Activity of Serum and Salivary $\alpha$-Amylase in Habitual Adult Tobacco Consumers

Moutawakilou Gomina*, Leila Badirou and Simon Ayeleroun Akpona

Training and Research Unit, Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Parakou, Benin

Abstract

Introduction: Tobacco consumption alters many biological parameters, as well as $\alpha$-amylase activity. This research work aims to study the activity of serum and salivary alpha-amylase in habitual adult tobacco consumers.

Methods: We carried out a cross-sectional descriptive and analytical study on 234 adults (54 smokers, 60 snuffers, 60 chewers and 60 tobacco non-consumers). Serum and salivary alpha-amylase was measured using kinetic enzymatic method. ANOVA, and Kruskal-Wallis test were used to compare averages according to cases. Linear regression enabled to establish relationships between duration of consumption, quantity of tobacco consumption, as well as serum and salivary $\alpha$-amylase activity.

Results and conclusion: The mean activity of serum (UI/L) and salivary (10^4 UI/L) alpha-amylase was respectively 110.53 $\pm$ 73.35 and 17.34 $\pm$ 17 for smokers, 109.69 $\pm$ 58.20 and 9.90 $\pm$ 9.44 for snuffers, 92.63 $\pm$ 48.94 and 5.61 $\pm$ 5.38 for chewers and 120.14 $\pm$ 71.99 and 8.73 $\pm$ 6.14 for tobacco non-consumers. A significant difference was observed as regards salivary alpha-amylase between smokers and chewers ($p=0.001$), and between sniffers and chewers ($p=0.002$). The mean activity of serum and salivary alpha-amylase was substantially higher in tobacco non-consumers than in chewers ($p=0.01$ and 0.02, respectively). Correlation was lower and significant in chewers between mean activity, salivary alpha-amylase and duration of tobacco consumption ($r=0.35$; inclination $p=0.006$). Serum and salivary alpha-amylase activity varies according to tobacco consumption mode. Subsequent studies are required to specify the mechanisms put at stake.

Keywords: Alpha-amylase; Serum; Saliva; Tobacco.

Introduction

Tobacco dependence is still the leading cause of avoidable mortality in the world [1]. The World Health Organization (WHO), estimated in 2005 that tobacco use killed 5 million of people at global level, half of which in the developing countries. According to forecasts for 2025-2030, the death toll is expected to rise to 10 million people, including 7 million in the developing countries [2].

Throughout the world, more than one billion of persons smoke everyday, i.e. about one quarter of the adults [3]. Tobacco dependence prevalence is 74% in male subjects and 11% in female ones [3,4]. Whereas tobacco dependence is on the decline in the developed countries, it keeps on increasing in the developing countries [5]. 82% of the smokers are living in the developing countries [3].

In 2009, a survey conducted by the Ministry of Health of Benin in cooperation with WHO revealed that prevalence of tobacco dependence at national level in youth from 10 to 19 years of age is 9.2%. The study on the comprehensive surveillance of tobacco dependence in youth from 13 to 15 years (GYTS) performed in 2003 in the Borgou and Alibori regions (Republic of Benin) showed a prevalence of 25.8%. In Borgou, out of one hundred persons, about eleven smoke tobacco and twenty-two consume smokeless tobacco either snuffed or chewed [6].

Tobacco is consumed in two forms: smoking tobacco through cigarette, pipe, narghile, cigar and smokeless tobacco consisting of snuff tobacco and chewing tobacco [7]. In Benin, tobacco is consumed smoked and smokeless for decades. If smoking tobacco is used throughout the country, smokeless tobacco (snuffed, chewed) represents the traditional consumption mode in vogue in the northern region. Chew is women's privilege and snuff is a habit specific to men.

Alpha-amylase (EC 3.2.1.1) in animals is a major digestive enzyme with optimum pH of 6.7-7.0. It is secreted by the salivary gland and pancreas, and so present in saliva and serum. Alpha-amylase is a calcium-containing metalloenzyme that hydrolyzes the a 1,4 linkages of starch to glucose and maltose [8]. Serum alpha-amylase is commonly measured in the diagnosis of pancreatic disorders. Salivary alpha-amylase has been used as a biomarker for stress that does not require a blood draw [9].

