

Protein phosphatase activities in the serum and saliva of healthy children

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ABSTRACT

The purpose of this study was to investigate the activities of the total acid phosphatase (TAP), tartrate-resistant acid phosphatase (TRAP), low molecular weight protein tyrosine phosphatase (LMW-PTP) and alkaline phosphatase (ALP) enzymes, as well as the possible correlation in the serum and in unstimulated whole saliva of children. Enzymatic activities were measured in pairs of concurrently obtained serum and salivary samples from 32 children in good oral and systemic health (16 of each sex) with a median age of 6.4 ± 3.3 years (range 1.08 – 12.92 years). All collections were made between the hours of 08:00 – 10:00 a.m. We used p-nitrophenyl phosphate as the substrate in the enzymatic assay for TAP, TRAP and LMW-PTP, and thymolphthalein monophosphate as the substrate for ALP. The enzymatic activities of all the studied enzymes were higher in serum than in saliva. The mean of enzymatic activities of serum TAP, TRAP, LMW-PTP and ALP were 36.51 ± 8.21 , 23.99 ± 5.73 , 11.16 ± 5.65 and 76.50 ± 17.32 U/L, respectively, while the mean salivary TAP, TRAP, LMW-PTP and ALP enzymatic activities were 9.60 ± 5.04 , 1.36 ± 0.87 , 5.65 ± 3.07 and 4.08 ± 1.83 U/L in this order. The TRAP revealed a positive linear correlation between its activity in the serum and saliva (Spearman $r = 0,4685$, $p < 0,05$). We concluded that the salivary TRAP has a potential to be

use as biomarkers of pathologies and states that modify its activity in the serum.

DESCRIPTORS

Acid phosphatase. Alkaline phosphatase. Saliva. Serum.

INTRODUCTION

Analysis of saliva composition can be used as a diagnostic tool for the localization and evaluation of various oral and/or systemic pathologies, mainly when the correlation between the salivary and blood concentrations is high^{20,21}. Easy storage and nontraumatic collection are an attractive aspect of saliva, especially to children, when repeated collections are required. The protein composition of saliva also reflects cellular signal processing that results from day-to-day environmental influences, as well as from acute or chronic stress⁹. Protein phosphatases reverse the covalent modifications of numerous cellular proteins imposed by the activation of protein kinases and, therefore, play key role in cell signaling metabolism, growth and differentiation. The activities of these enzymes have been evaluated in the saliva of children with alterations such as diabetes¹⁸ and cystic fibrosis²²; nevertheless, few authors have compared the enzymatic activities of blood and saliva^{20,22}.

Tartrate-resistant acid phosphatase (TRAP) belongs to a family of acid phosphatases that differ from the others isoforms of human acid phosphatase by being insensitive to tartrate and p-hydroxy mercury benzoate (p-HMB)¹⁷. Its most significant expression occurs in osteoclasts, making this enzyme a marker of bone resorption¹². Recent work showed that osteoblasts express TRAP activity in culture and this activity is modulated during osteoblastic differentiation⁵.

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The protein tyrosine phosphatases (PTPs) are signaling enzymes involved in the regulation of numerous cell functions, including growth, mitogenesis, motility, cell-to-cell interactions, gene transcription and immune response¹⁵. In humans, class II cysteine-based PTPs are represented by the members of the low molecular weight protein tyrosine phosphatase (LMW-PTP) family. LMW-PTP expression is upregulated in various human cancers. Moreover, reports are found in literature showing the implications of deregulated activity and expression of LMW-PTPs in common diseases, including allergy, asthma, obesity, myocardial hypertrophy and Alzheimer's disease^{2,8}.

Human alkaline phosphatases (ALP) constitute multiple molecular forms of enzymes that are produced by many tissues, mainly by bone, liver, intestine and placenta, and are excreted by bile¹⁹. The seric dose of these enzymes is particularly useful in investigating bone diseases²³. While elevated levels are found in bone diseases, characterized by the increase in osteoblastic function²⁷, their levels are diminished in cases of chronic malnutrition¹ and hypophosphatemia²⁸.

The literature lacks studies analyzing TRAP and LMW-PTP activities in unstimulated whole saliva in children. These phosphatases are normally expressed in low concentrations, but the increase or significant reduction in their expression may occur as part of pathophysiological processes, enabling them to be used as serologic and histologic markers of diseases^{16,24}.

