

# The effect of photodynamic therapy on the salivary flow rate, IgA concentration and C-reactive protein levels in active smokers: a case-control study

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**Abstract. – OBJECTIVE:** This study aimed to evaluate the effect of photodynamic therapy (PDT) on the salivary flow rate, secretory immunoglobulin A, and C-reactive protein levels in active smokers.

**PATIENTS AND METHODS:** The present study is a prospective case-control study. Twenty active smokers were allocated to two groups randomly of ten participants each: the experimental group was irradiated while the control was exposed to sham irradiation by turning off the equipment. In the experimental group, methylene blue mediated PDT was applied both intra- and extra-orally over the major and minor salivary glands using a diode laser. 780 nm wavelength and 4 J/cm<sup>2</sup> of energy were used to irradiate the 10 points of major salivary glands (6 for parotid and 2 for submandibular glands and 2 for sublingual glands). On the other hand, 660 nm was used to apply 10 J/cm<sup>2</sup> of energy over the minor salivary glands at numerous points. The samples of the stimulated and unstimulated saliva were collected from both groups to assess the SFR. ELISA method was used to assess the level of salivary IgA levels, statistical analysis was done using a One-way ANOVA, and a *p*-value of <0.05 was considered significant.

**RESULTS:** The results showed a significant increment in salivary and secretory immunoglobulin A levels of subjects undergone photodynamic therapy. C-reactive protein levels were significantly decreased in subjects exposed to irradiation.

**CONCLUSIONS:** The present study concludes that photodynamic therapy significantly improves the salivary flow rate, secretory Immunoglobulin A, and oral health quality of life in smokers. The inflammatory salivary marker C-reactive protein, which is usually raised in smokers, is also reduced.

#### Key Words:

Photodynamic therapy, Salivary flow rate, Iga, Crp, Ohip, Smokers.

## Introduction

Saliva is an alkaline, viscous translucent fluid product of the acinar cells of the salivary glands. It plays a fundamental part in the maintenance of physiological and microbiological balance within the oral cavity<sup>1</sup>. The principal role of saliva is the protection of oral mucosa by serving as a lubricating mucoid secretion. Its secondary functions include starch digestion by catabolic action of enzymes, cleansing of the oral cavity, facilitation in speech, neutralizing acidic environment owing to its buffering capacity, and antimicrobial action<sup>2</sup>.

Globally, cigarette smoking ultimately results in approximately seven million deaths annually<sup>3</sup>. Saliva is the first biological fluid that is exposed to cigarette smoke that comprises of several toxic formulations responsible for cytotoxic, mutagenic, and carcinogenic alterations<sup>4</sup>. When the harmful effect of cigarettes impacts the salivary glands, the first to be affected is the parotid gland whose role is the secretion of watery saliva. The loss of its function is compensated by submandibular and sublingual glands which secrete thick mucus saliva<sup>5</sup>. Evidence suggests that cigarette smoke obliterates protective macromolecules of saliva, enzymes, and proteins, thus compromising the protective function of saliva. Subsequently, the exposure of saliva to cigarette smoke acts as a harbinger of carcinogenesis and malignancy<sup>6</sup>.

One of the consequences of smoking is the diminished quantitative flow of saliva. The evidence indicates that smoking is one of the most influential extrinsic factors that cause a reduction in salivary secretions<sup>7</sup>. Macgregor comprehensively reported that long-term smoking significantly reduces sal-

ivary flow rate (SFR) and subsequently increases the risk of dry mouth and xerostomia<sup>8</sup>. Within the salivary secretions, secretory immunoglobulin A (sIgA) constitutes the predominant immunoglobulin isotype that acts as the initial line of defense of the host against the colonizing and invading pathogens bathed by external secretions<sup>9</sup>. Previous studies<sup>10</sup> have conclusively proven the detrimental effect of smoking on the IgA concentration of saliva that compromises its protective function. Giuca et al<sup>10</sup> conclusively established the inextricable dose-dependent relationship between smoking and decreased sIgA levels in the saliva.

