

# Comparison of Salivary Peroxidase in HIV Positive Patients and Healthy Controls: A Case-Control Study

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## Abstract

**Background and Aim:** Saliva, as an easy diagnostic tool, has various defense mechanisms such as immunological, enzymatic and antioxidant defense systems. Salivary peroxidase is one of the most important salivary antioxidants. Human immunodeficiency virus (HIV) positive and acquired immunodeficiency syndrome (AIDS) patients have decreased antioxidant and increased oxidative stress levels. Therefore, the aim of this study was to compare the level of salivary peroxidase in HIV positive patients and healthy controls.

**Materials and Methods:** In this case-control study, 49 HIV positive patients and 49 healthy controls were selected as the case and control group, respectively. Demographic, clinical, and paraclinical information was recorded in a checklist. Next, 5 mL of unstimulated whole saliva was collected during 5 min in sterile Falcon tubes. Peroxidase activity of the saliva was measured by the Gelycol method. Data were analyzed by SPSS 11 via t-test and Chi-square test.

**Results:** The salivary peroxidase level was slightly higher in the case group in comparison with the healthy control group, but this difference was not statistically significant ( $P=0.157$ ). Dental status of the case group was significantly different from the control group ( $P=0.029$ ). The mean age of the case group was also significantly higher than the control group ( $P=0.005$ ). In the case group, females had a higher frequency than males.

**Conclusion:** The results showed slightly, but not significantly, higher level of peroxidase in the study group than the healthy controls. Minimal change in salivary peroxidase in HIV positive patients may be related to the early stage of their disease and good function of the immune system.

**Key Words:** Saliva, Peroxidases, HIV, Antioxidants

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Received: 22 Aug 2019

Accepted: 29 Dec 2019

➤ **Cite this article as:** Ahmadi-Motamayel F, Vaziri-Amjad S, Davoodi P, Goodarzi MT, Poorolajal J, Abdolsamadi J. Comparison of Salivary Peroxidase in HIV Positive Patients and Healthy Controls: A Case-Control Study. *J Islam Dent Assoc Iran.* 2020; 32(1-2):30-36.

## Introduction

Acquired immunodeficiency syndrome (AIDS) is a life-threatening disease caused by the human immunodeficiency virus (HIV) that weakens the immune system and progressively leads to AIDS

(1), a state in which the immune system cannot defend against the virus (2).

Antioxidants are present in all body fluids such as the saliva, and can deactivate or stabilize free radicals (3) and delay or prevent oxidation of substrates (4). Total antioxidant capacity is the total activity of individual antioxidants together (5). When the production of reactive oxygen species exceeds the physiological threshold, an imbalance occurs between the antioxidants and reactive oxygen species, which leads to subsequent oxidative stress and cellular damage (4).

Saliva preserves the oral health and has various defense mechanisms such as immunological, enzymatic and antioxidant defense systems (6,7). Recently, saliva was used as an easy diagnostic tool for detection of systemic diseases (8). It has a positive correlation with serum, and its collection is easy and noninvasive (9). Antioxidants are classified as chain-breaking antioxidants, preventative antioxidants, and enzymes that control reactive oxygen species (10,11). The salivary antioxidant system is made of various enzymes (peroxidase, catalase, superoxide dismutase, glutathione peroxidase) and small molecules (uric acid, vitamins E and C) (12). Salivary peroxidase is one of the most important salivary antioxidants (13). Peroxidase controls the production of salivary glands and has an antimicrobial effect on oral bacteria (7). It is produced by the acinar cells (14) and controls oral bacteria to decrease dental caries by inhibition of bacterial enzymes (15,16).

Studies on salivary peroxidase in smokers have shown a lower level of peroxidase in this group, and inhibition of oral peroxidase activity by smoking (3, 17-19). To the best of our knowledge and according to our literature review, case-control studies on salivary peroxidase in HIV positive patients are limited. Therefore, the aim of this study was to compare the salivary peroxidase level in HIV positive patients and healthy controls.