OBJECTIVE

The purpose of this study was to evaluate the activities of the TAP, TRAP, LMW-PTP and ALP enzymes in serum and unstimulated whole saliva of children and the possible correlation between them.

MATERIAL AND METHODS

Thirty-two healthy children, aged 1 to 12 years, were submitted to clinical and oral exams, performed by a pediatrician and a pediatric dentist. Anamnesis was performed using the parents' reports and the children's health files. Only healthy, non medicated children, with good oral health were included in the study. The Research Protocol was approved by the Human Ethics Committee of the Faculdade de Odontologia de Araçatuba, Unesp – Universidade Estadual Paulista "Júlio de Mesquita Filho", Araçatuba/SP (Process Reference FOA 2003/558).

To minimize possible variations with reference to the circadian rhythm, the unstimulated whole saliva and blood were collected between 8:00 and 10:00 a.m., after 2 hours fast and oral hygiene with water, toothbrush and dental floss without fluoridated products. The salivary fluid present in the floor of the mouth was aspirated as described previously by Dezan *et al.*⁷ during 10 minutes. The saliva samples were centrifuged at 5.500 x g for 10 minutes, in a refrigerated centrifuge at 4°C, to remove cellular and food debris, squamous cells and undissolved contaminants. The supernatants were fractionated and stored at – 70°C for later analyses. Blood was collected immediately after saliva collection, by means of brachial vein puncture. The serum was obtained by centrifugation (1.500 x g) for 15 minutes, after 15 minutes rest at ambient temperature.

Total acid phosphatase (TAP), TRAP and LMW-PTP activities were determined using p-nitrophenyl phosphate (Sigma, St. Louis, USA) as the substrate as previously described^{5,6,10,11}. ALP activity was assayed as the release of thymolphthalein from thymolphthalein monophosphate with a commercial kit-based method described by Roy²⁶ (Labtest Diagnóstica, Lagoa Santa/MG, Brazil). One unit of enzymatic activity corresponds to the liberation of 1 µmol p-nitrophenol or 1 µmol thymolphthalein per minute. Enzymatic activity is expressed in units per litre (U/L).

The data were expressed as mean ± standard deviation of the mean. The data obtained presented a Gaussian distribution when submitted to the Kolmogorov-Smirnov (KS) test. The correlations among the enzymatic activities in whole saliva and in serum were analyzed by means of the Spearman's test. Statistical significance was assumed when $p < 0.05$. The statistical analyses were performed with the GraphPad Prism Version 3.00 software.

RESULTS

TAP, TRAP, LMW-PTP and ALP enzymatic activities (AE) were measured in pairs of concurrently obtained serum and salivary samples from 32 children with a median age of 6.4 ± 3.3 years (range 1.08 – 12.92 years). All enzymes analyzed were detected in both blood and saliva (Table 1). The enzymatic activities of all the studied enzymes were higher in serum than in saliva. The means ± sd of TAP, TRAP, LMW-PTP and ALP activities in serum were respectively: 36.51 ± 8.21 , 23.99 ± 5.73 , 11.16 ± 5.65 and

Table 1
Enzymatic activity of protein phosphatases in the serum and whole unstimulated saliva of healthy children

