

Evaluation of calcium concentration in saliva of Iraqi male smokers

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الخلاصة :

يعد الكالسيوم من العناصر الاساسية ضمن الاسنان والعظام وليس غريبا ان يكون الكالسيوم الاساسي في عدد من الامراض من بينها امراض الكلية و ترقق العظام وانسجة اللثة. يلعب اللعاب دورا اساسيا في تجانس وانتظام تجانس اللعاب وتنظيم مستواه ضمن الفم. تم شمول خمسة عشر عراقيا ضمن اعمار تتراوح من 49-52 سنة، يستهلك من 30-40 سيكارا لكل يوم وبمحتوى نيكوتين يبلغ 0.81-1.2 ملغ لكل سيكارا. تتضمن هذه الدراسة قياس مستوى الكالسيوم المذاب في اللعاب للاشخاص المدخنين و غير المدخنين و اظهرت النتائج ارتفاع مستوى الكالسيوم لدى المدخنين بشكل مميز وتغير المستوى بمعدل يبلغ 0.410 ± 0.538 ملي مول /لتر، وحصول انخفاض في الحامضية 7.12 الى 6.22 درجة هيدروجينية. يعزى سبب التغير الى الارتفاع في مستوى الكالسيوم الى انخفاض الاس الهيدروجيني لللعاب والذي يؤدي انتزاع الكالسيوم من الاسنان واطلاقه الى اللعاب.

Abstract:

Calcium is the major component of bones and teeth, and it is not surprising that disturbances in calcium metabolism have been implicated in most of the major chronic diseases, including renal disease, osteoporosis; and periodontal tissues Saliva is a secretion which plays an important role in initial digestive processes and in maintaining homeostasis of the oral cavity environment. Constant presence of saliva in the oral cavity helps to keep its tissues in healthy condition. Secretion of calcium in saliva depends on many physiological factors such as salivary flow rates and pH of oral environment.

Fifteen Iraqi males were considered in this study with age range 49-52 years old. They were smoking 30-40 cigarette per day with nicotine contain of about 0.81-1.2 mg per cigarette

Significant increasing in calcium concentration was observed in the smokers in comparison with the non-smokers with a mean changes 0.538 ± 0.410 SD. The pH values of saliva were decreased to 6.22 while for the non smokers was 7.12.

Our result predicted elevated levels of calcium concentration of the smoker's saliva than the non smokers. This increasing in concentration we attributed to decrease of pH of saliva which retrieving calcium from teeth or aging process that attributed decrease in skeleton density than release of calcium from bone and teeth to circulation or hormonal changes.

Introduction:

Saliva is a secretion plays an important role in initial digestive processes and in maintaining homeostasis of the oral cavity environment^[1]. Constant presence of saliva in the oral cavity helps to keep its tissues in healthy condition^[2]. The chemical complexity of saliva, have variety of compounds determines its properties and functions it performs in the organism. These include protective, buffering, immunological and digestive functions^[3].

Oral fluid contains plasma electrolytes such as potassium, sodium, chloride, bicarbonate, calcium and many other plasma constituents, such as enzymes, immunoglobulin, and DNA. The total volume of oral fluid produced by an adult may be in excess of 1000 mL/day with typical flows of 0.05 mL/min while sleeping, 0.5 mL/min while spitting, and 1 to 3 mL/min while chewing gum^[4-7].

The traditional methods of tobacco use are smoking, snuffing, chewing and dipping. It was in 18th century when it was discovered that smoking increases the activity of salivary glands. Indeed, this observation has been made by every one who begins smoking^[8]. Pan chewers secreted more saliva as compared to non-chewers on chemical but not on mechanical stimulation leading to concomitant decrease in enzyme and electrolyte contents^[9]. In long-term tobacco users, the taste receptors, a primary site for stimulation of salivary secretion, are constantly exposed to tobacco for long time thus presumably affecting the salivary reflex. Changes in salivary composition and flow rates may compromise the integrity of the soft and hard tissues in the oral cavity, because saliva functions include food and bacteria clearance, mastication and digestion, lubrication, antimicrobial defense, and buffering effect. Saliva is composed of water and organic and inorganic molecules, but a large intra- and inter-subject variability in composition is reported^[10].

This has led to concern among nutritionists that the majority of the United States population does not eat as much calcium as they need. Calcium appetite is

the motivation to seek out or choose calcium-containing items. This implies that animals are capable of detecting calcium or some marker for it ^[11].