### Materials and Methods

In this case-control study, sample size was calculated to be 49 in each group according to a

previous study (20). Forty-nine HIV positive patients from the behavioral consultation centers of Hamadan and Kermanshah provinces were selected as the case group. HIV infection was confirmed by two ELISA tests and one western blot test. Forty-nine healthy subjects with no HIV infection or other systemic diseases were selected from dental clinics in Hamadan and Kermanshah provinces to serve as the control group. Patients with the following conditions were excluded: living in other provinces, systemic diseases, pregnancy, and patients with both hepatitis and HIV infection. All HIV positive cases were examined by infectious disease specialists and were under medical treatment, if required. The CD4 count of patients was tested periodically. All clinical and paraclinical examinations and type, dosage, and duration of intake of medications, as well as CD4 count of patients were recorded in their confidential medical records. Written informed consent was obtained from all participants. The study protocol was approved by the Hamadan University of Medical Science Ethics Committee (D/P/16/35/4744).

Oral clinical examination was carried out by a senior post-graduate student of oral medicine under the supervision of an oral medicine specialist using a dental mirror and a dental explorer in a naturally lit room without radiography. Decayed, missing, and filled teeth (DMFT index) were recorded. Demographic, clinical, and paraclinical information, and patients' medication intake were all recorded in a checklist.

The patients were requested not to eat or drink for 90 min before the saliva sampling. Next, 5 mL of unstimulated whole saliva was collected during 5 min by in sterile Falcon tubes in the morning according to the method described by Navazesh (21). The samples were immediately placed on ice, stored at 4°C, transferred to a laboratory, and were frozen at -80°C temperature.

The peroxidase activity of the saliva was measured by the Gelycol method as described by Pruitt et al (22). For this purpose, 1.0 mL of phosphate buffer (pH 7.0), 1.0 ml guaiacol solution and 1.0 mL of saliva sample were

mixed. Next, 1.0 mL of hydrogen peroxide stock solution was added, and the reaction was started. Absorbance at 470 nm (A) and time (T) were monitored. Linear regression analysis of the recorded data determined the initial rates (dA/DT). One unit of Prox was defined as the amount that yielded 1.0 dA for 1 minute. The activity of the enzyme was expressed as units per milligram of protein in the saliva. The protein content of the samples was determined using the Bradford method (22).

All data were analyzed by using Stata statistical software 11 (Stata Corp., College Station, TX, USA) at 0.05 significance level. We compared categorical variables using the Chi-square test and continuous variables using ANCOVA adjusted for age and sex.

### Results

In this study, there were 10 (20.4%) males and 39 (79.6%) females in the case group, and 26 (53.1%) males and 23 (46.9%) females in the control group ( $P=0.001$ ).

The Chi-Square test showed no significant difference between the case and control groups in cigarette smoking ( $P=0.591$ ) or substance abuse ( $P=0.204$ , Table 1).

Salivary peroxidase level was slightly higher in the case group in comparison with the healthy control group, but this difference was not statistically significant ( $P=0.157$ , Table 1). The DMFT index ( $P=0.029$ ) and age ( $P=0.005$ ) were significantly higher in the case group (Table 2).

### Discussion

In HIV infection, oxidative stress in CD4+ lymphocytes can alter lymphocyte function and induce HIV replication especially in immunodeficient patients resulting in disease progression (23). HIV positive and AIDS patients have decreased antioxidant and increased oxidative stress levels (17). Peroxidase is one of the most important salivary antioxidant enzymes and part of the salivary defense mechanism, which controls oral bacteria that form dental plaque (13). Salivary peroxidase has specific antibacterial activity by inactivating bacterial glycolytic enzymes and inhibiting hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). H<sub>2</sub>O<sub>2</sub> is

produced by the bacteria and leukocytes in the oral cavity (7). The salivary peroxidase activity decreases by smoking (24). Moreover, peroxidase has bacteriostatic and bactericidal properties as well as antiviral and antifungal activities. Peroxidase decreases the N-nitroso-compounds by oxidizing nitrite in presence of H<sub>2</sub>O<sub>2</sub> and inactivates some mutagenic and carcinogenic compounds. However, cigarette smoke inhibits the peroxidase activity of the saliva, and thus, peroxidase cannot protect against the carcinogenic effects of cigarette smoke (25).

In this study, the peroxidase level was slightly higher in HIV positive group but this difference was not statistically significant. Other studies reported a higher level of peroxidase in diabetic patients (26) and those with periodontal disease (27), and lower levels in smokers (13, 24) and asthmatic patients (28).

Oxidative stress is involved in the pathogenesis of more than 100 diseases (29,30) such as AIDS (31). It impairs lymphocyte function and CD4+ T-cell apoptosis, and induces HIV replication and disease progression (17,19,23,31).

