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Salivary alkaline phosphatase and acid phosphatase levels in gingivitis diagnosis and treatment

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Abstract

Background and objective: Gingivitis is the presence of clinical signs of inflammation in gingiva and is associated with teeth showing no attachment loss. Human saliva contains informative components that can be used as diagnostic markers for human diseases. This study is directed to evaluate salivary phosphatase enzymes levels in diagnosis and follow up the treatment of gingivitis.

Methods: Saliva samples were collected from 100 healthy persons, and 50 patients before treatment (scaling and polishing) and 20 days after the treatment. Their age ranged between 20-30 years. Alkaline phosphatase and acid phosphatase activities, some kinetics and thermodynamic parameters were measured.

Results: Salivary alkaline phosphatase and acid phosphatase activities elevated in gingivitis, while their levels returned to the control values after treatment. The Michaelis constant (Km) values for salivary alkaline phosphatase and acid phosphatase decreased in gingivitis, while the maximum velocity (Vmax) values increased. These values returned back to normal values after treatment. The changes in the thermodynamic parameters (ΔH^*, ΔG^* and ΔS^*) values of transition state for salivary alkaline phosphatase were non-significant, while their changes were significant for salivary acid phosphatase, except for ΔG^* value.

Conclusion: Salivary alkaline phosphatase and acid phosphatase levels can be relevant for diagnosis and the follow-up of gingivitis treatment.

Keywords: Gingivitis, Salivary enzymes, Kinetic and thermodynamic parameters.

Introduction

Plaque related gingivitis is a mild, reversible form of gum disease. In gingivitis, there is inflammation of the gum tissue which surrounds the teeth.[1] Thus, gingivitis is an inflammation of the gums characterized by reddened and puffy gums.[2] If it is left untreated, gingivitis can progress to a serious condition called periodontitis which is inflammation of the supportable tissue and bone.[2,3] Gingivitis is due to the long-term effects of plaque deposits. Plaque is a sticky material made of bacteria, mucus, and food debris that develops everywhere in the mouth. If plaque is not removed, it turns into a hard deposit called tartar. Plaque and tartar irritate and inflame the gum. Bacteria and the toxins they produce cause gingivitis, swollen, and tender.[4,5] Some risk factors for developing gingivitis are general illness, poor dental hygiene, pregnancy due to hormonal changes which increase the sensitivity of the gum and uncontrolled diabetes. Other factors like misaligned teeth, rough edges of fillings, and ill-fitting or unclean mouth appliances such as braces, dentures, bridges, and crowns can irritate the gum and increase the risk of gingivitis.[6-9] Clinical findings of gingivitis are gingival bleeding on probing, color changes in the gingiva and changes in gingival consistency.[10] Human saliva contains informative components that can be used as diagnostic markers for human diseases.[11,12] There are various enzymes in saliva. Saliva contains enzymes that begin the process of digestion. It aids our sense of taste, and it helps cleanse and protect the teeth, gums, and other tissues inside the mouth.[13] In case of

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periodontal infection, enzyme families will be released from stromal, epithelial, inflammatory or bacterial cells. The intracellular enzymes are increasingly released from the damaged cells of periodontal tissues into the gingival crevicular fluid and saliva. These relevant enzymes are: Aspartate and Alanin aminotransferases (AST and ALT), Lactate dehydrogenase (LDH), Gamma glutamyl transferase (GGT), Creatine kinase (CK), Alkaline phosphatase (ALP) and Acid phosphatase (ACP).[14] This work is designed to evaluate the influence of gingivitis on salivary ALP and ACP activity and to assess the salivary levels of these enzymes as indicators for diagnosis of gingivitis and to follow up its the treatment (scaling and polishing).

