

# GINGIVAL CREVICULAR FLUID IMMUNOGLOBULINS IN SMOKERS WITH CHRONIC PERIODONTITIS

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## ABSTRACT

*Tobacco smoking is regarded as one of the most significant risk factors in the development and progression of periodontal disease. Studies have shown an altered host antibody response to antigenic challenge in smokers with periodontitis. The study was undertaken to estimate the gingival crevicular fluid (GCF) immunoglobulin G, A, M (IgA, IgG and IgM) levels in smokers and non smoker with periodontitis. Patients with a periodontal probing depth  $\geq 4$ mm and clinical attachment loss  $\geq 2$ mm in at least 30% of the teeth were diagnosed as chronic periodontitis group. Subject who smoked minimum of 10 cigarettes per day for not less than two years were included in smoker periodontitis group. GCF samples were obtained from the patient using filter paper and the immunoglobulin levels were estimated by the radial immunodiffusion assay. Gingival crevicular fluid immunoglobulins were found to be relatively lower in smokers compared to non-smokers. The IgA levels showed significant reduction in smokers. Current observations indicate that cigarette smoking may be associated with the suppression of B-cell function and immunoglobulin production. The alteration of antibody levels further explains the potential mechanism by which smoking exacerbates periodontal disease. Further studies at molecular level may highlight the specific mechanism by which tobacco can interact with cells of the immune system and its impact on periodontal disease process.*

**Key words:** Periodontitis; smoking; GCF; immunoglobulins; antibody levels.

## INTRODUCTION

Smoking is a major risk factor in the development and progression of periodontal diseases<sup>1</sup>. The effect of smoking on the extent and prevalence of periodontal destruction has also been shown to be dose dependent. Measures of the association of smoking status and periodontal destruction have been rather strong and consistent across studies and populations<sup>2</sup>. The evidence argues for the fact that cigarette smoking is a highly significant risk indicator for periodontitis<sup>3</sup>. Smoking can mask the early signs of periodontal disease by suppressing the inflammatory response. This can be a diagnostic problem, especially in young people with early periodontitis. Gingival pockets tend to be greater in the anterior segments and maxillary lingual sites. There is often recession in maxillary and mandibular anterior segments. The marginal gingiva tends to be thickened and fibrotic with rolled margin.

Periodontitis progress rapidly in smokers and have a low response to treatment. Treated smokers tend to exhibit attachment loss over time while undergoing maintenance therapy. These findings are particularly interesting in view of analyses that have show that 85% to 90% of refractory periodontitis patients were current smokers<sup>4</sup>.

Nicotine may cause a vasoconstriction in the peripheral blood vessels and thus may reduce the clinical signs on gingivitis<sup>5</sup>. The induced vasoconstriction could contribute to impaired gingival blood flow and decrease the amount of oxygen and blood constituents that reach the gingiva. The capacity to remove tissue waste products would also be reduced leading to tissue damage or a compromised immune response.

It is well documented that smoking can impair various components of the host response and immune

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system. These effects include the inhibition of neutrophil chemotaxis and phagocytosis<sup>6-8</sup>, inhibition of cellular immunity, and suppression of local antibody production<sup>9</sup>. Cigarette smoke products such as nicotine can also incorporate into root surfaces and into fibroblasts which may inhibit the reattachment of fibroblasts to the root surface<sup>10</sup>. Smoking can not only impair the normal host response but also stimulate the destructive arm of the host response<sup>11,12</sup>. For example, cigarette smoke can stimulate the neutrophil oxidative burst with release of potentially tissue destructive oxygen species such as superoxide, hydrogen peroxide and hydroxyl radical<sup>13,14</sup>. These products of the oxidative burst not only have direct cytotoxic effects on cells of the periodontium, but may also alter the tissue protease/antiprotease balance in favor of tissue destructive activity<sup>15,16</sup>.

The smokers have also lower amounts of GCF compared to non-smokers. A study done by Holmes<sup>17</sup> compared GCF in smokers and non-smokers with clinical healthy gingiva and GCF of smokers in areas physically exposed to smoke (maxilla palatal) and in areas not physically exposed to smoke (maxilla buccal). Smokers had a significantly less fluid flow than non-smoker. The effect of tobacco smoke on clinically healthy gingival may be through vasoconstriction rather than direct physical irritation.