C-reactive protein is an acute phase protein inflammatory marker synthesized primarily in the liver that is found in both serum and saliva. Due to the non-invasive nature of saliva, it is now a common practice to use it as a substitute for serum in order to assess the CRP levels to detect infectious or inflammatory conditions<sup>11</sup>. There is a paucity of data concerning the association of salivary CRP levels with smoking. Azar and Richard<sup>12</sup> reported significant CRP levels in active smokers compared to passive and non-smokers in 2011.

Photomodulation therapy involves controlling the molecular and cellular functions of the tissue irradiated by the laser light that produces a non-thermal healing effect. The potential therapeutic efficacy using photobiomodulation may be impacted by various properties such as wavelength, energy density, and penetration depth of the laser<sup>13-18</sup>. Photodynamic therapy (PDT) is a widely accepted treatment used to tackle a multitude of diseased conditions by inducing beneficial therapeutic changes<sup>19-26</sup>. PDT involves inducing non-thermal effects in an irradiated tissue with a low-powered laser in order to accelerate healing, relieve inflammation and pain and subsequently restore function *via* the released photon energy<sup>27</sup>. Reportedly, PDT has been employed to increase the quantity and quality of saliva in xerostomia-related conditions in a number of studies. According to the results of a study conducted by Vidović Juras et al<sup>28</sup> the application of PDT to the major salivary glands of xerostomia patients resulted in significant production levels of saliva. The study also reported an increment in the concentration of sIgA thereby, the anti-microbial potential of saliva<sup>28</sup>.

The aim of the current study was to comprehensively evaluate the effect of PDT on SFR, sIgA, and CRP levels in active smokers in order to establish PDT as a treatment modality that can holistically improve the salivary secretions both in terms of quality and quantity.

## Patients and Methods

### *Ethical Approval and Research Protocol*

The present study protocol was approved and conducted in agreement with the ethical standards of principles of the Declaration of Helsinki. All participants gave their informed consent in written form. The study protocol describing the aims and objectives of the clinical study was provided to all the recruited participants.

### *Study Groups*

The present study was a prospective case-control study. Twenty male active smokers were allocated to two groups randomly of ten participants each: the experimental group was irradiated while the control was exposed to sham irradiation by turning off the equipment.

### *Sample Selection*

All participants were healthy, with no acute or chronic illnesses. Subjects with a history of recurrent infections, atopic diseases, or any suspected immunological disorders and those who suffered from any type of xerostomia or had any oral inflammatory lesions were excluded from the study. A questionnaire was used to collect demographic data, medical history, dental health status, and duration and frequency of cigarette smoking.

### *Procedure*

In the experimental group, PDT was applied both intra- and extra-orally over the major and minor salivary glands using a soft tissue diode laser. 780 nm wavelength and 4 J/cm<sup>2</sup> of energy were used to irradiate the 10 points of major salivary glands (6 for parotid and 2 for submandibular glands and 2 for sublingual glands). On the other hand, 660 nm was used to apply 10 J/cm<sup>2</sup> of energy over the minor salivary glands at numerous points. Each patient received a total of applications, each lasting 120 seconds for 10 consecutive days.

### *Saliva Sample Collection*

The whole unstimulated and stimulated saliva were collected and quantified just before the first and after the tenth application of PDT as described in the previous protocol<sup>29</sup>. The sample was collected between 9-11 a.m., at least two hours after the last food or drink intake. Under resting conditions, while participants were sitting with their heads bent slightly forward, just after saliva swallowing, participants spat whole unstimulated saliva every 1 minute into calibrated containers (0.1 ml) for five minutes.

Later they were instructed to rinse their mouths with 50 ml of 1% ascorbic acid solution for one minute. After swallowing, participants spat whole stimulated saliva into other calibrated tubes for the next five minutes. Unstimulated and stimulated salivary flow rates were recorded for each participant and expressed in milliliters per five minutes.

### Enzyme-Linked Immunosorbent Assay (ELISA)

The samples of unstimulated whole saliva were frozen and stored at -20°C until used for sIgA determination. sIgA concentrations were measured by using a commercially available indirect competitive enzyme immunoassay kit (Salivary SIgA EIA kit, Salimetrics, State College, PA, USA). CRP in saliva was measured using Salimetrics' salivary CRP ELISA kit (State College, PA, USA) according to the manufacturer's instructions.