Methods

The work was performed in the period between February and August, 2008 in Hawler Medical University-College of Dentistry. The biochemical work was performed in the Department of Basic Sciences, while the oral examination for the subjects and the treatment (scaling and polishing) for the patients were performed in Department of Periodontology. The patients were diagnosed by specialized dentist according to gingivitis criteria including the usual clinical findings such as swelling of gingiva, bleeding on probing, bleeding on brushing and discoloration of gingiva.[6] The gingival index (GI) described by Loe and Silness (1963) was used for the diagnosis and assessment of gingival health condition,[15] while the scores and criteria for plaque index (PI) described by Silness and Loe (1964) were used[16]. Cigarette smokers**,** patient with systemic diseases and patient receiving medications were excluded. Saliva samples were collected from 100 healthy persons, and 50 patients with gingivitis before treatment and 20 days after the treatment (scaling and polishing only). Their age ranged between 20-30 years. The study design included the following steps:

1. Estimation of the salivary ALP and ACP levels (using kits and kits protocol of bioMerieux, Marcy-l'Etoile. France, and Randox Laboratories Ltd., U.K, respectively) in controls and patients with gingivitis before treatment (BT) and after treatment (AT).[17-19]
2. Studying some kinetic parameters of salivary phosphatases enzymes in gingivitis such as, Michaelis constant (Km), maximum velocity (Vmax) using Lineweaver-Burk plots (plots of 1/velocity (v) versus 1/ substrate concentration [*S*], the straight line has a slope of K_m/V_{max} and an intercept of $1/V_{max}$).[20,21]
3. Determination of the thermodynamic parameters for the transition state of the enzyme-substrate complex in gingivitis such as: activation energy (Ea*), enthalpy change (∆H*), free energy change and entropy change and comparing the values with those of controls to evaluate any change in the pathway of the enzyme-substrate complex formation. These parameters were calculated from the following equations:[21-23]

∆H* of transition state of enzyme substrate complexes were calculated from the following equation:

$$\Delta H^* = Ea^* - RT$$

While ∆G* values were found by using the following equation:

$$\Delta G^* = -RT \ln V_{max} + RT \ln (k^- T/h)$$

k^- & *h* are Boltzman and Plank's constant respectively

∆S* values were found from the following equation:

$$\Delta S^* = (\Delta H^* - \Delta G^*) / T$$

Statistical analysis

The statistical analysis of this study was performed using statistical package for social sciences (SPSS, version 16.0) . The statistical analysis tests that have been applied in this study were ANOVA, Chi-square test and Eta test.

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Results

The results showed that salivary ALP and ACP activities were higher in patients with gingivitis before treatment comparing with controls, while their levels decreased towards the control values 20 days after treatment (Table 1). The K_m and V_{max} values for the salivary ALP and ACP enzymes were estimated using Lineweaver-Burk plots (Figure 1 and 2). The results showed that the Km values for both salivary ALP and ACP decreased in gingivitis comparing with controls, while they returned to control values, after the treatment (Table 2). The Vmax values for both salivary ALP and ACP increased in gingivitis, but the values decreased again after treatment and they became close to that of control (Table 2).

Table 1: Salivary phosphatases enzymes levels in control (healthy) groups and gingivitis cases (BT) and (AT).

Enzyme parameter	Groups	Range IU/L	Mean IU/L	S.D.	p value
ALP	Control	0.66-15.36	5.41	±3.05	<0.001
	Gingivitis (BT)	1.75-38.41	10.59	±8.27	
	Gingivitis (AT)	1.10-18.00	5.49	±4.07	
ACP	Control	0.30-6.46	2.35	±1.51	<0.001
	Gingivitis (BT)	1.01-32.52	10.35	±8.60	
	Gingivitis (AT)	0.20-4.44	1.42	±1.12	

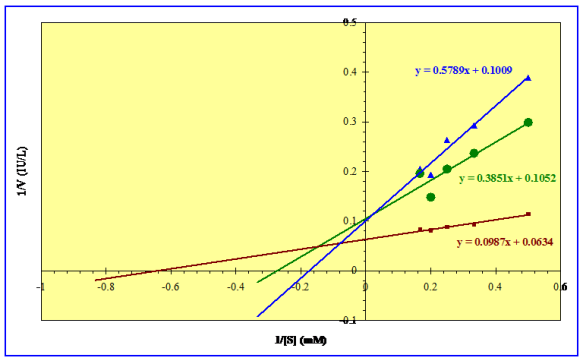

Figure 1: Lineweaver-Burk plots for salivary ALP enzyme in control (●), gingivitis before treatment (■) and gingivitis after treatment (▲).

