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Relationship between lactate dehydrogenase activity in saliva and oral health status

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ARTICLE INFO

Article history:
Accepted 24 April 2007

Keywords:
Lactate dehydrogenase
Oral health
Saliva

ABSTRACT

Objective: Lactate dehydrogenase (LDH) activity in serum increases as a marker of cellular necrosis. LDH activity in saliva could constitute a specific indicator of oral mucosal lesions with tissue breakdown including periodontal disease. The aim of this study was to determine LDH activity in saliva, assessing inter-individual variations with regard to the oral health status.

Materials and methods: An oral and perioral examination was performed on a study group of 175 volunteers, to assess the number of missing teeth, caries, fillings, dental prosthesis, periodontal status and temporomandibular joint condition. LDH activity was determined from stimulated whole saliva.

Results: After adjustment by age, gender, smoking habits, missing teeth and use of removable prosthesis, the multivariate analysis showed that increased LDH activity was associated with periodontal disease, specifically with the presence of calculus and pockets greater than 5 mm.

Conclusion: LDH activity in whole saliva could be useful as a biochemical marker of periodontal status.

1. Introduction

Within the cell, glucose is used principally for the production of pyruvate in the glycolysis pathway. Under aerobic conditions, pyruvate enters the mitochondrial matrix, where it is oxidised by the action of pyruvate dehydrogenase, being transformed into acetyl-CoA which, still under aerobic conditions, subsequently enters the citric acid cycle. In an anaerobic medium, pyruvate is reduced to lactate in a reversible reaction catalysed by lactate dehydrogenase (LDH), which uses nicotinamide adenine dinucleotide as a coenzyme. LDH is an enzyme detectable in cytoplasm in almost every cell of the human body, which becomes extracellular upon cell death. Therefore, its extracellular presence is always related to cell necrosis and tissue breakdown. Its serum activity non-specifically increases in many pathological conditions such as myocardial infarction, liver disease (being particularly high in toxic hepatitis with jaundice), megaloblastic anaemias, renal disease (especially in patients with tubular necrosis or pyelonephritis), malignant disease (Hodgkin's disease, cancer of the abdomen and lung, teratoma, liver metastases or leukaemia), progressive muscular dystrophy and pulmonary embolism.[1]

It has been reported that parotid and submaxillary–sublingual glands contributed very little to LDH activity in whole saliva.[2] The profiles of the LDH isoenzymes found in the healthy oral epithelium and those found in whole saliva are similar.[3] For this reason, it has been suggested that the main source of LDH in whole saliva was the oral epithelium, and not rather than the salivary glands the main source of LDH in whole saliva.[2]

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0003–9969/$ – see front matter
doi:10.1016/j.archoralbio.2007.04.008

LDH activity in saliva, being a cellular necrosis marker, could constitute a specific indicator of oral mucosa breakdown.

The existing literature about the levels of activity of LDH in saliva is scarce and shows variable results, depending on the diversity of the sampling, the handling and the methods of analysis used.[2,4–8]

The present paper aims to determine LDH activity in whole saliva, by assessing inter-individual variations with regard to the oral health status.

2. Materials and methods

One hundred and seventy-five patients were randomly recruited from those examined at a dental office in Santiago de Compostela (Spain), between 2004 and 2005. All of the individuals were volunteers, over 18 years of age and gave informed consent before participating in the study. The project was approved by the Ethics Committee of the Faculty of Medicine and Dentistry of the University of Santiago de Compostela.

The following exclusion criteria were applied: existence of systemic diseases involving an increase of serum LDH,[1] having received dental treatment 48 h prior to examination; presenting oral mucosa lesions, especially where tissue destruction is involved; having systemic diseases (such as diabetes, Sjögren's syndrome, or terminal nephropathy), or were subjected to certain therapeutic procedures, such as radiotherapy, which could induce a reduction in the salivary flow; pregnancy; other diseases or drugs affecting periodontal health.[9]

The following data were recorded: age, gender, tobacco consumption, recreational drugs consumption and medical background.[10] Each patient filled in a survey about his/her oral hygiene habits, previous oral diseases and dental treatment. All of the individuals were subjected to an oral and perioral exploration, to assess the number of missing teeth, caries, fillings, dental prosthesis type, size and structure, periodontal status and temporomandibular joint condition. Finally, all of the patients were subjected to a panoramic radiograph.