The aim of this study is monitoring the calcium changes with pH in smoker's saliva and the effects of this habit on calcium concentration in oral environment. Study the efficiency of calcium reaction with O-cresolphthalein complex in 8-hydroxyquinolinem for analysis of calcium in saliva as sophisticated, fast, precise and not expensive colorimetric method.

Material and method:

Subjects

A group of 15 male mainly from college of dentistry, Al Mustansiria University were considered in this study with age range of 49-52 years old. They were smoking average of 30-40 cigarettes per day with nicotine contains of about 0.8-1.2 mg per cigarette. Another fifteenth male's were considered as control with the same age period without smoking.

All considered smokers and control were free from any oral disease or any other pathogenic conditions.

Saliva sampling for tests:

Saliva specimens were collected into sterile graduated disposable plastic test tubes in conditions without stimulation, in period time between 9:00 and 10:00 hrs, after the last meal. Before collection of the test specimens of the saliva the subjects were rinsed their mouths with water. Saliva than was collected into plastic graduated test tubes with the volume scale accuracy to 0.5 ml. The volumes of the collected saliva were recorded after 5 min, then collecting of saliva was continued to reach the volume of 10 ml. During saliva collection test tubes were placed in a container with ice. Then the collected material was centrifuged at 5,000 rpm for 15 min, frozen at a temperature of -2°C and stored in such conditions until biochemical tests were preformed with period not more than 10 hours ^[12-14].

Method of analysis:

Calcium reacts with cresolphthalein complex in 8-hydroxyquinolinem to form a colored complex (purple color) that absorbs at 570 nm (550–580 nm). The intensity of the color is proportional to the calcium concentration. Color intensifiers and a stabilizer are present to minimize interference by other metallic ions ^[15].

The analysis has been preformed by using optima spectrometer–Japan with spectrum range 400-700 nm. The calcium kit were supplies by Biomegreb- Tunisia.

Results:

Table-1 represents the mean changes of calcium concentration for the 15 smokers with control. The collected data represent significant increase for the

smokers. Figure-1 represents the calibration curve for calcium estimation. Figure-2 represents the mean changes of calcium between the smokers and non-smoker. The figure reported elevation of calcium in smokers. Figure-3 represents the individual changes between the smokers and non smokers with standard deviation. Figure-4 represents the mean pH changes between the smokers and non smokers. The figure showed decrease of the saliva pH of the smokers.

Smokers mean Ca ²⁺ Concentration mmol / L±SD (N=15)	P<0.05	Control Ca ²⁺ Concentration mmol/L±SD(N=15)	P<0.05
0.538 ± 0.4101	0.005	0.323 ± 0.2857	0.001

Table-1: Calcium concentration between smokers and control Where N represents number of subjects.

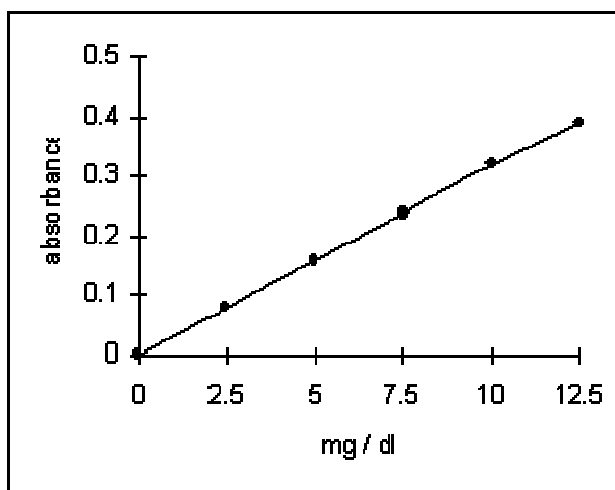


Figure 1: Calibration curve for calcium determination

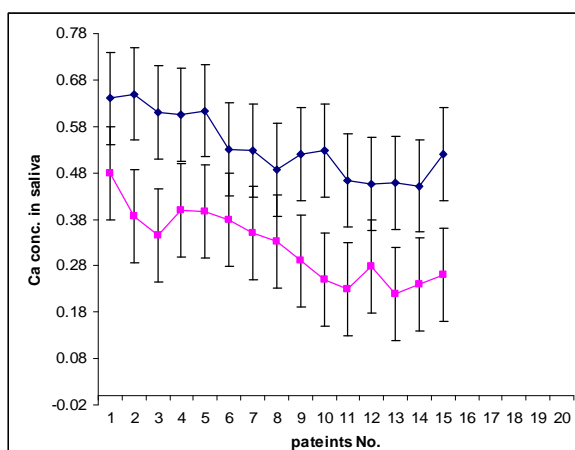


Figure-2: Mean calcium concentration changes in smokers and non smoker's subjects.