Patient code	Enzyme activity (U/L)							
	Serum				Saliva			
	TAP	TRAP	LMW-PTP	ALP	TAP	TRAP	LMW-PTP	ALP
1	31.62	19.94	9.63	77.48	3.52	0.22	0.37	2.66
2	26.66	18.89	8.96	55.02	3.55	0.39	0.47	5.95
3	24.27	16.85	3.15	63.72	14.00	1.50	9.59	5.26
4	35.62	23.76	9.58	68.71	11.27	1.91	6.69	2.10
5	39.69	30.51	9.83	79.48	9.06	1.83	4.87	3.40
6	43.01	23.15	19.38	74.59	6.58	0.68	4.34	2.85
7	40.45	26.12	15.51	64.73	13.04	1.59	8.54	3.85
8	46.35	38.29	10.31	54.47	17.27	1.81	10.53	8.69
9	33.90	25.87	6.63	112.58	3.77	0.58	2.52	2.75
10	35.28	23.15	10.25	96.37	6.68	0.92	3.91	6.77
11	33.46	21.40	8.62	102.34	6.32	1.31	3.66	1.50
12	33.12	25.39	12.95	90.57	16.05	2.26	10.72	4.86
13	25.06	14.24	11.99	52.36	3.88	0.51	2.59	2.37
14	23.60	16.32	0.93	79.73	10.78	0.99	7.84	4.14
15	31.54	32.42	5.73	56.11	16.50	2.99	8.04	1.74
16	35.54	24.01	12.10	77.50	10.54	1.58	6.57	4.95
17	41.49	18.74	20.79	81.10	1.91	0.41	1.02	4.94
18	25.62	18.96	5.37	64.02	6.24	0.96	4.31	5.91
19	52.78	20.76	30.51	92.21	12.68	1.71	7.17	5.62
20	37.44	23.62	10.73	72.92	5.22	0.73	3.32	1.82
21	47.36	27.75	15.62	67.15	16.01	2.77	7.00	2.90
22	51.32	34.66	13.85	73.10	10.41	1.33	6.61	6.18
23	43.62	24.44	12.02	73.09	19.10	4.03	8.68	2.38
24	47.30	22.81	19.66	62.30	9.66	0.78	7.57	2.11
25	28.99	20.87	8.60	58.07	2.30	0.41	0.69	7.04
26	35.51	27.72	8.65	78.97	2.43	0.41	0.88	4.46
27	33.15	22.87	8.90	113.58	15.00	2.06	9.25	2.79
28	22.25	17.30	5.28	60.10	11.59	1.42	7.55	1.72
29	43.29	30.31	12.50	119.26	18.12	2.50	9.64	4.59
30	31.35	16.12	6.32	81.24	5.68	0.69	3.59	3.82
31	42.84	26.49	14.02	78.90	10.29	1.36	6.83	6.24
32	44.72	33.90	8.76	66.29	7.86	0.85	5.49	2.50
Mean	36.51	23.99	11.16	76.50	9.60	1.36	5.65	4.03
SD	8.21	5.73	5.65	17.32	5.04	0.87	3.07	1.83

76.50 $\pm$ 17.32 U/L, and in saliva were: 9.60 $\pm$ 5.04, 1.36 $\pm$ 0.87, 5.65 $\pm$ 3.07 and 4.08 $\pm$ 1.83 U/L in the same sequence. Only scatter plot of the serum versus salivary TRAP enzymatic activities (Figure 1a) revealed a significant linear relationship (Spearman

correlation coefficient, $r = 0.4685$, $p = 0.0068$). We have not observed any statistically significant linear relationship between serum versus salivary TAP (Figure 1b), LMW-PTP (Figure 1c) and ALP (Figure 1d) enzymatic activities.