The noxious effects of tobacco dependence on human health are known. In fact, tobacco consumption is the cause of cardiovascular pathologies, pleuropneumonias and cancers [10]. In addition, tobacco consumption modifies several biological parameters, including alpha-amylase [11]. Previous studies carried on tobacco dependence's impact on the activity of serum and salivary alpha-amylase resulted in divergent viewpoints. For Nater et al. [12], smoking tobacco increases the value of alpha-amylase activity in serum and saliva. On the contrary, for other authors, smoking tobacco does not affect the value of serum and salivary alpha-amylase activity [13-15]. Nevertheless, investigations on the influence of smokeless tobacco consumption on alpha-amylase activity are very few. The research works done by Reddy et al. [16] found out an increase in salivary alpha-amylase activity in tobacco chewers.

Except these few works carried out all over the globe on the effect of

*Corresponding author: Moutawakilou Gomina, Training and Research Unit, Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Parakou, Benin, Tel: + 229 95 96 69 40; E-mail: elboutguero@yahoo.fr

Received August 23, 2013; Accepted September 28, 2013; Published October 01, 2013

Citation: Gomina M, Badirou L, Akpona SA (2013) Activity of Serum and Salivary $\alpha$-Amylase in Habitual Adult Tobacco Consumers. Biochem Anal Biochem 2: 140. doi: 10.4172/2161-1009.1000140

Copyright: © 2013 Gomina M, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
smoking and chewed tobacco on serum alpha-amylase activity, which are still arousing controversies, there are no studies on the impact of snuff tobacco on alpha-amylase activity. To the best of our knowledge, in Benin, except epidemiological and clinical studies on tobacco dependence [17,18], there are no studies on the impact of tobacco dependence on biological parameters in general, and on alpha-amylase activity in particular.

Our study aimed to: i) determine the activity of serum and salivary alpha-amylase in habitual adult tobacco consumers, ii) compare these activities between tobacco consumer populations, iii) compare these activities of tobacco consumers, with the ones of tobacco non-consumers, iv) investigate the relationship between serum and salivary alpha-amylase activity and duration of tobacco consumption, on the one hand, and quantity of daily tobacco consumption on the other hand.

Materials and Methods

Ethics

The institutional ethics committee approved this study.

Study site

Our study was conducted in the town of Parakou and in the village called Kpassa (District of Tchaourou) in the Republic of Benin, where the subjects were selected. The medical imaging unit and the biochemistry laboratory of Borgou Regional Hospital (CHD-B) were the facilities where ultrasound scans and samples handling were done, respectively.

Materials

The reagents used in the study were kits for the measurement of serum and salivary alpha-amylase. They were AMYLASE SL (ELITechClinical Systems laboratory, Sees, France).

The appliances used consisted of one Sivma centrifuge (MPW, Poland), one Microlab 300 spectrophotometer (Vital Scientific, Dieren-The Netherlands), and one Mindray ultrasound scanner (Digiprinçipe DP-8800 Plus, Hamburg, Germany).

Methods

Nature and time period of study: We carried out a cross-sectional study with descriptive and analytical purposes, which covered the period from 1st June to 31 August, 2012.

Study population: The subjects of this study were selected among the study setting's population. Before, they gave their well-informed consent after the reading and approval of study purposes.

Sampling

We did a two-degree randomized survey. The first degree consisted in selecting some areas in the town of Parakou on the one hand, and households in the village of Kpassa. For this purpose, we proceeded to drawing without replacement. The second degree concerned with the selection of the subjects who comply with our inclusion criteria. Any subject complying with these inclusion criteria, and who is present the day of data collection was systematically taken into account. This sampling technique enabled us to select 234 subjects distributed as follows (Table 1).

Were included in the study, as tobacco consumers, the adult subjects from both sexes, voluntary, aged 18 years old and more, habitual tobacco smokers, snuffers or chewers, and who freely agreed to participate to the study. As regards tobacco non-consumers, the inclusion criteria were: be aged 18 years old at least, not be an active or passive tobacco consumer and give one’s free and well-informed consent, or with impaired evolution of both glands.

Definition of concept

Was considered as habitual tobacco consumer, any subject who has consumed tobacco once per day, whatever the consumption mode is, during at least thirty days.

Data collection

A questionnaire was administered to each selected subject. Abdominopelvic (centered on pancreas, ovaries in women, liver and spleen) and salivary glands were scanned in each subject to eliminate any pathology affecting these organs likely to modify alpha-amylase activity. Blood sample was drawn and saliva collected from each subject, complying with the inclusion criteria. Different measurements were done on these blood and saliva samples. Two saliva and blood samples were collected at one month interval in each study subject.

