# The evaluation of lysozyme concentration and peroxidase activity in non-stimulated saliva of patients infected with HIV

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## Abstract

**Purpose:** The aim of the study was the comparison of lysozyme concentration and peroxidase activity in mixed, non-stimulated saliva of HIV-positive patients and healthy subjects.

**Material and methods:** The study was carried out in the group of 37 patients infected with HIV. The control group comprised of non-infected individuals, counterpart of the examined group. Mixed non-stimulated saliva, collected using expectoration method in the amount of 3-5 ml 2 hours after meal, was used for the study. Saliva samples were centrifuged, divided into portions 200 μl each, and stored at -80°C. Peroxidase activity was determined using the method by Mansson-Rahemtull et al. [14]. Lysozyme concentrations were determined with the use of radial immunodiffusion method, ready-made kits (Human NL Nanorid plate – The Binding Site Ltd., UK).

**Results:** Higher concentrations of lysozyme as well as peroxidase activity were observed in the group of patients with HIV as compared to the control group, and they were 35.08 μg/ml, 46.74 IU/l, 21.3 μg/ml, 37.73 IU/l, respectively. The difference was statistically significant only in case of peroxidase activity.

**Conclusions:**

1. HIV infection triggers immune mechanisms, that are manifested by the increase in salivary enzymes responsible for local non-specific resistance.
2. The immunological resistance decrease, manifested by the drop of the absolute number of CD4 lymphocytes T, is compensated by the increase in lysozyme concentration and peroxidase activity in non-stimulated saliva of HIV-positive patients.

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## Introduction

HIV attacks cells with an appropriate receptor on their cellular membrane surface. A molecule CD4 is the best known HIV-receptor.

The absolute number of CD4 lymphocytes T is an essential laboratory parameter to evaluate both the course of the infection and the efficiency of the treatment [1]. The number of CD4 lymphocytes T reflects the progress of the disease and shows the gradual dysfunction of the immune system in the course of HIV-infection [2,3].

The immune system of the oral cavity is a part of the systemic immune system. The interaction processes between non-specific (lysozyme, lactoferrin, and peroxidase) and specific elements of the immune system (A, G, and M immunoglobulins) that facilitate to create and maintain the homeostasis take part in the oral cavity. The development of opportunistic infections is caused by HIV disturbing the balance of the immune system [4,5]. Unlike specific factors, non-specific ones act without previous exposure to antigens. The saliva contains various specific and non-specific immunological components that are antibacterial, antiviral, and antimycotic/antifungal in their activities. Lysozyme and peroxidase are the most important non-immunological defense factors of the saliva.

Lysozyme is a glycoside hydrolase of bacteriolytic activity. Its source are the basal cells, mainly in salivary/sialaden leading out ducts, gingival crevice/sulcus fluid, and leukocytes. Lysozyme present in the fluid is assumed to be the blood serum filtrate [6]. The saliva from the parotid glands contains slight amounts of lysozyme while the sublingual glands secrete relatively more of it. It is produced mainly by the submandibular and sublingual salivary glands and shows antiviral and antimycotic/antifungal properties [7,8]. Lysozyme destroys bacterial cellular membranes by means of hydrolysis of 1,4-β glycoside between N-acetylmuramine acid and N-acetyl-D-glucosamine in pepti-

*Table 1*. **Lysozyme concentrations in mixed non-stimulated saliva depending on CD4 lymphocyte T number**

| CD4/μl lymphocyte number | Number of examined subjects | Lysozyme concentration (μg/ml) | Standard Deviation ±SD |
|---|---|---|---|
| CD4<200/μl (1) | 8 | 49.27 | 43.34 |
| CD4 200-499/μl (2) | 19 | 27.85 | 23.3 |
| CD4>500/μl (3) | 10 | 37.48 | 35.38 |
| Total(4) | 37 | 35.08 | 31.99 |
| Control group (0) | 37 | 21.3 | 21.88 |
| Statistical analysis (p<0.05) | | NS | |

*Table 2*. **Peroxidase activity in mixed non-stimulated saliva depending on CD4 lymphocyte T number**

| CD4/μl lymphocyte number | Number of examined subjects | Peroxidase activity (IU/l) | Standard Deviation ±SD |
|---|---|---|---|
| CD4<200/μl (1) | 8 | 54.37 | 2.71 |
| CD4 200-499/μl (2) | 19 | 46.84 | 12.07 |
| CD4>500/μl (3) | 10 | 40.45 | 10.13 |
| Total (4) | 37 | 46.74 | 11.13 |
| Control group (0) | 37 | 37.73 | 11.5 |
| Statistical analysis (p<0.05) | | 4v0, p<0.001 | |

dyloglycans [9]. It co-operates with other antibacterial systems (e.g. IgA) and causes bacteria aggregation [10]. The activity of lysozyme is intensified by proteases present in the saliva and the acid environment of the oral cavity modulated by monovalent anions, such as: bicarbonates, fluorides, chlorides, and thiocyanates. Lysozyme limits glucose assimilation by bacterial cells, which leads to lowered metabolism, adhesion, and aggregation [11]. Another property of lisozyme is the ability to bind with microorganism nucleic acids, which is essential as far as the homeostasis of the oral cavity is concerned [12].