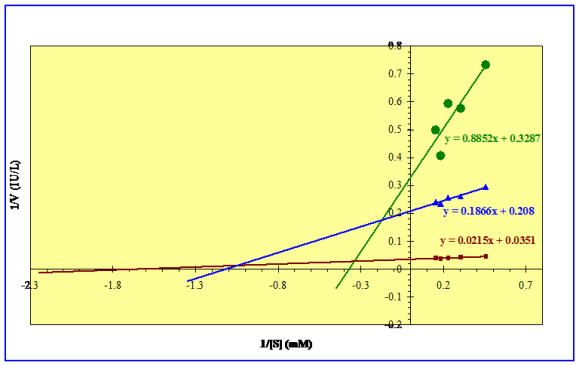

Figure 2: Lineweaver-Burk plots for salivary ACP enzyme in control (●), gingivitis before treatment (■) and gingivitis after treatment (▲).

Table 2: K_m and V_{max} values for salivary phosphatases enzymes in controls and gingivitis cases (BT) and (AT).

Enzyme parameter	K_m (mM)			V_{max} (IU/L)		
	Control	Gingivitis (BT)	Gingivitis (AT)	Control	Gingivitis (BT)	Gingivitis (AT)
ALP	3.66	1.55	5.73	9.50	15.77	9.91
ACP	2.69	0.61	0.89	3.04	28.49	4.80

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Figures 3 and 4 represent the Arrhenius plots for the salivary ALP and ACP respectively in controls and gingivitis cases (BT) and (AT). From these figures, the Activation energy (Ea*), Enthalpy change (ΔH*), Entropy change (ΔS*) and Free energy change (ΔG*) were estimated (Table 3). The results indicated that both ΔH* and ΔG* values for salivary ALP and ACP were positive in all the groups, while the ΔS* values were negative. In general, the results showed that, there were no observable changes in Ea*, ΔH*, ΔG* and ΔS* values for salivary ALP in gingivitis cases x comparing with controls. The Ea* value for salivary ACP reaction highly decreased in gingivitis (BT) when compared with that of controls and began to increase again in gingivitis (AT). Salivary ACP highly decreased in gingivitis cases (BT) when compared with controls, and began to increase again in gingivitis cases (AT). There was a slight change in ΔG* value for ACP in gingivitis (BT). The ΔS* value for ACP was very low in gingivitis (BT) comparing with controls and started to increase in gingivitis (AT) (Table 3).

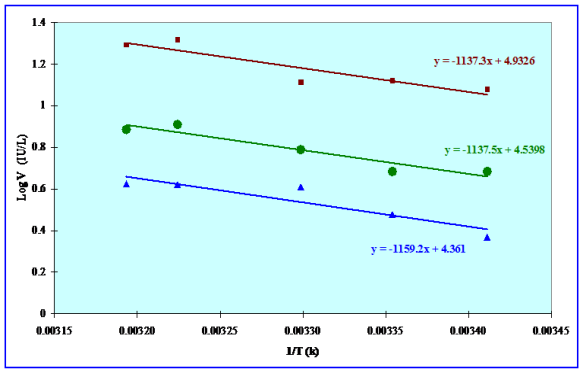

Figure 3: Arrhenius plots for salivary ALP enzyme in control (●), gingivitis before treatment (■) and gingivitis after treatment (▲).

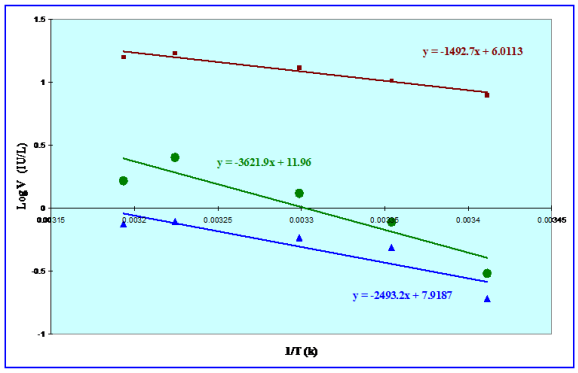

Figure 4: Arrhenius plots for salivary ACP enzyme in control (●), gingivitis before treatment (■) and gingivitis after treatment (▲).