The caries diagnosis in visible tooth surfaces was carried out with a mirror, explorer and with a specific caries detector (1% basic fuchsin in a propylene glycol solution).[11] Approximal caries diagnosis was established using bitewing digital radiographs.[12,13] Filled teeth were considered as those with any type of restoration. The extent of the restoration was not considered within the examination. The periodontal status was recorded using the ''Community Periodontal Index of Treatment Needs'' (CPITN).[14] The highest score in the mouth was recorded for each patient. The temporomandibular joint was classified either as normal or as pathological, according to a modified version of the WHO's proposal.[10]

In order to determine the salivary LDH activity in stimulated whole saliva, the patients themselves took out samples of their own saliva, after having received proper instruction and training on the collection method which was practiced immediately after patients woke up in the morning. In a non-invasive way, the 51.1534 Salivette® kit (Sarstedt Ltd., Nümbrecht, Germany) was used. The device consists of a cotton swab, which, after being chewed for 45 s, is placed in a conical-base centrifuging tube. The cotton roll was centrifuged at 5000 rpm for 10 min (''Centro 8'', JP SELECTA, Barcelona, Spain), with the purpose of extracting the saliva[15] and was stored at 4 °C until further analysis within 24 h. Patients were not allowed to eat, drink, smoke or brush their teeth 8 h prior to sampling.

LDH determinations were performed using a Cobas Mira Plus II® autoanalyzer (Roche Diagnostics GmbH, Mannheim, Germany) at 30 °C, in accordance with the recommendations of the French Society of Clinical Biology (Société Française de Biologie Clinique, 1982).[16,17]

The principle of the assay consists of the kinetic determination of LDH activity, based on the rate of NADH oxidation. We determined the oxidation rate, which is directly proportional to LDH activity, by measuring the decrease in absorbance at 340 nm. To standardise the expressions of enzyme activity, the units of enzyme activity have been defined as the quantity of enzyme that catalyses the reaction of 1 μmol of substrate per minute. The catalytic concentration is expressed as U/L or mU/L.

The LDH activity results were analysed in relation to the following variables: patients, age, gender, tobacco consumption, number of missing, decayed and filled teeth, use of dental prosthesis, periodontal disease, temporomandibular joint status and radiographic diagnosis of intrabony lesions related to dental structures.

Results were analysed using the statistic software SPSS version 10.0 (SPSS Inc., Chicago, USA). The relationship between a continuous variable (e.g. LDH activity) and a categorical variable of two criteria (e.g. gender) has been assessed applying a Student's *t*-test. To study the influence of a categorical variable (e.g. gender) on the mean values of a continuous variable (e.g. mean LDH activity), the analysis of variance (ANOVA) has been applied. In cases where several variables could be simultaneously compared (e.g. factors linked to the LDH activity in saliva), a multiple logistic regression model with Bonferroni correction was applied.

3. Results

In this series, 68.6% of the study group was comprised of females and 31.4% of males. The average age of the participants was 41.0 (95% confidence intervals, 95% CI, 30.6; 43.4). No significant age differences between patients of both genders were observed. A proportion of 21.1% were smokers. A 25.7% of the patients had a complete set of teeth, whereas 26.8% had $\geq$8 missing teeth. The percentage of individuals with 1–4 decayed teeth amounted to 55.4%, patients with at least 1 filling constituted 97.2%, 33.1% had >8 fillings and 17.7% had a removable prosthesis. The percentage of patients with CPITN = 0 was 43.4%, and the individuals presenting gingival bleeding and calculus amounted to 19.4% and 11.4%, respectively. Finally, the proportion of individuals who had at least one 4–5 mm periodontal pocket was 13.7%, and those who had at least one periodontal pocket >5 mm amounted to 11.4%. Temporomandibular joint disturbances and periapical radiolucent lesions were detected in 5.7% and 6.8% of patients, respectively. The demographic data, the results of the oral

