



Trade Science Inc.

ISSN : 0974 - 7427

Volume 5 Issue 5

BioCHEMISTRY

An Indian Journal

Regular Paper

BCAJJ, 5(5), 2011 [306-309]

Salivary α -amylase activity among cigarette smokers in Awka, Anambra state, Nigeria

B.N.Enemchukwu*, J.K.Emeh, K.I.Ubaoji, I.O.Igwilo

Department of Applied Biochemistry, Nnamdi Azikiwe University, P.M.B., 5025, Awka, Anambra State, (NIGERIA)

E-mail: achylesma@yahoo.com

Received: 2nd September, 2011 ; Accepted: 26th September, 2011

ABSTRACT

Alpha – amylase enzyme activity was determined in the saliva samples collected from two hundred and fifty (250) volunteers who were not known to have any disease of the buccal cavity. The volunteers comprise two hundred (200) smokers of different categories and fifty (50) non-smokers (control) randomly selected from Awka, Anambra State, Nigeria. All the volunteer subjects were within the age bracket of 23-40 years. The analysis of the saliva samples using buffered starch substrate solution revealed specific activities ranging from 1.27 to 2.32 U/mg for non smokers, 1.30 to 2.45 U/mg for light smokers (≤ 10 sticks/day) and 1.27 to 2.38 U/mg for heavy smokers (≥ 11 sticks/day). Mean values of 1.76 ± 0.30 U/mg and 1.77 ± 0.30 U/mg were obtained for light and heavy smokers respectively while the control group (ie non smokers) has a mean activity of 1.79 ± 0.33 U/mg. These mean values were subjected to a statistical test for differences in means using students t-test which revealed no significant difference at 95% level of confidence interval. From the findings in this study, it is evident that cigarette smoke does not alter salivary alpha-amylase activity and protein level. © 2011 Trade Science Inc. - INDIA

KEYWORDS

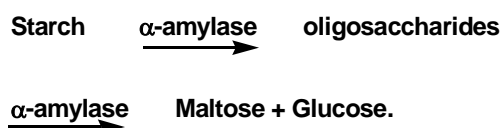
Alpha-amylase activity;
Saliva;
Cigarette smokers;
Pancreas.

INTRODUCTION

Alpha-amylase (α -1, 4-glucan 4-glucanohydrolase E.C. 3.2.1.1) is an enzyme that degrades starch to oligosaccharide and then to glucose and maltose by hydrolyzing α -1,4glucan bonds^[1]. It is the major form of amylase found in humans and other mammals^[2]. The enzyme also known as ptyalin is found in saliva and pancreatic secretions where they serve an obvious role in polysaccharide digestion. More surprisingly, α -amylase is also found in whole blood, sweat, urine and tears

possibly for antibacterial activity^[3].

In digestion, the role of α -amylase is primarily the first reaction of the process, generating oligosaccharides that are then hydrolyzed by other enzymes.



Salivary amylase pH in the mouth is slightly acidic at 6.8, within which the enzyme amylase is able to function. Amylase present in the stomach is deactivated due

to the presence of hydrochloric acid, also known as gastric acid which creates an acidic environment in which amylase is denatured. In gastric juice adjusted to pH 3.3, ptyalin was totally inactivated in 20 minutes at 37°C. In contrast, 50% of amylase activity remained after 150 minutes of exposure to gastric juice at pH 4.3^[4].

α -amylase determination has been recognized as an important diagnostic tool for many years because elevated levels of the enzyme are associated with pancreatic disorders as well as other diseases^[5-7].

The test for amylase is easier to perform than that for lipase, making it the primary test used to detect and monitor pancreatitis. In certain cases, levels of amylase may be elevated or reduced, inducing symptoms that require pathological investigation. Generally, abnormal levels of amylase may be linked to pancreatic problems. Some of the situations that may cause abnormal amylase levels in blood are as follows: Pancreatitis which is caused by inflammation of the pancreas where there are elevated amylase levels in blood, which may be due to damage to the cells that produce amylase. This may be acute or chronic. Chronic pancreatitis is often associated with alcoholism, though it can also be caused by trauma to the pancreas^[8]. Pancreatic cancer can also lead to an increase in amylase levels, as can gall bladder attacks and any blockages in the pancreatic duct. Cystic fibrosis is another cause of elevated amylase levels that may be seen with pancreatic disorders.

Increased plasma levels of amylase in humans are also found in, salivary trauma (including anaesthetic intubation), mumps which occur due to inflammation of the salivary glands and renal failure, due to reduced excretion^[9]. In human physiology, α -amylase of pancreatic and salivary origin have their respective isoforms that behave differently on isoelectric focusing, and can therefore be separated in testing using specific monoclonal antibodies. Salivary α -amylase has also been used as a biomarker for stress that does not require a blood draw^[10].

The salivary amylase gene has undergone duplication during evolution, and DNA hybridization studies indicate that many individuals have multiple tandem repeats of the gene. The number of gene copies correlates with the levels of salivary amylase, as measured by protein blot assays using antibodies to human amylase. Gene copy number is associated with apparent

evolutionary exposure to high starch diets^[11].

