levels of pro-inflammatory mediators both in patients with periodontal disease and in obese patients is well documented (i.e. pro-inflammatory cytokines). However, there is weak evidence of the relation between both pathologies and cytokines concentration both in serum as in gingival fluid (i.e. Interleukin 6 and Gamma Interferon). These two cytokines were strongly associated with severe or destructive periodontal disease.[13–15] The objective of our exploratory study is to measure the concentration of pro-inflammatory cytokines such as Interleukin 6 (IL6) and Gamma Interferon (INF-γ) in the gingival fluid of the obese patients with different clinical periodontal status.

## Materials and methods

Twenty one obese adults, both male and female and with a BMI>30Kg/m$^2$ were recruited for this exploratory cross-sectional study. The study protocol was approved by Ethics Committee-Universidad Abierta Interamericana. Exclusion criteria: 1-Patients who received antibiotics or anti-fungal medication 30 days prior to the study; 2-Patients with orthodontia, either permanent or removable. Baseline mesurements, including previous disease (i.e.

diabetes, hypothyroidism), clinical parameters and gingival fluid samples were taken to all subjets. Clinical measurements were taken at 6 sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual). The periodontal indexes (probing depth, periodontal attachment loss, Loe's index) were measured. "Healthy" were considered the patients with no hemorrhage while probing, probing depth <2mm, attachment loss <2mm, absence of mobility, plaque index 0 or 1, gingival index 0 to 1. Those who did not apply as health periodontal status were considered having periodontal disease that will be divided in three groups: mild, moderate, severe. Patients were indicated to use a mouthwash (sterile physiological solution 0.98%) before taking the simple, then it were obtained at the junction of the buccal and mesial surface. A gingival fluid sample was obtained with paper cones (Oraflow Inc, NY, USA) during 10 seconds and it was placed in a mico centrifuge tuve (Eppendorf tubes) with 0.5ml of physiological solution and stored at -70˚C until cytokine quantification. IL6 and INF-γ in gingival fluid samples were determined with ELISA Human IFN-Gamma and Human IL-6, Quantikine; RD Systems Kits). We define concomitant diseases to all those chronic disease that already were diagnosed at the time of study (i.e. diabetes, hypothyroidism). Statistics: Values for the citokines were presented as averaged and SD. The differences in the distribution of males and periodontal status were examined using the Chi-square test and the level of citokines was examined using ANOVA test. The ROC curve was used to determine the Cut-off value of interleukins concentration (Ad Hoc analysis).