Collecting biological samples

The blood samples (4 ml) were taken in the morning through superficial venous puncture on dry tubes, in subjects who had been on empty stomach for 10 hours. Saliva (5 ml) was also collected in the morning, on empty stomach.

The day before taking samples, we got into touch with the target individuals. We first asked them not to eat after 8 p.m. (local hour). Then, we explain them the saliva collection conditions before giving them small sterile bottles. Finally, we notified them our coming back on the next day at 6 a.m. (local hour) to collect blood and saliva samples.

Processing biological samples collected

The blood samples collected were centrifuged at 500×g during five (5) minutes in order to get sera. The latter will be used the same day to determine alpha-amylase activity. Saliva samples collected were also centrifuged at 1000×g during five (5) minutes, and supernatant fluid was used to determine alpha-amylase activity during a maximum of six (6) hours after collection.

Determination of alpha-amylase activity

It was applied to both samples of serum and saliva of each subject collected at one month interval. Alpha-amylase activity was determined through kinetic enzymatic method, which uses CNP-G3 (2-chloro-4-nitrophenyl-alpha-maltotriosid) [19] as substrate.

Study variables

The study variables were serum alpha-amylase activity, salivary alpha-amylase activity, quantity of tobacco smoked, sniffed or chewed daily, duration of tobacco consumption.
Data analysis

Data were analyzed with Excel and Epi Info 3.5.3 softwares. The quantity of daily tobacco consumption was determined by weighing one stick of ordinary cigarette, one ordinary snuff and one ordinary chew. The value of serum and salivary alpha-amylase activity in each subject was obtained by calculating the average of the values of the two determinations performed at one month interval. The qualitative variables were described using ratios, and quantitative variables were described using averages with their standard deviations. ANOVA test or Kruskal-Wallis test served to compare averages according to case. We used linear regression to investigate statistically significant relationships between duration of consumption, quantity of daily tobacco consumption and serum and salivary alpha-amylase activity. If p<0.05, it was considered as statistically significant.

Results

Smokers had the highest values of salivary alpha-amylase activity, whereas chewers had the lowest values; the difference noted was significant (p<0.001) (Table 2).

There was a significant difference for salivary alpha-amylase between smokers and chewers (p<0.001), and between snuffers and chewers (p=0.002) (Table 3).

The mean activity of serum alpha-amylase was higher in tobacco non-consumers than in consumers, with significant difference between chewers and non-consumers (p=0.01). Salivary amylase activity was lower in chewers than in non-consumers, with significant difference (p=0.02) (Table 4). In smokers, serum alpha-amylase activity rises as soon as duration of tobacco consumption becomes more important, with a correlation which is however poor and insignificant. The activity of serum and salivary alpha-amylase in snuffers declines as soon as duration of tobacco consumption becomes more important with, however, a poor and insignificant correlation (r=0.055; inclination p≥0.63). In chewers, serum and salivary alpha-amylase activity rises as soon as duration of tobacco consumption becomes more important, with, however, a poor and significant correlation for salivary alpha-amylase (r=0.35; inclination p=0.006) (Table 5).

The activity of serum and salivary alpha-amylase in tobacco consumers groups (smokers, chewers and snuffes) increased as soon as the quantity of daily tobacco consumption is more important, however, a poor and insignificant correlation (r=0.005; inclination p≥0.26) (Table 6).

Discussion

We studied the activity of serum and salivary alpha-amylase in habitual adult tobacco consumers. However, our research work has some limits. Actually, we were not able to perform a case-control study due to the difficulty in matching individuals according to age and sex. Nevertheless, the representative sample, the sampling mode and the statistical methods used allowed us to get results which contribute to set up a database.

The mean activity of serum alpha-amylase of tobacco non-consumers was lower than in consumers, with significant difference between chewers and non-consumers (p=0.01). The activity of serum alpha-amylase of tobacco consumers groups (smokers, chewers and snuffes) increased as soon as the quantity of daily tobacco consumption was more important, however, a poor and insignificant correlation (r=0.005; inclination p≥0.26) (Table 6).