There could not be a single study that directly measures the effects of cigarette smoke on the digestion of starch by amylase. But few studies are available which can monitor the effects of cigarette smoking or the effects of nicotine on salivary amylase activity or levels^[12]. It has been known from one study that the level of amylase activity in the saliva of people who smoke cigarette was not significantly different from that present in saliva of non smokers^[12]. Another study found that acute administration of nicotine to non smokers was associated with increased salivary amylase activity and protein levels^[9].

Therefore by determination of salivary alpha-amylase activity among cigarette smokers, it would be possible to ascertain clearly the effects of cigarette smoking on the activity of salivary alpha-amylase enzyme.

EXPERIMENTAL

Two hundred and fifty (250) volunteers (who were not known to have any disease of the buccal cavity) were randomly selected from Awka, Anambra State, Nigeria. The volunteers comprise one hundred (100) light smokers (≤ 10 sticks/day) and one hundred (100) heavy smokers (≥ 11 sticks/day). And fifty (50) non smoker subjects were used as control. All the subjects were within the age bracket of 23 – 40 years. About 1.0ml of saliva was collected from each subject using specimen sample bottles and diluted to 1:100 with physiological saline (0.85% NaCl) using the technique proposed by Jose and Marriana^[13]. The specimens were stored at 0°C to 4°C until analysis which could keep them stable for at least one year^[8]. The starch substrate used in the analysis was subjected to thorough washing in order to reduce the viscosity^[14] using dilute NaOH (0.25%) and then allowed to dry at room temperature. Then, the buffered starch substrate solution was prepared using 15g of the dried starch suspended in 100ml of the buffered solution, and then another 900ml of the buffer which was heated to boiling point was added to the starch suspension, forming a gel. The pH of the buffer was about 6.9 but the final pH of the starch gel was 7.0 because of the alkalinity of the purified starch.

Then glucose standard curve was calibrated using

Regular Paper

a glucose oxidase Kit (GOD/PAP Kit) containing a working glucose standard and a buffered glucose oxidase reagent. With this glucose standard curve, the amount of the reducing sugar (glucose) in the buffered starch substrate was determined. Also protein standard curve was calibrated using a standard Bovine Serum Albumin (BSA) in which 0.5g of BSA was weighed and dissolved in one litre of distilled water giving a standard BSA concentration of 500 μ g/ml. This curve is then used to estimate protein concentration of the saliva samples^[15,16].

ALPHA-AMYLASE ENZYME ASSAY

Routine analysis of the saliva samples were carried out with the buffered starch substrate using the saccharogenic method of determination of human alpha-amylase^[6] releasing glucose. The glucose standard curve was used to deduce the concentration of glucose released by each subject.

RESULTS AND DISCUSSION

The analysis of the saliva samples for different group of subjects revealed specific activities ranging from 1.27 to 2.32U/mg for non smokers, 1.30 to 2.45U/mg for light smokers (≤ 10 sticks/day) and 1.27 to 2.38U/mg for heavy smokers, (≥ 11 sticks/day). The summary of mean saliva α -amylase specific activities as shown in table 1 were 1.76 ± 0.30 U/mg, 1.77 ± 0.30 U/mg and 1.79 ± 0.33 U/mg for light smokers, heavy smokers and non smokers respectively. These mean values were subjected to a statistical test for difference in means using the students t-test which revealed no significant difference at 95% level of confidence interval (table 4).

α -amylase of salivary origin as observed in this study almost share identical catalytic characteristics with those from other sources. In this study, the observed range of α -amylase activity varies from 83.25U/L to 185.92 U/L for light smokers (≤ 10 sticks/day), 81.40 U/L to 185.92 U/L and 81.40 U/L to 185.92 U/L for heavy smokers (≥ 11 sticks/day) and for non smokers respectively. Salivary α -amylase as observed in this study showed maximum stability for 7 days or more when stored between 0 $^{\circ}$ C to 4 $^{\circ}$ C as recorded for alpha-amylase of pancreatic and urinary origin^[8]. Results from

statistical analysis conducted on the different groups showed that the differences between the mean values for those subjects were not significant. Furthermore, the mean values of specific activities as drawn in a bar chart in figure 1, showed a non significant disparity indicating that cigarette smoking may not account for the incidence of pancreatitis, pancreatic cancer and other related diseases in people who smoke cigarette.

It is obvious therefore from this study that cigarette smoke does not inhibit amylase digestion of starch, in accordance with earlier studies of Nagaya and Okuno^[12], although Maier *et al.*^[9] reported that acute administration of nicotine to non-smokers was associated with increased salivary amylase activity and protein level.

CONCLUSION

It can be concluded, therefore from the findings in this study, that cigarette has no effect on salivary α -amylase digestion of starch and that the amount of amylase activity in saliva of people who smoke cigarette was not significantly different from that present in the saliva of non-smokers.