### Statistical Analysis

The data were incorporated into Word Excel and computed using a specialized statistical software [SPSS Version 20, (IBM Corp., Armonk, NY,

USA)]. Normality testing was initially performed using Kolmogorov-Smirnov test. All parameters were compared using the analysis of variance and Bonferroni post-hoc adjustment tests. The resulted *p*-value was set at less than 0.05.

## Results

The salivary flow rates obtained from the cases and controls before and after the procedure were reported along with the corresponding differences between the flow rates measured before and after each exposure.

According to the results, PDT irradiated group showed a quantitative increment in SFR in the cases. The quantity of unstimulated saliva gradually increased during the study, from 0.6±0.3 mL/5 min just before the first PDT, and after the 10<sup>th</sup> LLLT 0.9±0.6 mL/5 min. The quantity of stimulated saliva was 1.4±0.6 mL/5 min at the beginning and it increased after the 10<sup>th</sup> PDT to 2.3±1.0 mL/5 min. This increase in the cases was statistically significant (Figure 1).

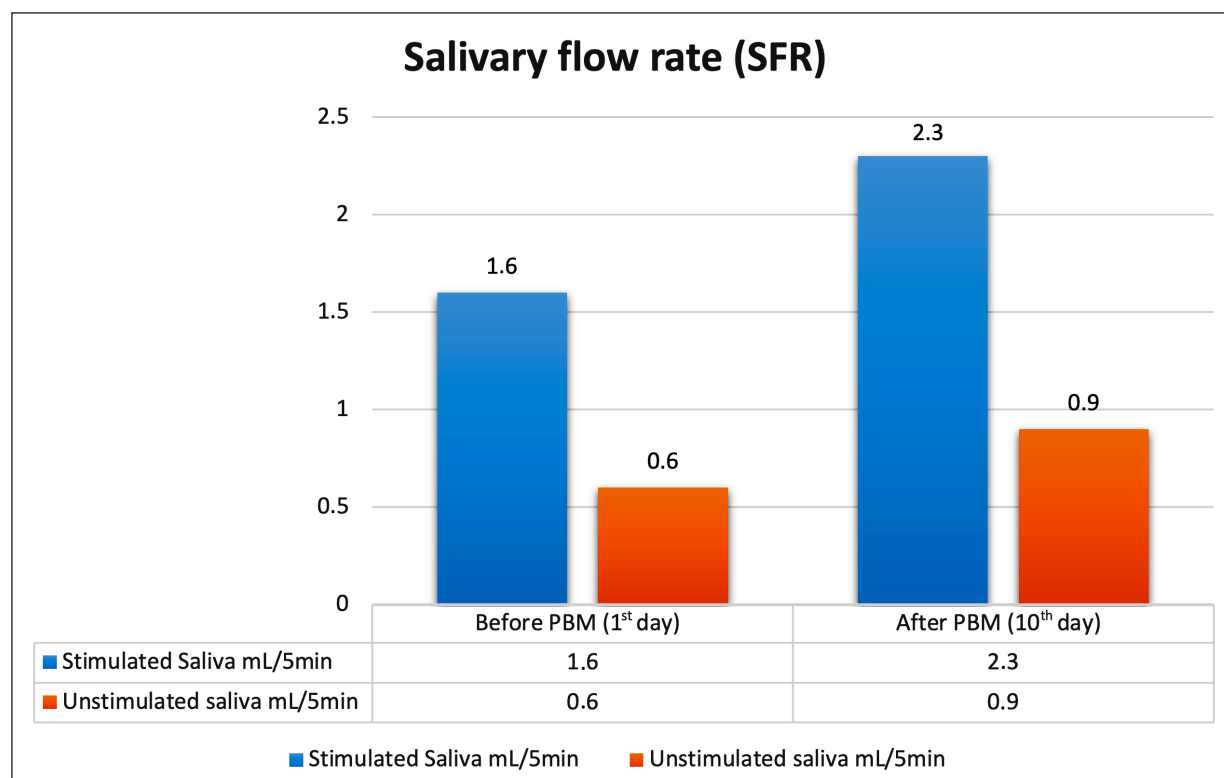


Figure 1. Salivary flow rate in cases before (1<sup>st</sup> day) and after (10<sup>th</sup> day) photodynamic therapy application.

**Table I.** Comparison between salivary IgA levels in both cases and controls before (1<sup>st</sup> day) and after (10<sup>th</sup> day) photodynamic therapy application.