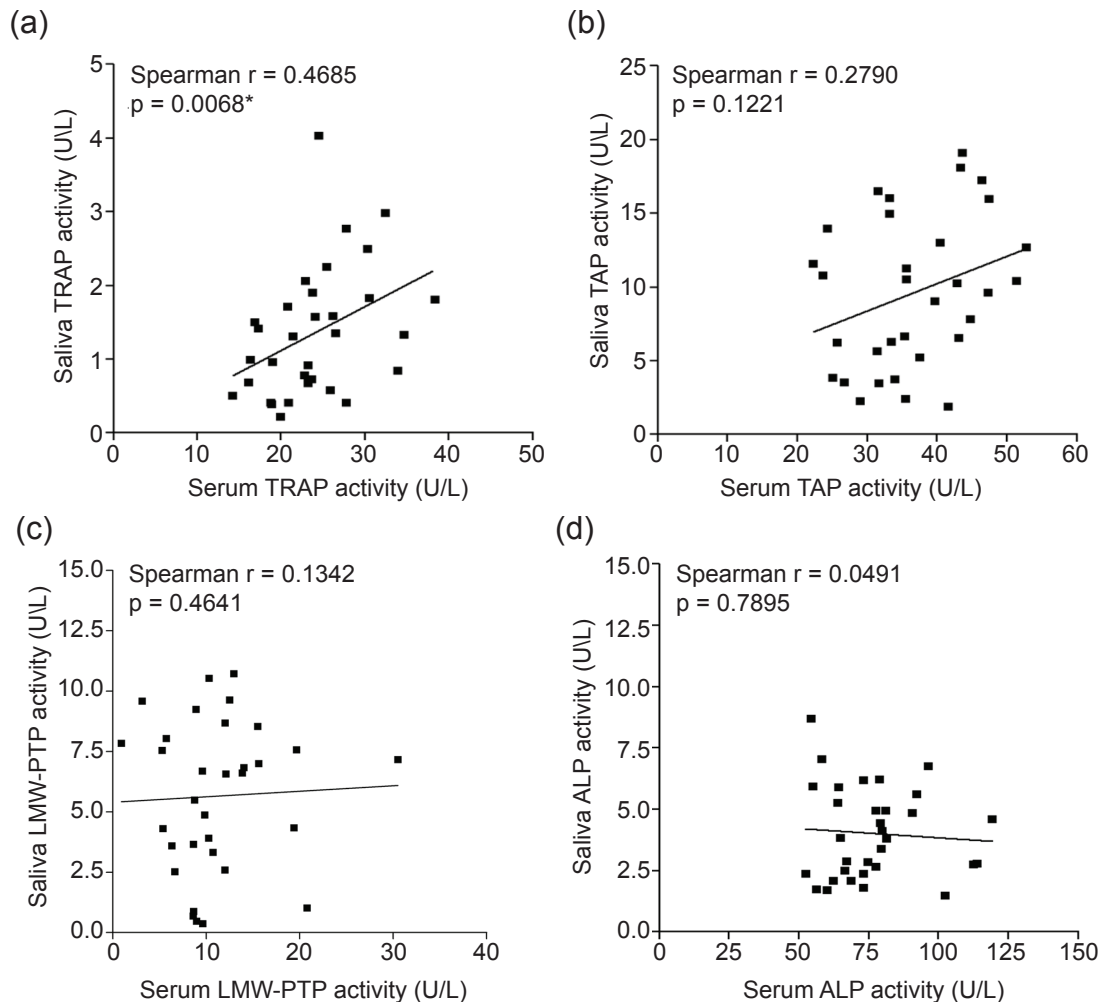


Figure 1 - Relationship between serum and unstimulated whole saliva of enzymatic activities (U/L). (a) Tartrate-resistant acid phosphatase (TRAP); (b) total acid phosphatase (TAP); (c) low weight molecular protein tyrosine phosphatase (LMW-PTP); and (d) alkaline phosphatase (ALP) in health children, as determined by Spearman correlation coefficient, * $p < 0.05$.

DISCUSSION

The advantages of saliva in relation to serum as diagnostic fluid are easy handling, reduced blood-borne infection, non invasive method and subsequently more cooperation from children. This work is the first to investigate the activities of TRAP and LMW-PTP in saliva, and shows that there is a moderate positive correlation between serum and saliva TRAP activities.

Whole saliva is a complex mixture of secretion from the three paired major salivary glands (parotid, submandibular and sublingual) and from many minor salivary glands. In addition, it may contain gingival crevicular fluid, blood, leukocytes, desquamated cells, bacteria, dental plaque, virus and food debris. The presence and origin of certain salivary enzymes is still controversial. Nagler *et al.*²⁰ described two

fundamental processes that modulate saliva composition: first, the glands and oral sources may be the primary sites in which the various components are produced, or they may be merely the sites of passage through which various components are passively diffused or actively transported from the blood; second, various processes take place in the oral cavity after the secretion of saliva that alter its composition.

The demand to perform oral hygiene without the use of fluoridated dentifrice was made to prevent inhibition of the osteoclastic fraction of TRAP by fluoride¹³. To avoid the interference of brushing in the salivary protein concentration, related by Hoek *et al.*¹⁴, the samples were collected a minimum of two hours after the children performed oral hygiene. To minimize variations in salivary flow resulting from the dietary status, collections were made after the children had fasted for 2 hours.

