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RELATIONSHIP OF SALIVARY PEROXIDASE ACTIVITY TO CD4+ LEVEL IN HIV/AIDS PATIENTS

(HUBUNGAN AKTIVITAS PEROKSIDASE SALIVA TERHADAP TINGKAT CD4 PASIEN HIV/AIDS)

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Abstract

Human whole saliva contains peroxidases (human peroxidase and myeloperoxidase) which are part of the innate host defence mechanism in oral cavity. The primary function is to catalyse the oxidation of thyocyanate, in the presence of hydrogen peroxidase, that resulting in the end product of wide antimicrobial activity. Patient with HIV/AIDS is often decreased in salivary flow rate, and this condition may also change the salivary composisition. These changes are contributed in oral lesions of HIV/AIDS patients. The aim of this study was to evaluate the salivary peroxidase activities of HIV/AIDS patients. The study involved 98 patients with HIV/AIDS of whom 55 were men and 43 were women. Unstimulated whole saliva was collected and all the patients received a complete oral examination. The saliva samples were analysed with Bradford assay (with Bradford reagent) and salivary peroxidase measurement with microplate reader and read at 450 nm wave length. The results showed that the mean concentration of total salivary protein and peroxidase in this study population was 1169µg/ml (SD±186.1) and 6.101unit/mg Protein (SD±1.26) respectively. It showed a tendency that the lower the CD4 level, the higher the total protein concentration as well as salivary peroxidase activity, however the difference was not statistically significant (p>0.05). Patients' data on age, sex, salivary flow rate and pH did not significantly influencing the total salivary protein concentration as well as the peroxidase activity (p>0.05). This study showed that salivary peroxidase activity have a correlation with the total salivary protein concentration. There was a tendency of higher total protein concentration and salivary peroxidase activity found in patients with lower CD4 counts but in those with more acidic saliva. In conclusion, salivary peroxidase activity have a correlation with the total salivary protein concentration in HIV/AIDS patients.

Key words: salivary peroxidase activity, HIV/AIDS

INTRODUCTION

HIV/AIDS has become serious global health problems. The prevalence and incidence of HIV/AIDS patients were increased all the time. [1-3] Indonesia is one of the fastest epidemic growth in the world.[4] Oral health is an essensial factor that has correlation with systemic health, including in patient with HIV/AIDS infection. Good nutritions are needed to maintain the stability or increasing the immune conditions, thus proper oral health is a necessity. The oral manifestations of HIV/AIDS patients are also the important issues that must be overcome to improve the quality of life of patients. Based on the research, 70-90 % HIV/AIDS patients, had at least one oral manifestation during their course of life. [5, 6]

Bacteria, is one of the etiologies of oral opportunistic infection in HIV/AIDS patients. The changes of saliva function and composition in HIV/AIDS infected patients are contributing factors to the oral opportunistic infection. [7]

Saliva is an essential oral fluid that is taken for granted to maintain oral homeostasis. [8-10] Various salivary enzymes have antibacterial and antifungal function. The enzymes were innate immune system in oral cavity. Salivary peroxidase is one of the enzymes that has antibacterial function. [9, 11] The aims of this research were to evaluate and analyse the relationship between salivary lactoperoxidase activety versus CD4+ count in HIV/AIDS patients,

and their correlations with contributing factors.

MATERIALS AND METHODS

The cross sectional study was composed of 98 HIV/AIDS patients; 55 men and 43 women, in accordance with inclusion criteria, who came to POKDISUS AIDS RSCM from January-February 2011 (consecutive study). Diagnosis of HIV/AIDS was determined by the internist in RSCM. Only patients without antifungal therapy were included in this study. All participants provided informed consent to the research protocol that had been reviewed and approved by the Ethic Committee of Faculty of Dentistry Universitas Indonesia. The participants age range from 20 to over 50 years. Patient records were used to obtain CD4+ count. Unstimulated whole saliva was collected and all the patients received a complete oral examination.

Unstimulated whole saliva samples were collected from each subject, according to spitting method. Saliva collection time was 9.00-13.00. Participants were asked to fast for 1 h before the test. Participants were asked to lean the head forward, kept the eyes open, minimized the movement of the tongue and the lips. Saliva was collected for 5 minutes (spitting every minute in 5 minutes) into the tube through the tunnel. Salivary flow rate and pH were analyzed, using universal indicator, and immediately stored in portable freezer. In the laboratory, this saliva was centrifuged with 1000 rpm, 4^0C in 5 minutes. The supernatant was frozen and stored in a -80^0C until protein and enzyme lactoperoxidase analized. The saliva samples were analysed with Bradford assay (with Bradford reagent: Super-Bradford Protein Assay Kit CWBIO version: 03092009; technical manual no: M0013), and read the absorbance by microplate reader at 640nm wave length. Supernatant was taken 50 µl, added with guaiacol 50 µl, buffer fosfat 100 µl, and H_2O_2 2 µl, mixed in microwell plate. Salivary peroxidase absorbance was read with microplate reader at 450 nm wave length.