IgA levels (mg/5 min)	Before PDT (1 <sup>st</sup> day)	After PDT (10 <sup>th</sup> day)	p-value
Cases	236.1±125.8	287.7±171.6	<0.05
Controls	236.1±125.8	234.1±124.6	<0.05

The quantity of sIgA in unstimulated saliva secreted in 5 min just before the PDT was 236.1±125.8 mg/5 min. Immediately after the 10<sup>th</sup> LLLT it was significantly higher (287.7±171.6 mg/5 min) in the cases (Table I).

The CRP levels showed significant decrement after PDT was applied. The CRP concentration before the start of the PDT was recorded at 118.1±17.8 in both cases and controls. After the 10<sup>th</sup> day, 81.7±17.6 of CRP levels were reported in the cases, indicating a significant decrease after PDT (Table II).

## Discussion

The present study reported significant results in SFR, sIgA, and CRP levels between cases and control groups. The case group exposed to irradiation recorded better quantities of saliva flow and protective antibodies in the saliva. A significant decrease in CRP levels was seen in cases as compared to controls after the PDT.

Both stimulated and unstimulated saliva reported significant levels of increment in the cases after the end of the 10-day period thereby, indicating an overall increase in the SFR. The results of a study by Terlević Dabić et al<sup>30</sup> showed PDT at a wavelength of 830 nm increased unstimulated salivary flow rate significantly in contrast to the stimulated salivary flow rate that did not increase significantly after the procedure in patients of drug-induced hyposalivation. Lončar et al<sup>31</sup> concluded that utilization of PDT on the salivary glands in patients with xerostomia not only raised the salivary flow but also induced regenerative effects in salivary glands additionally. The present study also incorporated the same parameters in PDT and met with successful results.

Our present study recorded significant increase in sIgA levels of the cases after 10 days of the application of PDT. Similar results were reported by Juras et al. that showed that PDT significantly increased sIgA in patients of mouth dryness after a month of the procedure<sup>28</sup>. In another study by Dostalova et al<sup>32</sup> positive association was found between concentration of salivary sIgA and lysozyme in the saliva after PDT application at 830 nm of wavelength in patients undergone third molar surgery. It can be speculated that the increase of sIgA is that PDT catalyzes the differentiation of B lymphocytes, into plasma cells that subsequently contribute to increased levels of immunoglobulin<sup>33</sup>.

The effect of PDT on the salivary CRP levels haven't been explored before. Our study shows a significant decrease in CRP levels of smokers who have undergone PDT. This specific result demonstrates that, in addition to improving the flow rate and increasing the sIgA levels, PDT also has an anti-inflammatory effect on the salivary composition. These anti-inflammatory effects are represented by lower levels of CRP after PDT, that are usually raised in the smokers.

## Conclusions

The present study concludes that PDT significantly improves the SFR and sIgA. The inflammatory salivary marker C-reactive protein, which is usually raised in smokers, was also reduced. Therefore, with substantial results in hand, we believe that PDT as a treatment modality can holistically improve the salivary secretions both in terms of quality and quantity in active smokers.

**Table II.** Comparison between salivary CRP levels in cases and controls before (1<sup>st</sup> day) and after (10<sup>th</sup> day) photodynamic therapy application.

CRP levels (mg/5 min)	Before PDT (1 <sup>st</sup> day)	After PDT (10 <sup>th</sup> day)	p-value
Cases	118.1±17.8	81.7±17.6	<0.05
Controls	118.1±11.8	119.1±12.6	<0.05

### Conflict of Interest

The authors declare that they have no conflict of interests.

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### Authors' Contributions

Conceptualization, MSH, MAK, MLM, methodology, MSH, SMK, SS, MA, formal analysis, MLM, MSH, MAK data curation, MAK, SS, MA, writing-reviewing, and editing, MSH, MAK, supervision, MSH, MAK, project administration, SMK, SS, MA. All the authors have read and agreed to the published version of the manuscript.

### Data Availability

Data are available on reasonable request.

### Informed Consent

All participants gave their informed consent in written form.

### Ethics Approval

The study was approved by the Ethical Committee of King Khalid University College of Dentistry (No. KKUCOD/ETH/2022/229).

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