It has been suggested that the humoral immune response has a protective role in the pathogenesis of periodontal disease<sup>18</sup>. Alterations in specific IgG and IgA responses both locally at inflamed sites and systemically have relevance in disease progression<sup>19,20</sup>. It has been shown that gingival crevicular fluid (GCF) levels of IgG may be reduced in active and deep periodontal pockets when compared with other sites in chronic periodontitis patients<sup>20</sup>. These findings, and more recent findings for IgA levels in GCF, suggest that GCF immunoglobulins may indicate 'high risk' sites for periodontitis<sup>21</sup>. GCF antibodies are both serum-derived<sup>22, 23</sup> and locally produced by the abundant plasma cells of the diseased periodontal tissue<sup>24,25</sup>.

There is strong evidence that smoking affects the innate and immune host responses<sup>26,27</sup>. The effects of cigarette smoking on total serum IgG, IgA and IgM were reported in several studies<sup>11, 12, 28</sup>. However no

studies have been undertaken to estimate the gingival crevicular fluid immunoglobulins in smokers with periodontitis. Hence the present study is undertaken to estimate the GCF immunoglobulins levels in smokers with periodontitis and to compare with non smokers.

## MATERIALS AND METHODS

### Study population

A total of 30 smoker and 30 non-smoker male subjects, aged between 20 to 35 years old, were enrolled in the study. They were selected from the patients attending the outpatient clinics of the College of Dentistry Clinics. Approval of the Ethics committee was obtained from the College of Dentistry Research Center (CDRC), King Saud University, Riyadh, Saudi Arabia.

Only patients with a periodontal probing depth  $\geq 3$ mm in at least 30% of the teeth, and no gingival recession were included in the present study. Periodontal diagnosis was assessed according to the classification of American Academy of Periodontology<sup>29</sup>. Smoking status was determined based on the daily consumption; minimum of 10 cigarettes per day for not less than two years<sup>30</sup>. The following criteria were also used to exclude subjects in the study: (1) suffering from any given chronic medical condition, including diabetes, viral, fungal or bacterial infections, (2) suffering from aggressive periodontitis, periodontal abscess, or necrotizing ulcerative gingivitis or periodontitis, (3) received periodontal treatment or antibiotics within the preceding three months.

### Clinical Periodontal Examination

The medical history obtained by a written questionnaire and by interview was recorded. For each patient, a complete set of examination of extra oral and intraoral full-mouth clinical parameters were documented. One clinical examiner performed all the clinical measurements. Calibration exercises for probing measurements were performed in five patients before the actual study. Periodontal probing depth (PPD), clinical attachment level (CAL), plaque index (PI), and bleeding on probing (BOP) were measured with a calibrated probe (Williams markings) at mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and

disto-lingual of each tooth. The smoking history was assessed through a standardized interview and self-reported questionnaire.

### Gingival Crevicular Fluid (GCF)

The gingival crevicular fluid (GCF) was collected by using Periotron System (Periotron 8000<sup>a</sup>, Harco Electronics Ltd., USA). The area was isolated with cotton rolls, supragingival plaque was removed, and the clean surface dried with a gentle stream of air. A sterile dry filter paper strip (Periopaper, Ora Flow Inc, Amityville, NY, USA) 15 mm x 3 mm, were carefully inserted into the gingival crevice of the appropriate sites and allowed to remain for 30 seconds. Strips contaminated by bleeding were discarded. GCF was collected from eight teeth # 16, 11, 24, 26, 36, 32, 44 and 46 were examined at 6 sites <sup>31</sup>. The strips were then placed into a sealed plastic tube containing 50 ml PBS (phosphate-buffered saline) 0.05% Tween® 20 (Polysorbatum 20, Apoteksbolaget, Sweden). The tubes were then frozen at -70° C until assayed.