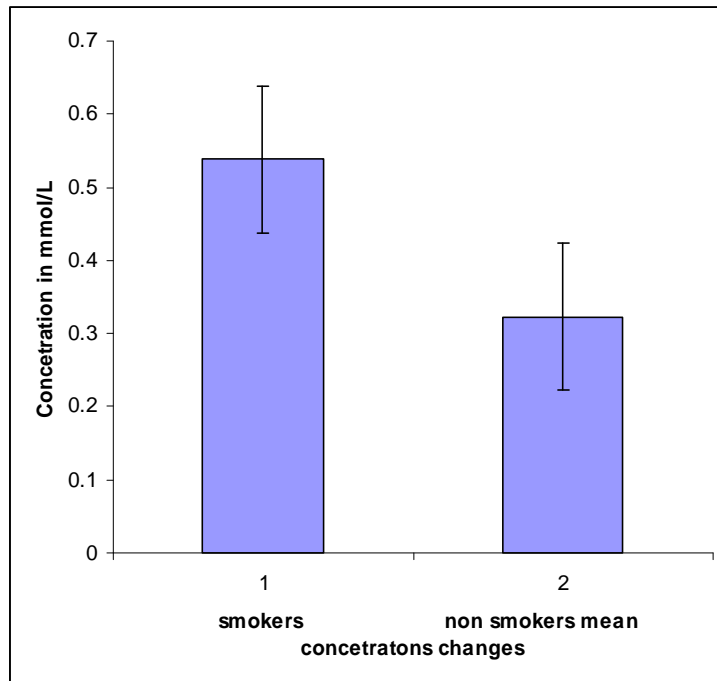


Figure-3: Individual changes in calcium concentration between smokers and non smokers (the upper curve represent smoker while the lower curve represent non-smokers).

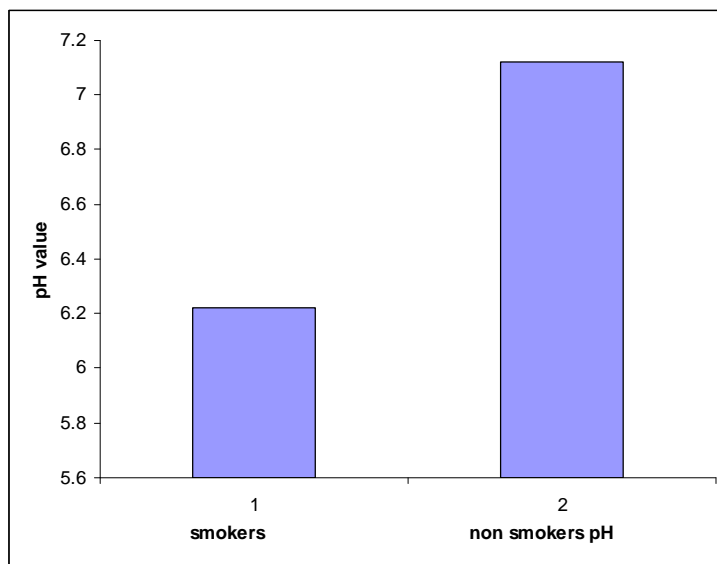


Figure-4: The mean pH changes between smokers and non-smokers

Discussion:

The saliva chemical composition evaluation is greatly affected by the chemical substances that administrated by different activities such as drinking or food. Cigarette smoking could release more than 3000 different compounds with wide Varsity of changes in oral cavity. To eliminate these effects of these factors on the salivary chemical composition, all analysis in this research were preformed at the same time in a day and designed to be at least ten to eleven hours after the last intake of food or drink with careful mouth wash and cleaning considerations.^(16, 17) in this research it had been rejected any subjects admitted meal with a rich calcium or medications included calcium in its compositions. Subjects considered in this study were smoking close values of nicotine amounts and number of cigarettes admitted per day. This step was designed to avoid any variation in nicotine administration on the calcium concentration. However, all Smokers and control were free from oral or systemic diseases, or any medications at the time of the study. Samples were collected in plastic tubes instead of glass to decrease the absorption of calcium to class surfaces. This factor could affect potentially on the level of calcium for the collected saliva^[14].

Our result predicted significant increase ($p < 0.005$) of calcium in the saliva of the investigated smokers. This elevation could be attributing to the following factors first; smoking lowered the pH in the oral cavity for the all investigated males, which may accelerate the removing calcium from the tooth lattice and release the calcium to saliva^[18, 19]. Second, in ageing, decrease in skeletal bone density, frequently detected in elderly people, and associate with increase the amount of calcium in saliva and other physiological fluids^[20-23]. All collected data were not reported in any research included the relation of smoking with calcium concentration in saliva.