Table 1 – Demographic data and oral findings of the study group

	n	%
Gender		
Male	55	31.4
Female	120	68.6
Age		
18–24	32	18.2
25–44	83	47.4
45–64	45	25.7
>64	15	8.5
Smoker		
No	138	78.9
Yes	37	21.1
Missing teeth		
0	45	25.7
1–4	52	29.7
5–8	31	17.7
>8	47	26.8
Decayed teeth		
0	43	24.6
1–4	97	55.4
5–8	29	16.6
>8	6	3.4
Filled teeth		
0	5	2.8
1–4	54	30.9
5–8	58	33.1
>8	58	33.1
Teeth replaced by ceramo-metalic fixed prosthesis		
0	138	78.8
1–2	20	11.4
>2	17	9.7
Removable prosthesis		
No	144	82.2
Yes	31	17.7
"Community Periodontal Index of Treatment Needs" (CPITN)		
Healthy	76	43.4
Bleeding	34	19.4
Calculus	20	11.4
4–5 mm pocket	24	13.7
>5 mm pocket	21	11.4
Temporomandibular joint		
Normal	165	94.2
Disturbance	10	5.7
Periapical radiolucent lesion		
No	163	93.1
Yes	12	6.8

health survey and the oral examination are summarised in Table 1.

The stimulated saliva volume was >0.7 mL/min in all patients (range 0.8–1.7 mL/min), and was therefore superior to the lower limit for the normal whole stimulated salivary flow rate. The mean LDH value recorded in the study was 1573 U/L (95% CI: 1392; 1753). To ensure the LDH was of salivary origin, the electrophoretic profile of all the samples was performed. The mean percentage of the LDH_5 isoenzyme in the saliva was 59%, whereas that proportions reported in the erythrocytes and serum are 3–5%. In the age category, a statistically significant increase in the mean LDH value in individuals above 64 was noticed, with regard to the other age groups ($P = 0.02$). In female patients, the mean level of LDH in stimulated saliva was 1621 U/L (95% CI: 1380; 1861) as opposed to a mean activity of 1469 found in males (95% CI: 1228; 1710) ($P = 0.76$). The mean salivary LDH activity value activity for smokers was 1767 U/L (95% CI: 1313; 2222), whereas it showed lower values for non-smokers, the mean being 1521 U/L (95% CI: 1325; 1717). Differences between these two groups were not statistically significant ($P = 0.79$). No relationship was established between the number of missing teeth and the salivary LDH activity, except for individuals with >15 missing teeth, who presented mean LDH activity values greater than those with no missing teeth (2499 and 1358 U/L, respectively; $P < 0.01$). Neither the number of caries nor that of fillings was found to affect LDH activity in stimulated whole saliva. Likewise, we did not notice any significant differences in LDH regarding temporomandibular joint disturbances or intrabony radiological lesions.

The existence of a fixed prosthesis did not condition salivary LDH activity values. LDH activity in patients with a removable prosthesis attained a mean value of 2152 U/L, whereas those with no removable prosthesis showed a mean LDH activity value of 1448 U/L. This difference was statistically significant ($P = 0.004$).

Individuals with healthy periodontium (CPITN = 0) showed a mean LDH activity value of 1107 U/L (95% CI: 904; 1310), in contrast to the mean value of 1930 U/L (95% CI: 1670; 2191) detected in patients with periodontal disease (CPITN $\geq$ 1). This difference was statistically significant ($P < 0.0001$). Patients with calculus and/or >5 mm periodontal pockets (CPITN = 2, 3 and 4) showed salivary LDH activity values significantly higher than those of patients without periodontal disease ($P < 0.01$, $P = 0.02$ and $P < 0.001$, respectively).