Statistical analysis using GraphPad Prism 5 software to compare the results with other parameter such as age, sex, smoking habits, CD4 counts, oral hygiene status, salivary flow rate, pH were performed using Student *t*-test and One-way ANOVA continued with Newman-Keuls Multiple Comparison Test to observe correlation between parameters tested with *P* value <0.05 for result set for the results to be statistically significant.

RESULTS

The study involved 104 patients with HIV/AIDS of whom 57 were men and 47 were women. Tabel 1 described the frequency characteristic distribution in HIV/AIDS patients based on gender, age, risk factors of transmission, and CD4+ counts. This demographic data was obtained from anamnesis and confirmed the patient's medical record books, which was then recorded on research examination sheet. The CD4+count was based on the last conducted and record in the medical record books.

Tabel 1. Characteristic of HIV/AIDS sample based on gender, age, risk factor of transmission and CD4+ counts (N=98)

Characteristic of sampel	N	%
Gender		
Men	55	56
Women	43	44
Age(year)		
20-30	25	25.5
31-40	65	66.3
41-50	4	4.1
>50	4	4.1
Risk factors of tranmission		
IVDU	22	22.4
Homosexual	3	3.06
Heterosexual	60	61.2
IVDU and homoxual	0	0
IVDU heterosexual	7	
Others	6	
CD4+ (sel/ µl)count		
<200	22	22.4
201-500	59	60.2
>500	17	17.4
Salivary flow rate		
< 0,19	28	28.6
>0,2	70	71.4
pH Saliva		
Highly acidic	12	12.3
Moderate acidic	46	46.9
Healthy saliva	40	40.8

Based on gender, appears the tendency of lactoperoxidase activity in 43(43.88%) female (6.23 unit/mg protein) slightly higher compared with 55 (56.12%) men (5.99 unit/mg protein), but this was not statistically significant (Tabel 2).

Table 2. Relationship between gender and mean salivary protein concentration & lactoperoxidase

Gender	Mean salivary Protein concentration (µg/ml)	Mean salivary lactoperoxidase	p
Men	1168.34±185.25	5.99±1.25	> 0,05
Women	1169.80±189.41	6.23±1.28	

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By age, the concentration of salivary concentration and lactoperoxidase activity also was not significantly different (Tabel 3).

Table 3. Mean salivary protein concentration based on age

Age (year)	Protein Concentration (ug/ml)	SD	N	p
20-30	1134.951	181.42	25	> 0,005
31-40	1189.56	190.04	65	
41-50	1086.81	174.52	4	
>51	1062.50	38.01	4	

CD4+ count indicates the immunosupression level of HIV/AIDS patients. The salivary protein concentration tended to be lower than the salivary concentration in patients with lower CD4+ count. This trend was not statistically significant. The clinical examination of HIV/AIDS patients showed the oral hygiene were not good, with focus infections(radices, deep caries, periodontal disease) and smoking habit were also noted. This indicated the existence of the oral mucosa defence system increases with the existence of both physical and chemical exposure from cigarette. Kanehira T stated that the level of salivary thiocyanat (SCN^-) in smokers about twice more than in non smokers.

Table 4. Salivary protein concentration based on CD4+ count classification (ug/ml)

CD4+ count classifycations (cell/µl)	Salivary protein (ug/ml)		N
	Mean	SD	
CD4 (<200)	1191.829	218.50	22
CD4 (201-500)	1178.383	174.63	59
CD4 (>500)	1106.793	177.88	17

The mean of salivary peroxidase activity compared with CD4+count showed the declining trend of peroxidase activity in samples with CD4+ >500 cell/µl. Samples with CD4+<200cell/ µl had a higher tendency of peroxidase activity. There was no significant relationship between CD4+ level with peroxidase activity.

Table 5. Mean salivary peroxidase activity based on CD4+ count classification(cell/ µl)

CD4+countclassification (cell/ µl)	Salivary peroxidase		N
	Mean	SD	
CD4+ (<200)	6.22	1.32	22
CD4+ (201-500)	6.15	1.37	59
CD4+ (>500)	5.77	0.70	17

Salivary protein concentration was higher in salivary flow <0.2 ml/minute, with an average was 1219.09 ug/ml. Compared with Salivary flow rate >0.2ml/minute (70 samples), the average was 1148.78 ug/ml. However this difference was not statistically significant. The relationship between salivary flow rate and peroxidase activity, described the tendency of increasing peroxidase activity in salivary flow rate <0.2 ml/minute, this difference was not statistically significant (Table 6).