Antioxidant level is lower in AIDS patients than healthy controls (19). Administration of antioxidants in combination with antiviral drugs increases the efficacy of medications and enhances their immunological and antiviral effects (19,23).

AIDS is a fatal infectious disease in which the HIV leads to severe immunosuppression (1,32). Antioxidants can deactivate or stabilize free radicals before they damage the cells (3). Saliva is easy to obtain and there are positive correlations between serum parameters and saliva (9).

Salivary peroxidase as an important salivary antioxidant has different subtypes (16). Level of salivary peroxidase was  $3.81 \pm 2.34$  IU/mL in the case group and  $3.17 \pm 2.09$  IU/mL in the control group in our study, with no significant difference ( $P=0.157$ ).

Although the total antioxidant capacity is higher in unstimulated whole saliva (33,34), one study showed no significant difference in salivary peroxidase in stimulated and unstimulated saliva of young healthy subjects (35). In a study

**Table 1.** Characteristics of the patients (HIV+) and controls (HIV-)

Variable		Controls		Patients		Chi-Square
		Number	Percentage	Number	Percentage	P-value
<b>Substance abuse</b>	No	41	47.13	46	52.87	0.204
	Yes	8	66.67	3	33.33	
<b>Cigarette smoking</b>	No	37	48.5	40	51.95	0.591
	Yes	12	54.55	9	45.45	

**Table 2.** Comparison of salivary peroxidase levels between HIV positive cases (n=49) and healthy controls (n=49)

Variable	Control		Case		P value†
	Mean	SD	Mean	SD	
Peroxidase (IU/mL)	3.17	2.09	3.81	2.34	0.219
DMFT	9.6	6.26	12.95	7.85	0.302

† Adjusted for age and sex using ANCOVA; SD: Standard deviation

by Lemos et al, (36) the mean value of total salivary peroxidase function was 238.9  $\mu\text{m}/\text{mL}$  in resting condition and 175.3  $\mu\text{m}/\text{mL}$  in stimulated saliva with no significant difference. Peroxidase is present in body fluids and saliva. Peroxidase is mainly secreted by the parotid glands and is identified in the whole saliva. Salivary peroxidase has the tendency to adhere and concentrate on the dental plaque and has better function in this area (37). Salivary peroxidase is produced by acinar cells (38). Inhibition of bacteria and prevention of dental caries are among the most important functions of salivary peroxidase in dental plaque (39). In studies by Abdolsamadi et al, (3), Kanehira et al, (18) and Garg et al, (40) the mean level of salivary peroxidase was significantly lower in the smoking group. One study suggested that smoking had no additive effect on oxidative stress, as no differences were found in oxidative stress and antioxidant capacity between clinically stable smoking and nonsmoking males with HIV/AIDS (17). Saliva peroxidase level accounts for 0.01% of the total salivary proteins (41).

There is no similar study on HIV patients in the literature to compare our results with. Absence

of a significant difference in salivary peroxidase levels between HIV positive patients and HIV negative controls in our study may be due to the fact that most patients had high CD4 counts (389.9 cells/ $\mu\text{L}$ ) indicating that they were in early stages of the disease and the function of the immune system had not declined significantly, and they had not entered the AIDS stage.

Short-term, high-dose treatment with N-acetylcysteine and vitamin C leads to immunological and virological effects and has therapeutic value in HIV positive patients and those with advanced immunodeficiency (23).

The antioxidant system is related to many factors including antioxidant potency and level, the amount of production of free radicals, genetics, diet, smoking status, physical activity, hormones, stress, age, menstrual cycles, and pregnancy (25,42). Thus, future studies are required with consideration of these factors in salivary peroxidase assay. Other studies on patients in a different stage of HIV progression and immunosuppression as well as AIDS patients are required to assess the changes in salivary peroxidase level in these patients.

## Conclusion

The results showed that the salivary peroxidase in the study group was slightly higher than that in the control group but this difference was not significant. Minimal change in salivary peroxidase of HIV positive patients can be related to early stage of the disease and their good immune system.

## Acknowledgments

This study was part of a thesis submitted to the Oral Medicine Department. We would like to appreciate the consultation centers of Hamadan and Kermanshah provinces, Vice-chancellor of Education, and Vice-chancellor of Research and Technology of Hamadan University of Medical Sciences.

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