

Comparison of Salivary pH, Buffering Capacity and Alkaline Phosphatase in Smokers and Healthy Non-Smokers

Retrospective cohort study

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مقارنة بين درجة الحموضة اللعابية وكفاءة معادلة الحموضة والفوسفاتاز القلوية في المدخنين وغير المدخنين الأصحاء دراسة مجموعة استيعادية

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ABSTRACT: Objectives: Saliva contains alkaline phosphatase (ALP)—a key intracellular enzyme related to destructive processes and cellular damage—and has buffering capacity (BC) against acids due to the presence of bicarbonate and phosphate ions. Smoking may have deleterious effects on the oral environment due to pH changes which can affect ALP activity. This study aimed to evaluate the salivary pH, BC and ALP activity of male smokers and healthy non-smokers. **Methods:** This retrospective cohort study took place between August 2012 and December 2013. A total of 251 healthy male non-smokers and 259 male smokers from Hamadan, Iran, were selected. Unstimulated whole saliva was collected from each participant and pH and BC were determined using a pH meter. Salivary enzymes were measured by spectrophotometric assay. **Results:** Mean salivary pH (7.42 ± 0.48 and 7.52 ± 0.43 , respectively; $P = 0.018$) and BC (3.41 ± 0.54 and 4.17 ± 0.71 ; $P = 0.001$) was significantly lower in smokers compared to non-smokers. Mean ALP levels were 49.58 ± 23.33 IU/L among smokers and 55.11 ± 27.85 IU/L among non-smokers ($P = 0.015$). **Conclusion:** Significantly lower pH, BC and ALP levels were observed among smokers in comparison to a healthy control group. These salivary alterations could potentially be utilised as biochemical markers for the evaluation of oral tissue function and side-effects among smokers. Further longitudinal studies are recommended to evaluate the effects of smoking on salivary components.

Keywords: Saliva; Alkaline Phosphatase; Acids; Buffers; Smoking.

المخلص: أهداف: يحتوي اللعاب على الفوسفاتيز القلوي (ALP)، وهو إنزيم أساسي خلوي مرتبط بتلف الخلايا والعمليات الحيوية الضارة. يعادل ALP الأحماض بسبب وجود أيونات البيكربونات والفوسفات. قد يكون للتدخين آثار ضارة على بيئة الفم نتيجة لتغيرات درجة الحموضة التي يمكن أن تؤثر على نشاط ALP. هدفت هذه الدراسة إلى تقييم درجة الحموضة اللعابية، وكفاءة معادلة الأحماض، ونشاط ALP في المدخنين وغير المدخنين من الذكور الأصحاء. منهجية: هذه دراسة استيعادية أجريت في الفترة ما بين أغسطس 2012 وديسمبر 2013. تم اختيار 251 من غير المدخنين و 259 من المدخنين الذكور من همدان، إيران. تم جمع اللعاب الكامل الغير محفز من كل مشارك وتم تحديد درجة الحموضة وكفاءة معادلة الأحماض باستخدام جهاز مقياس درجة الحموضة. تم قياس الإنزيمات اللعابية بواسطة الفحص الطيفي. نتائج: معدل الرقم الهيدروجيني اللعابي (7.42 ± 0.48 و 7.52 ± 0.43 ; $P = 0.018$) وعلى التوالي، 3.41 ± 0.54 و 4.17 ± 0.71 ; $P = 0.001$) كانت أقل ذات دلالة إحصائية لدى المدخنين مقارنة بغير المدخنين. كان معدل مستويات ALP: 49.58 ± 23.33 وحدة دولية/لتر للمدخنين و 55.11 ± 27.85 وحدة دولية/لتر لغير المدخنين ($P = 0.015$). خاتمة: وجد انخفاض ملحوظ لدرجة الحموضة، وكفاءة معادلة الأحماض ومستويات ALP بين المدخنين بالمقارنة مع مجموعة المراقبة الغير مدخنة. من المحتمل أن تستخدم هذه التغيرات اللعابية كعلامات كيميائية حيوية لتقييم وظيفة الأنسجة الفموية والآثار الجانبية للتدخين. ينصح مستقبلاً بإجراء دراسات طولية لتقييم آثار التدخين على المكونات اللعابية.