Table 6. Comparition between salivary flow rate and peroxidase activity

Salivary flow rate (ml/minute)	Salivary Peroxidase activity (unit/mg protein)	SD	N
<0.2ml/menit	6.13	1.46	28
>0.2ml/menit	6.09	1.19	70

Salivary pH was measured in this research. There are (12.3%) salivary samples with highly acidic, (46.9%), sample moderate acid saliva and remaining (40.8%) health salivary sample. In comparison between acidity saliva and salivary protein concentration showed there was increasing salivary protein concentration in acidic saliva compared with healthy saliva, however this trend was not statistically significant.

Tabel 7. Relationship between salivary pH and protein concentration

Salivary pH classification	Mean Salivary Protein Concentration	SD	N
High acid	1171.13	178.3	12
Moderate acid	1170.48	189.15	46
Healthty saliva	1166.62	189.15	40

When compared pH saliva in HIV/AIDS patients with salivary peroxidase activity, acidic salivary sample had higher salivary peroxidase activity than normal salivary sample. This trend was not statistically significant.

Table 8. Relationship between salivary pH with peroxidase activity

Salivary pH classification	Mean Salivary Protein Concentration (unit/mg protein)	SD	N
High acid	6.09	1.92	12
Moderate acid	6.16	1.26	46
Healthy acid	6.03	1.04	40

DISCUSSION

Salivary peroxidase system (SPS) is a non specific immune system in saliva, consisting of peroxidase and thiocyanat ion (SCN^-) and hydrogen peroxide (H_2O_2). Peroxidase and thiocyanat are normal component contained in saliva, while hydrogen peroxidase derived from micro organism in oral cavity. [12] Oral bacteria produced H_2O_2 during anaerobic glycolysis. At sufficient concentrations, H_2O_2 is cytotoxic to mammalian cell lines, including human epithelial cells and gingival fibroblasts. It has been previously suggested that one of the important roles of human peroxidases is to detoxify H_2O_2, to prevent oral mucosa damage. [13] SPS has effect that can inhibit the growth of micro organism. [14] Peroxidase is an oxidation catalyst reaction of thiocyanat ion with hydrogen peroxidase which would be hypothiocyanat acid (HOSCN) or hypothiocyanat anion ($OSCN^-$), which is antibacterial. [9]

$$SCN^- + H_2O_2 \xrightarrow{\text{peroxidase}} OSCN^- + H_2O$$

The H_2O_2 was produced by microorganisms was used in the oxidation of SCN^- to produced $OSCN^-$ (antibacterial agent) which in turn reduced the amount of micro organism. The decreasing in the number of microorganisms decreased the production of H_2O_2.. [9] It is predicted that in HIV/AIDS patients living with good oral hygiene, the lower Salivary peroxidase activity.

In this study, patients with CD4> 500 cell/ µl have lower salivary peroxidase activity than patients with CD4+ < 500 sel/ µl, but that is only the trend of the research. The result showed that there was no significant correlation between salivary peroxidase activity and the CD4+count in HIV/AIDS patients. Based on the result of the clinical examination, patients with CD4+> 500 cell/ µl, have better oral hygiene than patients with low CD4+ count. Based on level of education, patients with CD4+>500cell/µl have a higher level of education and better job than patients with lower CD4+ count. Thus, HIV/AIDS patients with CD4+>500cell/ µl estimated to have awareness to maintain better oral health.

Linetal stated in their study, salivary flow rate was decreased in early stages of HIV infections. Salivary composition was altered as well. [8,10,15] In our study, patients with salivary flow rate >0.2 ml/minute are more than patients with Salivary flow rate <0.2 ml/minute. This is probably because the number of samples is much less, and no data when patients were detected with HIV/AIDS. In our study, the relationship between salivary flow rate and peroxidase activity, described the tendency of increasing peroxidase activity in salivary flow rate <0.2 ml/minute.[8] In hypofunction of salivary gland, which is salivary flow rate is less than normal, it is allowing the occurance of opportunistic infections is greater than normal salivary flow rate (>0,2ml/-minute). The growth of micro organism increased the H_2O_2 level, and as one of the most important functions of Salivary peroxidase is the control of oral micro organisms thus it is predicted, in hyposalivation, the peroxidase activity increased. In this result study, the salivary peroxidase activity was trend to be increased in lower salivary flow rate. It can be concluded that salivary peroxidase activity might have a correlation with the total salivary protein concetration. There was tendency of higher total protein concentration and salivary peroxidase activity found in patients with lower CD4 counts but in those with more acidic saliva.

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