Peroxidase is secreted by acinous cells of the salivary glands and it is thought to have the most important role in the oral cavity healthy condition. However, myeloperoxidase, produced by leukocytes, has a greater impact in inflammatory conditions with the presence of dental plaque. Peroxidase, together with naturally occurring thiocyanates (SCN-) and hydrogen peroxide ($H_2O_2$) in the saliva, is a so-called system of salivary peroxidase [13]. It catalyzes the oxidation of salivary thiocyanates by hydrogen peroxide, whose source are oral microorganisms as well as leukocytes and salivary glands. Hypothiocyanic acid (HOSCN) at the low pH and hypothiocyanic anion (OSCN-) at the neutral reaction are the end products of the reaction. It also oxidizes bromides and iodides to hypobromides and hypoiodides [10]. Additionally, myeloperoxidase catalyzes Cl-oxidation. Both enzymes catalyze oxidation of organic compounds, especially phenols. The reaction products react to a wide spectrum of bacteria, viruses, and fungi that occur in the oral cavity. They inhibit the growth, glucose uptake and the production of acids by *Streptococcus* mutans. Peroxidase prevents $H_2O_2$ accumulation and potential cytotoxic level using the compound produced in oxidation in the oral cavity by numerous streptoccocal strains and in host cells. On the other hand, organic compound oxidation prevents their mutagenic action.

## Material and methods

The study was carried out in the group of 37 patients (11 women and 26 men, aged 19-65, mean age 32 years) infected with HIV, hospitalized in the Teaching Hospital of Infectious Diseases, the Medical University of Białystok. Patients were divided into 3 groups, according to immune disturbances, and a division criterion was laboratory tests concerning the number of CD4 helper lymphocytes in peripheral blood. The control group comprised of non-infected individuals, counterpart of the examined group. The patients were informed about the aim of the study and they gave their consent. The study was carried out after the approval of the Bioethic Committee of the Medical University of Białystok.

Mixed non-stimulated saliva, collected using expectoration method in the amount of 3-5 ml 2 hours after meal, was used for the study. Saliva samples were centrifuged, divided into portions 200 μl each, and stored at -80°C.

Peroxidase activity was determined using the method by Mansson-Rahemtull et al. [14]. Lysozyme concentrations were determined with the use of radial immunodiffusion method, ready-made kits (Human NL Nanorid plate – The Binding Site Ltd., UK).

The statistical analysis concerning the differences of examined parameters was performed using the analysis of variances (for the variables of normal distribution) or Kruskal-Wallis test (for other variables). In case of significant difference between groups, post hoc analyses were conducted to compare all pairs of groups using t Student test with Bonferroni alteration/amendment (for variables of normal distribution) or Dwass-Steele-Critchler-Flieger test (for other variables).

## Results

Mean lysozyme concentrations in the saliva of patients with HIV were higher than in controls, however, they did not reach the level of statistical significance. All groups showed a wide distribution of results, which is confirmed by standard deviation of mean lysozyme concentrations (*Tab. 1*). The lowest mean lysozyme concentration was determined in the group of patients with the absolute number of CD4 lymphocytes T at the level of 200-499/μl. The deterioration of immunological condition of the examined group, manifested by CD4 amount<200/μl, was accompanied by the increase in lysozyme concentrations (*Tab. 1*).

*Tab. 2* presents the mean value of peroxidase activity in examined groups. HIV+ patients showed markedly higher, confirmed statistically, activity of the examined enzyme as compared to the control group. The immunological condition of particular patients did not affect peroxidase activity in the significant manner but a gradual elevation of its activity was observed together with CD4 lymphocyte T absolute number drop.

## Discussion

HIV infection is responsible for the immune system impairment, which leads to the progressive CD4 lymphocyte T damage and, as a result, the development of AIDS. Frequent and persistent opportunistic infections in the oral cavity are its consequence. Both specific and non-specific immune mechanisms participate in the defense against pathogenic influence of microorganisms. The non-specific system of secretory resistance of the saliva has its functions based on proteins, which have antiviral, antibacterial, and antimycotic/antifungal properties (lactoferrin, lysozyme, peroxidase, SLPI, cystatine, histatine, defensine, staterine, proline-rich proteins) [15-17].

Our study revealed that lysozyme concentrations in the saliva of HIV+ patients were non-significantly higher in comparison with the saliva of healthy subjects, which is in accordance with other authors' reports [18,19]. On the other hand, studies by Tsang and Samaranayake showed statistically significant higher concentrations of the enzyme in mixed saliva of infected patients as compared to the controls. Lysozyme concentrations varied depending on the immunological status of examined patients and in patients with HIV (absolute CD4 number below 200/μl) was twice higher than in the controls. According to Müller et al. [18], high levels of lysozyme in the saliva of patients with HIV is a result of increased secretion by parotid gland and not the passive transport of the enzyme from blood. It is confirmed by the increase in lysozyme concentration in a salivary gland, in which stimulated local synthesis is a response to cytokines generated by lymphocytes [20].

Peroxidase is another enzyme responsible for maintaining the balance of oral cavity ecosystem. Our results point to the higher peroxidase activity in non-stimulated saliva of HIV+ patients in comparison with non-infected individuals. In the group of infected patients, a negative correlation between peroxidase activity and the immunological status of the examined group was observed. The results of our study are in accordance with the studies by Vučićević-Boras et al. [20], who showed the significantly elevated levels of peroxidase in patients with AIDS with the comparison to healthy subjects and suggested that is a sign of increased antibacterial saliva activity.

Our study presented higher levels of lysozyme contents and higher peroxidase activity in the saliva of patients with HIV as compared to the healthy group. The parameters were increased together with the lowering of the immunological status, expressed with the absolute number of CD4 lymphocytes. Thus, it confirmed the observations of Laibe et al. [5] that there is a relationship between non-specific and specific immune resistance.

## Conclusions

1. HIV infection triggers immune mechanisms, that are manifested by the increase in salivary enzymes responsible for local non-specific resistance.
2. The immunological resistance decrease, manifested by the drop of the absolute number of CD4 lymphocytes T, is compensated by the increase in lysozyme concentration and peroxidase activity in non-stimulated saliva of HIV-positive patients.

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