Table 3: Thermodynamic parameters of transition state for controls and gingivitis cases (BT) and (AT).

Salivary enzyme	Groups	Ea* (cal.mole^{-1})	Δ H* (cal.mole^{-1})	Δ G* (cal.mole^{-1})	Δ S* (cal.mole^{-1}.deg^{-1})
ALP	Control	5198	4584	16786	-39
	Gingivitis (BT)	5198	4583	16474	-38
	Gingivitis (AT)	5298	4684	16760	-39
ACP	Control	16552	15938	17488	-5
	Gingivitis (BT)	6822	6208	16110	-32
	Gingivitis (AT)	11394	10780	17206	-21

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Discussion

The obtained results of salivary ALP and ACP in gingivitis have shown that the activities of these enzymes elevated in gingivitis. This elevation may be due to their release by stromal, epithelial, inflammatory or/and bacterial cells into gingival crevicular fluid and consequently into saliva (salivary ALP and ACP associated with cell injury and cell death)[24]. The changes in enzymatic activity of salivary ALP and ACP reflect metabolic changes in the gingiva and periodontium in inflammation.[24,25]

The activities of the examined salivary phosphatases enzymes started to decrease in patients after treatment (AT); including scaling and polishing when compared with those of gingivitis before treatment (BT). Our study showed that treatment with scaling and polishing was effective to decrease the activities of ALP and ACP in saliva after 20 days. This may be due to:

a- The treatment of the inflammation, which reduces the gingival damage.

b- The role of scaling is the removal of plaque which consists mostly of bacteria, which may be a source for ALP and ACP in gingival crevicular fluid and consequently in saliva.[26,27] Therefore, their release may decrease after treatment (scaling and polishing).

This study showed that the K_m values for both salivary ALP and ACP decreased in gingivitis, while they began to increase again after treatment (AT). This decrease in K_m values in gingivitis means that the affinity of the enzymes for their substrates was affected by the disease, and the chemical structure (ionic state) of the active sites became more suitable for the substrate for binding.[18] The V_{max} values for both salivary ALP and ACP in patients with gingivitis increased when compared with controls; this means that the disease also affect the catalytic efficiency of the enzymes.[28,29] The Ea* values for salivary ACP reactions decreased significantly in patients with gingivitis comparing with that of controls. Thus in patients with gingivitis, the enzyme reaction rate is faster when compared with controls, and the number of colliding molecules with this low Ea* is higher in gingivitis, so more enzyme-substrate complexes (ES*) are formed.[30] The positive values of ΔH^* indicates that, the enzyme reactions are Arrhenius-behavior and endothermic reactions[31]. The decrease in ΔH^* for ACP reaction indicates that the heat content of the enzyme reactions is smaller in gingivitis (BT) than that of controls and gingivitis (AT) due to the disease. The positive charged values for the free energy change (ΔG^*) for the enzyme-substrate reactions indicate that the active complex; *ES** formations required input of energy.[32] The (ΔG^*) value for salivary ACP was slightly less in patients with gingivitis comparing with that of controls and patients after treatment, therefore, the input energy required in patients with gingivitis for the formation of *ES**, is slightly less than that in controls and patients after treatment. The Entropy of a system is a measure of its "disorder" or "randomness". The negative entropy change (ΔS^*) for a reaction means that the products are more ordered than the reactants (*ES** is more ordered than *E* and *S*).[33] From the thermodynamic parameters, one can conclude that gingivitis will affect the mechanism of *ES** complex formation pathway in salivary ACP enzyme but not in salivary ALP. Finally, all changes in K_m, V_{max}, activation energy Ea* and the other thermodynamic parameters are due to the change in the enzymatic activity, binding site, catalytic efficiency and the mechanism of *ES** formation. These changes reflect metabolic changes in the gingiva (gum) in inflammation (gingivitis).

Conclusion

Gingivitis changes the activities, kinetic, and thermodynamic parameters of salivary ALP and ACP, therefore; affecting metabolic state in the gingiva. The changes of these parameters return to control values after treatment. Thus salivary ALP and ACP may be used as biomarker for gingivitis treatment responses and follow up.

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Conflicts of interest

The authors report no conflicts of interest.

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