Table 2: Average values (± standard deviation) of serum and salivary alpha-amylase activity of the study population.

| Activity | Smokers (n=54) | Snuffers (n=60) | p (0.05) | P (0.01) | P (0.02) |
|----------|----------------|----------------|----------|----------|----------|
| Serum α-amylase (UI/L) | 109.53 ± 73.35 | 109.69 ± 58.20 | 0.23 | 0.63 | 0.38 |
| Salivary α-amylase (UI/L) | 17.34 ± 17.00 | 9.90 ± 9.44 | 0.059 | 0.055 | 0.005 |

Table 3: Comparison of serum (UI/L) and salivary (10⁴ UI/L) alpha-amylase activity between tobacco consumers groups.

| Activity | Smokers (n=54) | Non-consumers (n=60) | % increase | % decline | p (0.05) |
|----------|----------------|---------------------|------------|-----------|----------|
| Serum α-amylase (m ± SD) | 110.53 ± 73.35 | 120.14 ± 71.99 | 8.00 | 0.48 | 0.04 |
| Salivary α-amylase (m ± SD) | 17.34 ± 17 | 8.73 ± 6.14 | 98.62 | 0.04 | 0.005 |

Table 4: Comparison of serum (UI/L) and saliva (10⁴ UI/L) alpha-amylase activity between tobacco consumers and non-consumers.

| Activity | chewers (n=60) | Smokers (n=54) | Snuffers (n=60) | r² | P (inclination) |
|----------|----------------|----------------|----------------|----|----------------|
| Equation Y | 0.5977×71.359 | 1213.1×10672 | 70.83×98.532 | -940.95×189377 | -0.3097×114.1 | -1553.6×121098 |
| r² | 0.039 | 0.1219 | 0.0082 | 0.0027 | 0.003 | 0.0284 |
| P (inclination) | 0.13 | 0.006 | 0.51 | 0.71 | 0.67 | 0.19 |

Table 5: Correlation between serum and salivary alpha-amylase activity and duration of tobacco consumption (in year).
consumers and smokers in our study is respectively higher and lower than the one reported by Onyeson et al. [20] (68.3 ± 11.3 and 131.2 ± 17.0 UI/L). On the contrary, it is higher than the one reported by El Garen et al. [21] (15.42 ± 4.97 UI/L and 22.79 ± 9.15 UI/L). The mean activity of salivary alpha-amylase of tobacco non-consumers and smokers is much higher than the one noted by Onyeson et al. [20] in their research work (133.5 ± 8.6 UI/L and 241.8 ± 23.6 UI/L). These differences noted at the level of salivary alpha-amylase activity may be caused by several factors that the different studies did not take into account. Some of those factors, such as physical exercise and stress, activate the sympathetic nervous system [10,22]. Others are known for their influence on the other stress systems, e.g. sex, age, sleepiness and wake up time [10]. Actually, salivary alpha-amylase activity has an increased nyctohemeral rythm with high values in the hour following wake up, and keeps on increasing till the evening [10]. In addition, stress and increased age elevate salivary alpha-amylase activity [10].

The mean activity of serum and salivary alpha-amylase was higher in smokers than in chewers with a significant difference at the level of salivary alpha-amylase. This result confirms the one of Girja et al. [23], who reported that chewed tobacco substantially reduces salivary alpha-amylase activity compared with smoking tobacco. Tobacco non-consumers showed a mean activity for serum amylase higher than the one of the study carried out by Gallegari and Lami [10], and the ones conducted by Nasrallah and Martin [24]. On the contrary, our result is different from the one reported by Onyeson et al. [20], El Garen et al. [21] and Badlin et al. [25] who found out that serum alpha-amylase activity was more elevated in smokers than in tobacco non-consumers with a statistically significant difference. We noted a rate of decline of 8%. On the contrary, Onyeson et al. [20] found out a rate of increase of 84.3%. In our investigative study, the mean activity of salivary alpha-amylase was lower in tobacco non-consumers than in smokers with significant difference. Our result agrees with the one of Onyeson et al. [20]. An increase of 98.62% in the salivary alpha-amylase activity was noted in smokers compared to non-consumers. There is a discrepancy between this observation and previous studies which suggest a significant inhibition of salivary alpha-amylase activity after smoking a stick of cigarette [26]. In vitro studies showed those 3 hours after exposure to cigarette smoke, one observes a significant reduction of 33.8% of alpha-amylase activity in saliva [23]. Some research works report that unsaturated aldehydes contained in the cigarette smoke cause, in conjunction with reactive nitrogen species, the inhibition of salivary alpha-amylase by diminishing its activity [11,27,28]. The mean activity of salivary alpha-amylase was lower in chewers compared with tobacco non-consumers, and a significant difference is noted (p=0.02). Reddy et al. [16] made the same remark. They reported that salivary alpha-amylase concentrations are lower in tobacco chewers, this is probably associated with an increase in salivary flow [29]. It is due to the fact that chewing compresses tooth periodontal membrane [30], and thus, activates the mechanoreceptors of the same membrane [31,32]. The activation of these mechanoreceptors induces salivary hypersecretion, which comes with a decline in salivary alpha-amylase activity through dilution effect [33]. One or many of the following factors had been mentioned by Reddy et al. [16], in the increase of salivary flow in tobacco chewers: nicotine or tobacco effect on other chew components, hyperplasia of salivary glands or chronic hypertrophy of muscles of mastication. On the contrary, for Gregory et al. [34], smokeless tobacco consumers experience a decline in salivary flow which may be significant. According to Taylor [35], the mechanism associated with the decline in salivary flow rate is probably due to the harmful effects of nicotine on exocrine glands secretion. Nicotine has an initial stimulating effect, followed by an inhibition of saliva production. It is evident that nicotine, the major component of tobacco, is also responsible for salivary stimulation in chronic consumption of chewed tobacco [34]. Nevertheless, chewed tobacco may have many other additional factors that influence salivary flow. The precise substance which increases salivary flow and the way it is induced, are not clearly known.