After adjustments by age, gender, smoking habits, missing teeth and use of removable prosthesis, the multivariate analysis showed that only the presence of calculus ($P < 0.01$) and >5 mm periodontal pockets ($P < 0.001$) had a significant impact on LDH activity (Tables 2 and 3).

Table 2 – Multivariate analysis of salivary LDH activity according to age, gender, tobacco habits, and use of removable prosthesis

	Mean LDH	95% CI	P
Age			
18–24	1294	(880; 1709)	
25–44	1425	(1205; 1644)	0.659
45–64	1659	(1274; 2045)	0.536
>64	2636	(1757; 2514)	0.155
Gender			
Male	1469	(1228; 1710)	
Female	1621	(1380; 1861)	0.290
Smoker			
No	1521	(1325; 1717)	
Yes	1767	(1331; 2222)	0.210
Removable prosthesis			
No	1448	(1048; 1848)	
Yes	2152	(1389; 2763)	0.489

Table 3 – Multivariate analysis of salivary LDH activity by periodontal disease

Periodontal disease	Mean LDH	95% CI	P
CPTIN = 0			
Healthy	1107	(904; 1310)	
Disease	1930[a]	(1670; 2191)	0.0001*
CPTIN = 1			
Bleeding	1469	(1144; 1794)	0.120
CPTIN = 2			
Calculus	1801	(1241; 2361)	0.011*
CPTIN = 3			
4–5 mm pocket	1669	(1246; 2091)	0.069
CPTIN = 4			
>5 mm pocket	2782	(2004; 3559)	0.001*

[a] 1930 is the mean value of the following four groups (bleeding, calculus, 4–5 mm pocket and >5 mm pocket).
* Statistically significant.

4. Discussion

Little information is available related to LDH activity in whole saliva and age, and it has been pointed out that the values for parotid saliva found in adults (19–48 years old) and young people (11–17 years old) are similar.[7] In this study, an increase in salivary LDH activity in individuals older than 64 was observed, in accordance with other findings reported for serum samples.[18] LDH activity in whole saliva was slightly higher in females than in males. This finding matched results achieved in serum LDH of previous studies.[18]

Significant variations in salivary LDH activity between smokers and non-smokers were not found. Nagler et al.[19] detected a reduction of 41–57% in salivary LDH activity after a 3-h exposure to cigarette smoke, but these studies could not reproduce the biological conditions of the oral cavity in the *in vitro* experimental model designed. Zappacosta et al.[20] also found a significant inhibition of salivary LDH activity after smoking a single cigarette, but the recovery time of the enzymatic activity was not evaluated and the patients in the study were not allowed to smoke 8-h prior to sampling. The high salivary LDH activity found in individuals with a removable prosthesis can be explained by the histological changes (inflammation and ulcers),[21] and the high prevalence of periodontal disease found in removable prosthesis bearers.[22]

In the present study, the values of salivary LDH activity in individuals with periodontal disease were significantly higher than those obtained in patients with a healthy periodontium. Most of the literature that focuses on the LDH as a diagnosis marker for periodontal disease has been carried out in samples of gingival crevicular fluid.[23–30] The literature regarding LDH activity levels in whole saliva as a possible marker of periodontitis is scarce.[3,5,31,32] Only one paper by Sornin et al.[3] has been found referring to LDH activity in whole saliva in individuals with and without periodontal disease. In accordance with the present study, the results pointed out that the LDH activity value was higher in patients with periodontal disease, although the authors did not specify the diagnosis criteria applied nor the severity of the periodontal disease in the participants.

Conclusively, with the application of a standardised method for saliva collection, storage and handling, and the French Society of Clinical Biology analytical protocol, the LDH in whole saliva could be useful as a biochemical marker of the periodontal status assessed with the CPITN index. However, further studies are needed to determine if salivary LDH activity may represent a marker of current periodontal disease activity.

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