References:

- 1- Edgar, M.; Dawes, C. and O'Mullane, D. (2004). Factors influencing salivary flow rate and composition In *Saliva and dental health*; 3rd. ed. Br Dent, J, pp. 32–49.
- 2- Mandel, I.D. (1987) . The function of saliva, *J. Dent Res* 66: 623-627.
- 3- Humphrey, S.P.; Williamson, R..T.(2001). A review of saliva: Normal composition, flow, and function. *J Prosthet Dent* 85: 162-9.
- 4- Herrera, J.L.; Lyons, I.I.M.F.and Johnson, L.F. (1988). Saliva; its role in health and disease. *J. Clin. Gastroenterol* 10:569-578.
- 5- Salvolini, E.; Di, Giorgio, R.; Curatola, A.; Mazzanti, L. and Fratto, G. (1998). Biochemical modifications of human whole saliva induced by pregnancy. *Br J Obstet Gynaecol*, 105:656-660.
- 6- Streckfus, C.S. and Bigler, L.R. (2002). Saliva as a diagnostic fluid. *Oral Diseases*; 8: 69-76

AJPS, 2012, Vol. 11, No.1

- 7- Guyton, A.C.; Hall, J.E.(2000).In: Textbook of Medical Physiology. Harcourt A Health Sciences Company. Philadelphia. WB Saunders and company. 738-53.
- 8- Lawrence, H.P. (2002). Salivary markers of systemic disease: noninvasive diagnosis of disease and monitoring of general health. *J Can Dent Assoc*, 68:170-174.
- 9- Pedersen,A.M.; Bardow, A.; Jensen, S.B.and Nauntofte, B. (2002). Saliva and gastrointestinal functions of taste, mastication, swallowing and digestion. *Oral Dis*, 8:117-129.
- 10- Thylstrup, A.and Fejerskov, O.(1994). Textbook of Clinical Cariology Copenhagen: Munksgaard;
- 11- Gonzalez, M.; Montes, de, Oca, L.and Jiménez, G. (2001). [Changes in saliva composition of pregnant and non-pregnant patients]. *Perinatol reprod hum*, 15:195-201.
- 12- Gitelman, H.J. (1967). An improved automated procedure for the determination of calcium in biological specimens. *Anal Biochem*, 18:521-531.
- 13- Daly, J.A.and Ertingshausen, G. (1972). Direct method for determining inorganic phosphate in serum with the "CentrifiChem". *Clin Chem*, 18:263-265.
- 14- Tietz, N.W. (1976). Fundamentals of clinical chemistry, W.B sanders, Philadelphia. PA, , 897
- 15- Biomaghreb instruction manual, calcium profile enzymatic colorimetric method test, (2002).
- 16- Sreebny, L.M.(2000) .Saliva in health and disease: an appraisal and update. *Int Dent J*, 50:140-161.
- 17- Sewón, L.; Laine, M.; Karjalainen, S.; Doroguinskaia,A.and Lehtonen-Veromaa ,M. (2004).Salivary calcium reflects skeletal bone density of heavy smokers. *Arch Oral Biol.*;49:355–8.
- 18- Sato ,T.P.(2002).A pH curve of human resting saliva sampled with a small paper slip and its medical application. *Pathophysiology* 8:283-290.
- 19- Dawes, C.(2004). Factors influencing salivary flow rate and composition. In *Saliva and oral health* 3rd edition. Edited by: Edgar M, Dawes C, O'Mullane D. London: British Dental Association;32-49.
- 20- Dawes, C.(1974).The effects of flow rate and duration of stimulation on the concentrations of protein and the main electrolytes in human submandibular saliva. *Arch Oral Biol.*;19: 887–9.
- 21- Sewón, L.and Mäkelä,M. (1990). A study of the possible correlation of high salivary calcium levels with periodontal and dental conditions in young adults. *Arch Oral Biol.*35(Suppl):211–2.
- 22- Percival, R..S.;Challacombe,S.J.and Marsh, P.D. (1994) .Flow rates of resting whole and stimulated parotid saliva in relation to age and gender. *J Dent Res.*; 73:1416–20.
- 23- Yeh, C.K.;Johnson, D.A.and Dodds, M.W. (1998). Aging. Vol. 10. Milano: Impact of aging on human salivary gland function a community-based study; pp. 421–8.