Serum alpha-amylase activity increases in smokers as soon as duration of tobacco consumption becomes more significant, with a correlation, which is however, poor and insignificant (Table 5). This result agrees with the one of El Garen et al. [21], who observed insignificant poor correlations between serum alpha-amylase activity and duration of tobacco consumption (r=-0.159 and inclination p>0.5). Onyeson et al. [20] made the same observation. On the contrary, the mean activity of salivary alpha-amylase declines as soon as duration of tobacco consumption becomes more significant with a correlation, which is however, poor and insignificant (Table 5). Our result is similar to the one reported by Onyeson et al. [20] who noted that duration of tobacco consumption did not significantly influence salivary alpha-amylase activity. Activity of serum and salivary alpha-amylase in chewers rises as soon as duration of tobacco consumption becomes more significant; correlation is however poor and significant for salivary alpha-amylase (r=0.35; inclination p=0.006). Our result confirms the one of El Garen et al. [21] for serum alpha-amylase, whereas the results found by Reddy et al. [16] are contrary to the ours as regards salivary alpha-amylase (duration of tobacco mastication did not influence salivary alpha-amylase activity).

The activity of serum and salivary alpha-amylase increased with the quantity of daily tobacco consumption, with a correlation, which is, however, poor and insignificant in smokers (Table 6). This outcome is identical with the one of Onyeson et al. [20], who reported that the quantity of daily tobacco consumption did not significantly influence the activity of serum and salivary alpha-amylase. The activity of serum and salivary alpha-amylase increased in chewers, as soon as the quantity of daily tobacco consumption is more significant, with a correlation which is, however, poor and insignificant (Table 6). This finding agrees with the one of Reddy et al. [16], who stated that the daily quantity of chewed tobacco did not significantly influence salivary alpha-amylase activity. The values of salivary alpha-amylase activity collected in

| Equation Y | Salivary α-Amylase | Serum α-amylase | Salivary α-Amylase | Serum α-amylase | Salivary α-Amylase | Serum α-amylase | Salivary α-Amylase | Serum α-amylase | Salivary α-Amylase |
|------------|-------------------|----------------|-------------------|----------------|-------------------|----------------|-------------------|----------------|-------------------|
| y = 1.39x+83.83 | -0.14x+111.78 | 0.012x+17.23 | 0.58x+107.02 | -0.26x+11.08 |
| r² | 0.010 | 0.0009 | 0.0002 | 0.0003 |
| P (of inclination) | 0.40 | 0.77 | 0.92 | 0.94 |
| Interpretation | Correlation: poor or nil |
| Degree of significance | NS | NS | NS | NS |
chewers enable us to state the following hypothesis: the more salivary alpha-amylase activity declines, the longer duration of chewed tobacco consumption is.