### Radial immunodiffusion (RID) assay

Estimation of the immunoglobulins GAM (IgG, IgA and IgM) concentrations were determined by radial immunodiffusion assay. Low concentration immunodiffusion plates (NANORID™ kits, The Binding Site Inc, CA 92121, USA) were used to determine the concentration of crevicular fluid immunoglobulin concentrations. The plates were incubated for 72 hours. After the required diffusion time, ring diameters were measured using the digital RID palate reader. The concentrations of the immunoglobulins were recorded from the plotted calibration curve.

### Statistical analysis

Statistical analysis of data was performed with GraphPad InStat® software ¶ The mean and standard deviations GCF immunoglobulin levels (IgG, IgA, IgM) of the subjects (non-smoker periodontitis and smoker periodontitis) were analyzed. Differences between the two study groups analyzed using Student's *t*-test. P-values <0.05 were considered significant.

## RESULTS

The mean age of the smoker group and non-smoker group were  $34.61 \pm 8.42$  and  $34.55 \pm 7.72$  years, respectively. The periodontal probing depth and clinical attachment levels were measured at six sites and the mean values were calculated for each subject in the smoker and non-smoker group with periodontitis (Table 1). The mean periodontal probing depth for the smokers with periodontitis was  $5.61 \pm 0.21$  mm and for the non-smoker  $5.32 \pm 0.22$  mm. The smoker group had a significantly higher periodontal probing depth compared to non-smokers ( $p < 0.02$ ). The clinical attachment loss was significantly higher ( $p < 0.03$ ) among smokers ( $3.61 \pm 0.83$  mm) compared to non-smokers ( $3.15 \pm 0.51$  mm).

The GCF immunoglobulins IgG, IgA and IgM estimated by the radial immunodiffusion assay are presented in table 2. The mean levels of IgA was  $17.87 \mu\text{g/ml}$  in non smoker group and  $13.00 \pm 4.83 \mu\text{g/ml}$  in non-smoker subjects. The IgA antibody showed significantly lower levels in smoker group compared to non-smokers ( $P < 0.05$ ). Though the mean GCF Ig G showed lower levels in smokers ( $21.97 \pm 9.01$ ) compared to non smokers with periodontitis ( $25.07 \pm 9.27$ ) it was not

Group(n=30)	PPD mm (Mean $\pm$ SD)	CAL mm (Mean $\pm$ SD)
Non-Smoker	$5.32 \pm 0.22$	$3.15 \pm 0.51$
Smoker	$5.61 \pm 0.21$	$3.61 \pm 0.83$

TABLE 1: THE PROBING POCKET DEPTH (PPD) AND CLINICAL ATTACHMENT LEVEL (CAL) AMONG SMOKER AND NON-SMOKER PERIODONTITIS GROUPS

Groups (n=30)	Ig A Mean $\pm$ SD ( $\mu\text{g/ml}$ )	Ig G Mean $\pm$ SD ( $\mu\text{g/ml}$ )	IgM Mean $\pm$ SD ( $\mu\text{g/ml}$ )
Non Smoker	$17.87 \pm 4.61$	$25.07 \pm 9.27$	$1.11 \pm 0.84$
Smokers	$13.00 \pm 4.83$	$21.97 \pm 9.01$	$0.99 \pm 0.76$