## Results

Baseline characteristics of the patients: 48% were men, the mean age was 46 years and the BMI 34, 84 +/- 2, 93. Almost all (90%) had periodontal disease which was, mild in 9 (43%), moderate in 3 (14%) and severe in 7 (33%). Two patients showed healthy periodontal status. Eight patients (38%) had concomitant diseases: Celiac Disease (CD) 4, Diabetes 1 and Hypothyroidism 3. The severity of the periodontal disease in the patients with hypothyroidism was: mild in 1 patient, moderate in 1 and severe in 1; in the patients with CD most were severe (moderate in 1 patient and severe in 3 patients) and in the diabetic patient was severe. In subjects with and without periodontal disease, the mean cytokines concentration between groups in gingival fluid were similar for IL6 (2,06 +/- 0,96 vs. 1,08 +/- 0,39 pg/ml respectively; p=0.10) while the mean INF-γ concentration showed statistically significant differences (2,73 +/- 1,13 vs. 1,38 +/- 0,11pg/ml; p<0.01 respectively). The mean concentration of IL6 and INF-γ between sex were similar (IL6: 2,11 +/- 1,09pg/ml vs. women: 2,02 +/- 0,88pg/ml; p=0,83 and INF-γ: 2,89 +/- 1,42pg/ml vs. women 2,59 +/- 0,84pg/ml; p=0,57). In patients with concomitant diseases the INF-γ was statistically significant higher than those without (3, 48 +/- 1, 13 vs. 2, 19 +/- 0,79pg/ml; p=0.009) and the mean IL6 concentrations also showed statistically significant differences between groups (2,66 +/- 0,91vs 1,63 +/- 0,77; p=0.01) (Table 1) (Table 2). In hypothyroid patients the mean IL6 concentration was significantly higher than those without (3, 23 +/- 0, 93 vs. 1, 63 +/- 0, 77pg/ml; p=0.009) and similar differences were seen in the mean INF-γ concentration (3,55 +/- 1,51 vs. 2,58 +/- 0,94pg/ml, respectively; p=0.04). On the other hand the mean IL6 concentration were similar in those patients with CD and those without (2,40 +/- 0,90 vs 1,63 +/- 0,77pg/ml; p=0.12), while the mean INF-γ concentration were different between groups mentioned (3,47 +/- 0,73 vs. 2,19 +/- 0,79pg/ml; p=0.01) (Table 3) In obese patients with periodontal disease we found that those with concomitant diseases (Hypothyroidism, Diabetes, Celiac Disease) had a higher prevalence of severe disease than those without (20,8% vs. 8,3% respectively; p<0.01). On the other hand, in patients with severe periodontal disease compared to the those with mild or moderate status, we found not only a higher concentration of INF-γ (3,93+/-0,32 vs. 2,03 +/-0,13, respectively; p<0.01) but also higher concentrations of IL6 in gingival fluid (2,64+/-0, 33 vs. 1,72+/-0, 24, respectively; p=0.04). In an Ad-hoc analysis and looking for a predictive cytokine concentration value for concomitant disease in this kind of patients, we found the best cut-off point by using the ROC curve analysis (Area under the curve: 0,84) above which, the levels of IL6 in gingival fluid that might predict concomitant disease was: 1,66 pg/ml (Figure 1), giving a diagnostic value of: sensibility 87,5% (CI 95%: 64,6-110,4%), specificity 63,6% (CI 95%: 35,2-92,1%), PPV 64% (CI 95%: 35,2-92,1%), NPV 88% (CI 95%: 64,6-110,4%) and Likelihood Ratio: 2,41 (CI 95%: 1,43-4,04). The cut-off point confirmed by ROC curve analysis (Area under the curve: 0,84), above which the INF-γ levels in gingival fluid that might predict concomitant disease was: 2,33 pg/ml (Figure 2), giving a diagnostic value of: sensibility 100%, specificity 63,6% (IC95%: 35,2-92,1%), PPV 67% (IC95%: 40-93%), NPV 100% y Likelihood Ratio: 2,75 (CI95%: 1,76-4,34).

**Table 1** Cytokine concentration in patients with concomitant diseases

| Data | IL6 (pg./ml) | INF-γ (pg./ml) |
|---|---|---|
| n | 8 | 8 |
| Mean (pg./ml) | 2,66 | 3,48 |
| SD | 0,91 | 1,13 |
| Mínimum | 1,50 | 2,40 |
| Máximum | 4,16 | 5,76 |
| Range | 2,66 | 3,36 |

**Table 2** Cytokine concentration in patients without concomitant diseases

| Data | IL6 (pg./ml) | INF-γ (pg./ml) |
|---|---|---|
| n | 11 | 11 |
| Mean (pg./ml) | 1,632 | 21,918 |
| SD | 0,77 | 0,79 |
| Mínimum | 0,8 | 1,30 |
| Máximum | 3,4 | 3,60 |
| Range | 2,6 | 2,30 |

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**Table 3** Cytokine concentration according to gender and concomitant disease

| Cytokine* | Gender | | | Concomitant diseases | | | CD | | | Hypothyroidism | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Men | Female | p | Yes | No | p | Yes | No | p | Yes | No | p |
| IL6 | 2,11 +/- 1,09 | 2,02 +/- 0,88 | 0.83 | 2,66 +/- 0,91 | 1,63 +/- 0,77 | 0.01 | 2,40 +/- 0,90 | 1,63 +/- 0,77 | 0.12 | 3,23 +/- 0,93 | 1,63 +/- 0,77 | 0.009 |
| INF-γ | 2,89 +/- 1,42 | 2,59 +/- 0,84 | 0.57 | 3,48 +/- 1,13 | 2,19 +/- 0,79 | 0.009 | 3,47 +/- 0,73 | 2,19 +/- 0,79 | 0.01 | 3,55 +/- 1,51 | 2,58 +/- 0,94 | 0.04 |