كلمات مفتاحية: اللعاب؛ الفوسفاتيز القلوي؛ الأحماض؛ معادل الحموضة؛ التدخين.

ADVANCES IN KNOWLEDGE

- Smoking was found to significantly alter salivary pH and buffering capacity (BC), which can have many dangerous and deleterious effects on the oral mucosa.

APPLICATION TO PATIENT CARE

- This study found significantly lower pH, BC and alkaline phosphatase levels among smokers. These changes could potentially be used as biochemical markers to evaluate oral tissue function among smokers.
- Dental and medical practitioners should make patients aware of the effect of smoking on salivary components. The findings of this study could be used by healthcare workers to encourage patients to take part in smoking cessation programmes.

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SALIVA IS AN ORAL FLUID SECRETED FROM major and minor salivary glands for the primary purposes of digestion, lubrication and the protection of tooth integrity.^{1,2} Overall, saliva is composed of 99% water and 1% organic and inorganic molecules.¹ Important components of saliva include electrolytes such as bicarbonate, calcium, fluoride and phosphate; enzymes such as α -amylase, invertase and mucins; immunoglobulins (Igs) including IgA, IgG and IgM; lipids including neutral lipids, glycolipids and phospholipids; non-Igs such as histidine-rich proteins, lactoferrin and lysozyme; and proteins such as peroxidase, proline-rich proteins, agglutinins and statherin.¹ Alkaline phosphatase (ALP) is an intracellular enzyme present in the saliva and most tissues, organs and bones, including the epithelial, inflammatory cells, bacterial organisms and mineralising tissue cells.³⁻⁵ The enzyme is related to cell injury and death. ALP most commonly correlates with bone metabolism and is present in the osteoblast cell membrane and polymorphonuclear leukocyte granules.³ Destructive processes in the alveolar bone can lead to increased ALP activity.^{4,5}

A key factor in enzyme functionality is pH, with the optimum pH for ALP activity being ≈ 7.4 .⁶ Therefore, the maximum enzyme activity of ALP is related to the pH and concentration of phosphate esters existing in cells.⁶ Normal salivary pH values range from 7.4–7.6, depending on salivary calcium and phosphate concentrations.^{1,7,8} Due to the presence of bicarbonate/carbonate ions and, to a lesser extent, phosphate ions and proteins, saliva has a buffering capacity (BC) and can neutralise acids produced in the oral cavity or ingested.^{9,10} Oral health and integrity, demineralisation-remineralisation balance and dilution and antimicrobial activity are important to maintain good salivary BC.¹¹ High salivary pH and BC have been found to lead to better oral health outcomes and a lower incidence of dental caries.¹² Bagherian *et al.* reported lower pH and BC values among children without caries.¹³ In contrast, another study indicated lower resting salivary pH values among individuals with dental caries.¹⁴ However, changes in pH can also result in pathological changes to the teeth and oral cavity.¹⁰

Cigarette smoke contains harmful components such as pyridine alkaloids, aromatic hydrocarbons and combustion gases; these can lead to the development of various life-threatening diseases.^{15,16} Moreover, smoking has many side-effects which manifest in the oral mucosa, including delays in wound healing, periodontitis and premalignant and malignant oral lesions.¹⁶ Due to the deleterious effects of smoking on the oral mucosa, associated salivary changes and the

limited number of studies in this field, the aim of this study was to evaluate the salivary pH, BC and ALP activity of male smokers and healthy non-smokers in Hamadan, Iran.