TABLE 2: THE GCF LEVELS OF IMMUNOGLOBULIN G, A, M (IgA, IgG, IgM) IN SMOKERS AND NON SMOKERS GROUP

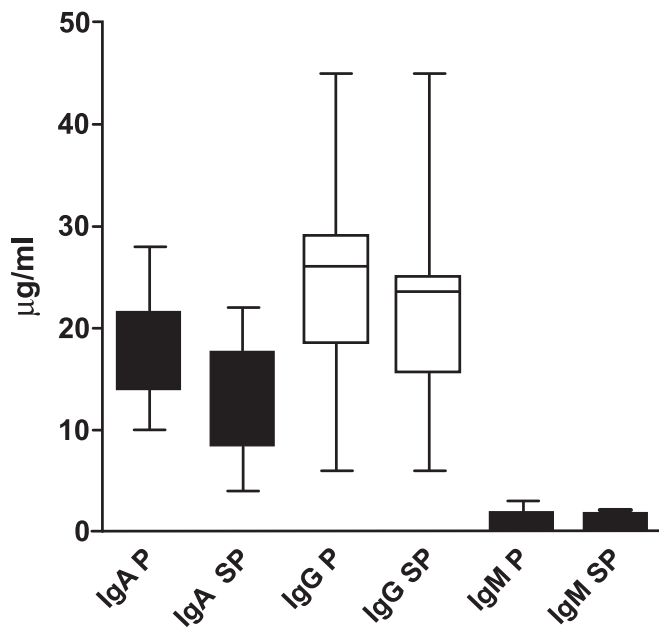


Fig 1: The GCF immunoglobulin levels in smokers and non smokers with periodontitis (P- non smokers with periodontitis, SP – Smokers with Periodontitis)

statistically significant. Ig M was detected in around 80 percent of the samples and it didn't show much variation in the smoker and non smoker group (Fig 1).

## DISCUSSION

Periodontitis is a common chronic infectious disease caused by periodontal bacteria affecting the supporting structures of teeth<sup>32, 33</sup>. The disease process finally leads to a loss of alveolar bone and teeth. Cigarette smoking is a significant risk factor for the initiation as well as progression of periodontal disease. The inhibition of inflammatory destructive effects of smoke on periodontal tissues coupled with the possible vasoconstrictive effects of tobacco products may also explain in part the greater reduction of bleeding on probing in former smokers<sup>34</sup>.

Studies have shown altered serum and gingival crevicular fluid inflammatory cytokine profiles, immune cell function and altered proteolytic regulation in smokers<sup>18, 35</sup>. The humoral immune response, especially the production of IgG and IgA, is considered to have a protective role in the pathogenesis of periodontal disease, but the precise mechanisms are still unknown. Systemic alterations of the cellular and humoral immune responses to periodontal pathogens

among smokers have been evaluated including immunosuppression, exaggerated inflammatory cell responses, impaired neutrophils, and reduced antibody production<sup>7 25</sup>.

It has been suggested that the humoral immune response has a protective role in the pathogenesis of periodontal disease and that alterations in specific serum IgG and IgA responses in gingival crevicular fluid (GCF) and peripheral blood either influence or are affected by the disease progression.<sup>36, 37</sup>

GCF is an important local defence mechanism of the periodontal pocket. In the healthy periodontal sulcus GCF is an exudate of serum origin and excreted in small amounts. With an increase of the severity of periodontal inflammation the amount of GCF excretion increases significantly<sup>38</sup>. In inflamed gingiva GCF is an inflammatory exudate derived from a number of sources, including serum, the connective tissue and epithelium through which GCF passes on its way to the gingival crevice. As fluid traverses the inflamed tissue, enzymes and other molecules as well as products of cell and tissue remodeling and degradation of host and bacterial origin are collected. The cellular components of GCF are 70-80% neutrophils, 10-20% monocytes/macrophages, 5% mast cells and 5% T-lymphocytes.

In the present study a reduction in immunoglobulins was noticed in smokers compared to non smokers. This observation may be indirectly correlating with the disease progression in smokers. It has been suggested earlier that a reduction in specific antibody in serum and consequently GCF in patients with existing disease can place them at risk for further disease progression, and this clearly indicates the important protective role of specific antibodies<sup>39</sup>. One of the mechanisms to explain the observation is that smoking decreases the proliferative capacity of T-cells and T-cell-dependent antibody responses which affects B-cell function and antibody generation<sup>8</sup>. It is possible that B-cells are functionally compromised by the reduced proliferative responses to oral pathogens, resulting in decreased production of serum antibodies<sup>27</sup>. In addition, it has been shown that alveolar macrophages from smokers exhibiting reduced expression as antigen-presenting cells<sup>14</sup>. This may eventually lead to a reduction in the humoral immune response to invading organisms in these patients.



The observations of the present study further emphasize the influence of smoking on the immune function and its implications in the pathogenesis of periodontal disease in smokers.

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