Activities of the all enzymes studied were detectable in both biological samples and were higher in serum than in saliva. The ratio of serum to salivary enzymatic activity for TAP, TRAP, LMW-PTP and ALP were 3.8, 17.66, 1.98 and 18.8, respectively. Nagler *et al.*²⁰ also had described ALP activity in serum higher in comparison with those in saliva, however the enzymatic activity was only 5.73 times higher; on the other hand, in the same work, the TAP activity in saliva was 9.135 higher in comparison with those in serum. The existence of correlation between specific components in whole saliva and in blood is the point of origin to validate its use as biological material for clinical laboratory analysis; nevertheless, the absence of a full correlation between the concentrations of a component of saliva and of blood does not necessarily deny its blood origin²⁰. In this work, only TRAP demonstrated correlation between its activities in the blood and whole saliva. This isoenzyme seems to be essential for demineralization of hard tissue in tooth roots and for digestion for demineralization of hard tissue³. Despite of our data, Nagler *et al.*²⁰ showed the correlation between the activities of TAP and ALP in the blood/whole saliva. This divergence in results may be related to the different age groups (6.4 *versus* 30.8 years) and different substrates (p-nitrophenyl phosphate and thymolphthalein monophosphate vs 1-naphthylphosphate and p-nitrophenyl phosphate) used in this study.

Another important aspect to be considered is the differences between the TRAP and LMW-PTP activities in relation to proportion of TAP activities. The isoenzyme TRAP accounts for approximately 65.70% of the TAP activity in the serum and this proportion diminished in the saliva to 14.13%, whereas LMW-PTP comprises 30.57% of the TAP

activity in serum and this proportion increased in the saliva to 58.90%. These data suggest that diffusion from the surrounding tissues through the salivary glands and the blood vessels may take place partially or processes like production of radical oxygen species occurring in the oral cavity could alter the enzymatic activity. It is known that the regulation of LMW-PTP activity can occur by a refined redox system due cysteine near the catalytic site and that hydrogen peroxide inactivates reversibly LMW-PTP⁴, while the regulation of TRAP activity occurs in a redox-dependent pathway, in which two ferric ions present in the enzyme determine its activity, being active when these ions are reduced and inactive when they are oxidized²⁵. The enzymatic assay of saliva obtained separately from each pair of glands such as parotid, submaxillary/sublingual could explain the contribution of these glands in the secretion of TRAP e LMW-PTP and the influence of the chemical composition of whole saliva in the enzymatic activities.

CONCLUSION

The activities of all the examined enzymes (TAP, ALP, TRAP and LMW-PTP) were detected in the total unstimulated saliva and serum of normal children, being higher in serum than in saliva. However, we observed different proportions of TRAP and LMW-PTP activities in relation to TAP activities. It was observed a moderate correlation between serum and saliva TRAP activities.

Further investigations on saliva obtained separately from parotid and submaxillary/sublingual glands would provide new insights on the use of salivary enzymes as biochemical markers of diseases and saliva as diagnostic tool in children.

RESUMO

Atividade de proteínas fosfatases no soro e saliva de crianças saudáveis

O objetivo deste estudo foi investigar as atividades das enzimas fosfatase ácida total (FAT), fosfatase ácida resistente ao tartarato (TRAP), proteína tirosina fosfatase de baixa massa molar (LMW-PTP) e fosfatase alcalina (FAL), como também a possível correlação no soro e na saliva total não estimulada de crianças. As atividades enzimáticas foram mensuradas simultaneamente no soro e na saliva de 32 crianças (16 de cada sexo) com boa saúde oral e sistêmica, com média de idade de $6,4 \pm 3,3$ anos (variando entre 1,08 – 12,92 anos). Todas as coletas foram realizadas entre 8 e 10 h da manhã. Foi utilizado como substrato p-nitrofenil fosfato para a análise de FAT, TRAP e LMW-PTP, e timolftaleína monofosfato para análise de FAL. As atividades enzimáticas de todas as enzimas estudadas foram maiores no soro que na saliva. As médias $\pm dp$ das atividades enzimáticas para FAT, TRAP, LMW-PTP e FAL no soro foram $36,51 \pm 8,21$, $23,99 \pm 5,73$, $11,16 \pm 5,65$ e $76,50 \pm 17,32$ U/L, respectivamente, enquanto para a saliva foram, na mesma sequência, $9,60 \pm 5,04$, $1,36 \pm 0,87$, $5,65 \pm 3,07$ e $4,08 \pm 1,83$ U/L. A TRAP mostrou uma correlação linear positiva entre sua atividade no soro e na saliva (Spearman $r = 0,4685$, $p < 0,05$). Conclui-se que a TRAP salivar possui potencial para ser utilizada como biomarcador de patologias e estados que alterem sua atividade no soro.

DESCRIPTORES

Fosfatase ácida. Fosfatase alcalina. Saliva. Soro.

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