Methods

This retrospective cohort study took place between August 2012 and December 2013 and included 251 male non-smokers and 259 male smokers. Participants in the smoking group were selected from smokers patients attending routine dental examinations at the Oral Medicine Department of the Hamadan Dental School who had been smoking at minimum of five cigarettes a day for at least five years. The control group included healthy non-smokers who attended routine dental examinations at the Oral Medicine Department of the Hamadan Dental School during the study period. New smokers and individuals with systemic diseases, a history of alcohol and tobacco consumption and/or drug treatment were excluded from the study. On the basis of a previous study assessing salivary enzymes and total antioxidant capacity among smokers and non-smokers, the ideal sample size was determined to be 258 for each group with a total sample size of 516 at a 95% significance level and with 90% statistical power.¹⁷

Prior to saliva collection, all participants were requested to avoid any oral stimuli for at least 90 minutes beforehand and smokers were asked to refrain from smoking for one hour. Unstimulated whole saliva was then collected according to a previously described method.¹⁸ A total of 5 mL of spat unstimulated saliva was collected from each participant in a sterile falcon tube. The specimens were immediately centrifuged (1,000 g for 10 minutes) at 4 °C. Squamous cells and cell debris were removed and the supernatant was isolated. Saliva samples were immediately frozen at -80 °C until the sample collection period was complete. After this, the pH of each saliva sample was determined using a pH meter (Hanna Instruments Inc., Ann Arbor, Michigan, USA).¹³ The salivary BC was then evaluated by the addition of 1 mL of 0.1 N of hydrochloric acid to 1 mL of saliva. The BC was calculated according to changes in pH and ranked via the Ericsson method as high (pH >5.5), moderate (pH 4.5–5.5) or low (pH <4.5).¹⁹ Salivary ALP levels were determined using a spectrophotometric assay kit (Pars Azmoon Inc., Tehran, Iran) by the addition of a P-Nitrophenyl phosphate solution. The production of P-Nitrophenol in the saliva was evaluated by measuring absorbance at 450 nm on a spectrophotometer.

Data were analysed using an independent t-test and Mann-Whitney U test at the 0.050 significance level by

Table 1: Age profile of male smokers and healthy non-smokers in Hamadan, Iran (N = 510)

	n (%)		P value
	Smokers (n = 259)	Non-smokers (n = 251)	
Age in years			
20–30	188 (72.6)	128 (51)	0.001
31–40	53 (20.5)	83 (33.1)	
41–50	18 (6.9)	40 (15.9)	
Mean ± SD	27.76 ± 6.54	30.98 ± 7.80	

SD = standard deviation.

Stata® data analysis and statistical software, Version 12 (StataCorp LP, College Station, Texas, USA). All values were reported as means ± standard deviation.

This study protocol was approved by the Ethics Committee of the Hamadan University of Medical Sciences & Health (#16/35/4749). Written informed consent was obtained from all participants after the aims of the study had been explained.

Results

Overall, participants in both groups ranged in age from 20–50 years old. The mean age was 27.76 ± 6.54 years and 30.98 ± 7.80 years for the smoker and control groups, respectively [Table 1]. Smokers had significantly lower salivary pH in comparison to non-smokers (7.42 ± 0.48 versus 7.52 ± 0.43, respectively; *P* = 0.018). Mean levels of salivary BC were also significantly lower among smokers compared to non-smokers (3.41 ± 0.54 versus 4.17 ± 0.71, respectively; *P* = 0.001). Additionally, mean ALP levels were significantly lower among smokers in comparison to non-smokers (49.58 ± 23.33 IU/L versus 55.11 ± 27.85 IU/L, respectively; *P* = 0.015). However, the latter difference was not significant according to the Mann-Whitney U analysis (*P* = 0.064) [Table 2].