**Conclusion**

This research work mainly aims to study the activity of serum and salivary alpha-amylase in habitual adult tobacco consumers. It has resulted in miscellaneous remarks. The activity of serum and salivary alpha-amylase is higher in smokers, followed by chewers and nonsmokers. Serum alpha-amylase activity is higher in tobacco non-consumers than in consumers. Salivary alpha-amylase activity is lower in chewers than in non-consumers. There is a poor and significant correlation between mean activity of salivary alpha-amylase and duration of tobacco consumption in chewers.

Therefore, the activity of serum and salivary alpha-amylase varies according to mode of tobacco consumption. Subsequent studies are required for specifying the mechanisms involved or put at stake.

**References**

1. Da Costa e Sylla VL (2005) Health consequences of the tobacco epidemic in West African French-speaking countries and current tobacco control. Promot Educ 4: 7-12.

2. Albert H (2005) Tobacco control in Africa. International Journal of Health Promotion and Education Supplement 4: 4.

3. Anderson P (2006) Global use of alcohol, drugs and tobacco. Drug Alcohol Rev 25: 489-502.

4. Bombard JM, Pederson LL, Nelson DE, Malarcher AM (2007) Are smokers only using cigarettes? Exploring current polytobacco use among an adult population. Addict Behav 32: 2411-2419.

5. Fakhfakh R, Hsairi M, Aouch N (2005) Epidemiology and prevention of tobacco use in Tunisia: A review. Prev Med 40: 652-657.

6. Ministry of Health (2011) Tobacco control. BENIN.

7. O'Loughlin J, Karp I, Koulis T, Paradis G, Difranza J (2009) Determinants of first puff and daily cigarette smoking in adolescents. Am J Epidemiol 170: 585-597.

8. Zakowski JJ, Bruns ED (1985) Biochemistry of human alpha-amylase isoenzymes. Crit Rev Clin Lab Sci 2: 283-322.

9. Noto Y, Sato T, Kudo M, Kurata K, Hirota K (2005) The relationship between salivary biomarkers and state-trait anxiety inventory score under mental arithmetic stress: A pilot study. Anesth Analg 101: 1873-1876.

10. Callegari C, Lami F (1984) Cigarette smoking and salivary amylase activity. Gut 25: 909.

11. Weiner D, Khankin EV, Levy Y, Reznick AZ (2009) Effects of cigarette smoke borne reactive nitrogen species on salivary alpha-amylase activity and protein modifications. J Physiol Pharmacol 60: 127-132.

12. Nater UM, Rohleder N, Schlott W, Ehlert U, Kirschbaum C (2007) Determinants of the diurnal course of salivary alpha-amylase. Psychoneuroendocrinol 32: 392-401.

13. Nagaya T, Okuno M (1993) No effects of smoking or drinking habits on salivary amylase. Toxicol Lett 66: 257-261.

14. Kivelä J, Parkkila S, Metteri J, Parkkila AK, Toivanen A, et al. (1997) Salivary carbonic anhydrase VI concentration and its relation to basic characteristics of saliva in young men. Acta Physiol Scand 161: 221-225.

15. AvÅ¥ar A, Daro O, Bodrumlu EH, Bek Y (2009) Evaluation of the relationship between passive smoking and salivary electrolytes, protein, secretory IgA, sialic acid and amylase in young children. Arch Oral Biol 54: 457-463.

16. Reddy MS, Naik SR, Bagga OP, Chuttani HK (1980) Effect of chronic tobacco-betel-lime “gudh” chewing on human salivary secretions. Am J Clin Nutr 33: 77-80.

17. Fayoni B, Josse R, Djivoh C, Gaschet X, Zohoun Th (1992) Tobacco use among health workers in the Oueme Region in Benin. Med Afr Noire 39: 516-520.

18. Fourn L, Ducu S, Seguin L (1999) Smoking and intrauterine growth retardation in Republic of Benin. J Epidemiol Commun Health 53: 432-433.

19. Gella FJ, Guberi G, Vidal R, Canalias F (1997) Determination of total and pancreatic alpha-amylase in human serum with 2-chloro-4-nitrophenyl-alpha-D-maltotrioside as substrate. Clin Chim Acta 259: 147-160.

20. Onyeson I, Osiona E, Ille EJ, Oueue OT (2012) Activities of alpha-amylase in serum and saliva of some Nigerian cigarette smokers. Life Sci 26: 28-30.

21. El Garen N, Abdel Aziz AT, Tawadrous G (1991) Serum activities of some pancreatic enzymes in cigarette smokers. Sci Med J Cai Med Synd 3: 33-44.