*Cytokine concentration in pg. /ml; IL6, interleukin 6; INF-γ, gamma interferon; CD, celiac disease

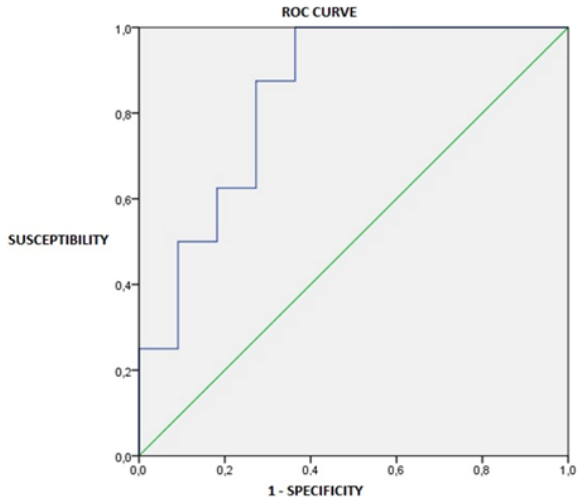


**Figure 1** ROC Curve for IL6 concentrations.

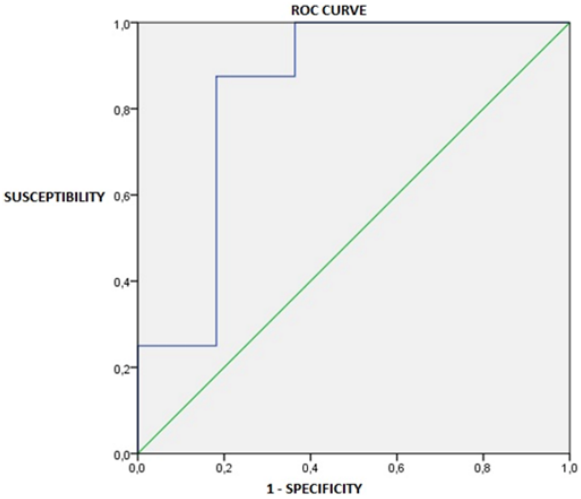


**Figure 2** ROC Curve for INF-γ concentrations.

## Discussion

The prevalence of periodontal disease in this obese patient population was 90% and the severe status less than 50%, this finding was different from the publication by Khan et al.,[16] where the prevalence was 74% and the severe form reached more than half of the participants. Probably because in our study we used a higher BMI than in the above mentioned study (30Kg/m$^2$ vs. 27, 5Kg/m$^2$). Our study sustains the strong association between obesity and periodontal disease, being obesity a potential risk factor for periodontal inflammation.[17] In our exploratory study the levels of cytokines concentration in gingival fluid (i.e. IL6 and INF-γ) showed differences depending on the severity of the periodontal disease, being higher not only in those patients with severe periodontal disease but also in those with concomitant disease (hypothyroidism, diabetes or celiac disease), this probably shows the higher impact in the production of these pro-inflammatory mediators in autoimmune diseases.[18,19] In this study the INF-γ levels were higher in the mentioned concomitant diseases, while the IL6 showed significant differences only in the patients with hypothyroidism. The hypothyroidism showed a significant difference in both cytokines concentration in the gingival fluid, unrelated to the severity of the periodontal disease, showing a possible association beyond that and perhaps obesity.[20] Another important finding in our study was the potential usefulness of IL6 and INF-γ concentrations in gingival fluid as predictors of concomitant disease in these kinds of patients. Both pro-inflammatory cytokines showed a high negative predictive value, making it unlikely the existence of concomitant diseases with levels below the cutoffs found (IL6: 1.66pg/ml and INF-γ: 2.33pg/ml). The limitation in our study is the sample size, especially in those patients with concomitant diseases, therefore it would be needed more studies to aim more information in this regarding.

## Conclusion

Even though the strong association between obesity and periodontal disease has been proved, the estimation of the persistence of the inflammatory response through the high levels of pro-inflammatory cytokines seems to be more related to the concomitant diseases with inflammatory origin disorders than with the association itself.

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## Conflicts of interest

The authors declare there is no conflict of interests.

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