Table 2: Salivary pH, buffering capacity and alkaline phosphatase levels among male smokers and healthy non-smokers in Hamadan, Iran (N = 510)

Variable	Mean ± SD (95% CI)		Mean difference ± SE (95% CI)	P value	
	Smokers (n = 259)	Non-smokers (n = 251)		Independent t-test	Mann-Whitney U
pH	7.42 ± 0.48 (0.48–7.37)	7.52 ± 0.43 (0.43–7.47)	0.10 ± 0.04 (0.02–0.17)	0.018	-
BC	3.41 ± 0.54 (3.34–3.47)	4.17 ± 0.71 (4.08–4.26)	0.77 ± 0.06 (0.65–0.87)	0.001	-
ALP in IU/L	49.58 ± 23.33 (46.72–52.43)	55.11 ± 27.85 (51.65–58.57)	5.54 ± 2.27 (1.07–10.00)	0.015	0.064

SD = standard deviation; CI = confidence interval; SE = standard error; BC = buffering capacity; ALP = alkaline phosphatase.

Discussion

The composition of saliva can act as a biomarker for the diagnosis of certain systemic diseases, determination of exposure to harmful substances and general assessment of health and disease status.² Since the second half of the twentieth century, saliva has been used diagnostically; in terms of biological testing, saliva has many advantages over serum, including easily accessible non-invasive sample collection. As such, saliva can play an important role in the early detection and monitoring of drug use.²⁰ Furthermore, various studies have shown that altered salivary pH, BC and ALP levels are associated with the formation or development of dental caries, gingivitis, periodontitis, human immunodeficiency virus (HIV) infection, diabetes, orthodontic tooth movements, cancer, abdominal inflammatory diseases and the early onset of menopause.^{3,4,12,13,21–29}

In the current study, salivary pH was lower among smokers in comparison to non-smokers; although the mean values found seem to reflect a minimal difference between the two groups (7.42 versus 7.52, respectively), even very small alterations in pH can influence salivary enzyme activity.⁶ Additionally, BC was significantly lower among smokers in the current study. In previous research, salivary pH and BC values were reportedly lower among HIV-positive patients; this reduction correlated with an increase in the degree of immunosuppression.²⁴ Salivary pH has also been reported to be lower and BC higher in post-menopausal women.²¹ Age may also have an effect on BC; in one study, BC was found to be higher among the elderly.²² However, no significant differences in pH and BC were noted among individuals with different stages of periodontal disease.²²

In the current study, ALP activity levels were significantly lower in the smoker group than the control group, which may indicate the effect of smoking on salivary enzymes. The lower ALP levels observed among

smokers may be explained by hyperkeratinisation of the oral and gingival mucosa, which could prevent the release of ALP in the saliva. In addition, these changes could also be due to the wide age range of participants in the current study. In contrast to the findings of the current study, some researchers have shown higher ALP levels in smokers.^{30,31} Another study found that smoking had no effect on ALP activity.²⁸ Kibayashi *et al.* noted significantly lower levels of salivary ALP and albumin in current smokers compared to non-current smokers; smoking was therefore considered a risk factor for periodontitis and a potential biomarker for the disease.²³ Higher salivary ALP levels have also been found among individuals with periodontal disease, pancreatitis and appendicitis, with treatment of the disease resulting in lowered enzyme levels.^{3,4,29} Therefore, non-invasive assessment of ALP levels may be useful in the diagnosis and prognosis of periodontal tissue function and treatment monitoring of abdominal inflammatory diseases.^{3,29}

Smoking has been found to cause periodontal disease and bone destruction.³² However, in the current study, smokers with periodontal disease were not included and specific side-effects of smoking on the oral mucosa were not evaluated. Further longitudinal studies among smokers with and without oral manifestations in comparison with a healthy control group in different age- and gender-matched populations are necessary to evaluate the effects of smoking on salivary components and oral conditions.

Conclusion

In the current study, salivary ALP activity levels, pH and BC were significantly decreased among smokers in comparison to non-smokers. These findings are probably due to the deleterious effect of smoking on the oral environment, including saliva. Salivary changes in smokers may therefore be potential biochemical markers of the functional condition of the oral tissues, which could consequently help in the diagnosis of various conditions and the evaluation of the effect of smoking on oral and dental health. Further longitudinal studies should be carried out to understand the true effects of smoking on saliva and its components.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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