22. Walsh NP, Blannin AK, Clark AM, Cook L, Robson PJ, et al. (1999) The effects of high-intensity intermittent exercise on saliva IgA, total protein and alpha-amylase. J Sports Sci 17: 129-134.

23. Ginja KP, Sundharam BS, Krishnan PA, Devi CS (2002) Biochemical changes of saliva in chewers tobacco smokers, alcohol consumers, leukoplakia and oral cancer patients. Indian J Dent Res 13: 102-107.

24. Nasrallah SM, Martin DM (1983) Serum isoamylase as a test for pancreatic insufficiency. Gut 24: 161-164.

25. Balldin G, Borgström A, Edelander A, Genell S, Hagberg L, et al. (1980) Elevated serum level of pancreatic secretory proteins in cigarette smokers after secretin stimulation. J Clin Invest 66: 159-162.

26. Zappacosta B, Persichilli S, De Sole P, Mordente A, Giardina B (1999) Effect of smoking one cigarette on antioxidant metabolites in the saliva of healthy smokers. Arch Oral Biol 44: 485-488.

27. Nagler R, Lischinsky S, Diamond E, Drigues N, Klein I, et al. (2000) Effect of cigarette smoke on salivary proteins and enzyme activity. Arch Biochem Biophys 379: 229-236.

28. Weiner D, Levy Y, Khankin EV, Reznick AZ (2008) Inhibition of salivary amylase activity by cigarette smoke aldehydes. J Physiol Pharmacol 59: 727-737.

29. Rooban T, Mishra G, Elizabphet J, Ranganathan K, Saraswathi TR (2006) Effect of habitual arecanut chewing on resting whole mouth saliva flow rate and pH. Indian J Med Sci 60: 95-105.

30. Linden RW, Millar BJ (1988) The response characteristics of mecanoreceptors related to their position in the cat canine periodontal ligament. Arch Oral Biol 33: 51-56.

31. Anderson DJ, Hector MP (1987) Periodontal mecanoreceptors and parotid secretion in animals and man. J Dent Res 66: 518-523.

32. Hector MP, Linden RW (1987) The possible role of periodontal mecanoreceptors in the control of parotid secretion in man. J Exp Physiol 72: 285-301.

33. Larson PS, Haag HB, Silvette H (1961) Tobacco experimental and clinical studies. Williams & Wilkins Co., Baltimore, USA.

34. Gregory RL, Kindle JC, Hobbbs LC, Malmstrom HS (1991) Effect of smokeless tobacco use in humans on mucosal immune factors. Arch Oral Biol 36: 341-347.

35. Zappacosta B, Persichilli S, De Sole P, Mordente A, Giardina B (1999) Effect of smoking one cigarette on antioxidant metabolites in the saliva of healthy smokers. Arch Oral Biol 44: 485-488.

36. Weiner D, Levy Y, Khankin EV, Reznick AZ (2008) Inhibition of salivary amylase activity by cigarette smoke aldehydes. J Physiol Pharmacol 59: 727-737.

37. Rooban T, Mishra G, Elizabphet J, Ranganathan K, Saraswathi TR (2006) Effect of habitual arecanut chewing on resting whole mouth saliva flow rate and pH. Indian J Med Sci 60: 95-105.

38. Linden RW, Millar BJ (1988) The response characteristics of mecanoreceptors related to their position in the cat canine periodontal ligament. Arch Oral Biol 33: 51-56.

39. Anderson DJ, Hector MP (1987) Periodontal mecanoreceptors and parotid secretion in animals and man. J Dent Res 66: 518-523.

40. Hector MP, Linden RW (1987) The possible role of periodontal mecanoreceptors in the control of parotid secretion in man. J Exp Physiol 72: 285-301.

41. Larson PS, Haag HB, Silvette H (1961) Tobacco experimental and clinical studies. Williams & Wilkins Co., Baltimore, USA.

42. Gregory RL, Kindle JC, Hobbbs LC, Malmstrom HS (1991) Effect of smokeless tobacco use in humans on mucosal immune factors. Arch Oral Biol 36: 35-51.

43. Taylor P (1980) Ganglionic stimulating and blocking agents. In: Gilman AG, Goodman LS, Gilman A Goodman, The pharmacological basis of therapeutics, 6th Ed., Macmillan Publishing Co, New York, USA 592-607.