ACKNOWLEDGEMENT

I wish to express profoundly my overwhelming joy and sincere appreciation of the ever ready assistance and encouragement constantly received from my wife Mrs. Ifeyinwa Enemchukwu.

May I acknowledge also the wonderful, invaluable and dynamic contributions of Dr Ezenwa Chukeze and Prof Emeka Ezeonu from Applied Biochemistry Department of Nnamdi Azikiwe University. Awka.

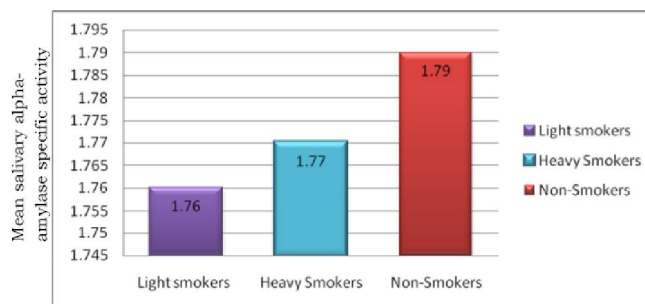


Fig. 1 variation in mean values of salivary alpha amylase enzyme specific activity among smokers and non-smokers (control)

REFERENCES

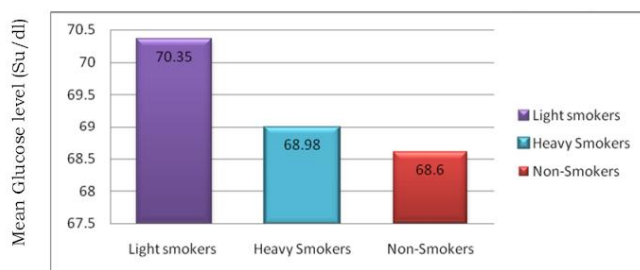


Figure 2 variation in mean values of glucose level among smokers and non-smokers (control)

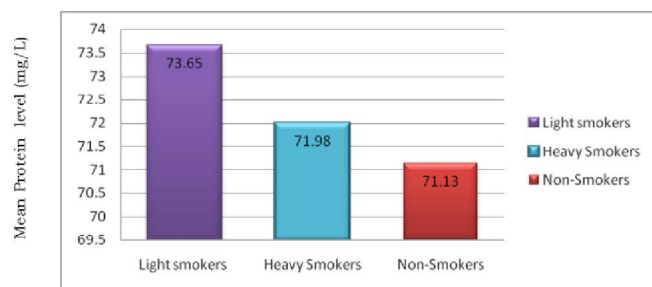


Figure 3 variation in mean values of protein level among smokers and non-smokercontrol)

I wish also to acknowledge with great thanks the invaluable encouragement, the very solid assistance and inspiration constantly received from Dr (Mrs.) Josephine O. Nebedum. She, like a mother, sister, colleague and companion so to speak has always motivated my zeal to successful completion of this prospective study. My unreserved thanks and sincere appreciation also goes to Mr. Joe Onwuatuwegwu, the director of Joe Medics Laboratories, Nnewi where some of the practicals in this study was carried, Miss Benedette Anajekwu, the technologist then with Joe Medics Laboratories, Nnewi.

- [1] B.P.Maureen; Stedman's Medical Dictionary (27th edition), Baltimore, Maryland, USA: Lippincott Williams & Wilkins, P.65, (2000).
- [2] D.Voet, J.G.Voet; Biochimie, (2nd edition), Bruxelles, De Boeck, P.1583, (2005).
- [3] G.Geerling, K.Honick, C.Schroder, C.Framme, P.Seig, I.Lauer, H.Pagel, M.Kirschstein, M.Seyfarth, Marx, Am, H.Laqua; Graefes Arch Clin.Exp.Ophthalmol, **238**, 45-52 (2000).
- [4] M.Fried, S.Abramson, J.H.Meyer; Digestive Diseases and Sciences **32**, 1097-1103 (1987).
- [5] R.L.Searcy, P.Wilding, J.E.Berk; Clin.Chim.Acta., **15**, 187-197 (1967).
- [6] R.J.Henry, N.Chiamori; Clin.Chem., **5**, 434-451 (1960).
- [7] D.S.Young, L.C.Pestaner, V.Gilbberman; Clin.chem., **21**, 10-15 (1975).
- [8] K.Lorentz; Frankfurt, TH-Books, Vertagesellschaft, pp192-202, (1998).
- [9] H.Maier, L.Jarczyk, G.Scherer, I.A.Born; Laryngo-Rhino-Otologie., **70**, 24-26 (1991).
- [10] Y.Noto, S.Tetsumi, K.Mihoko, K.Kiyoshi, H.Kazuyoshi; A pilot study, Anesthesia & Analgesia (United States, Lippincott Williams & Wilkins) **101**, 1873-1876 (2005).
- [11] G.H.Perry; Nature Genetics., **39**, 1256-1260 (2007).
- [12] T.Nagaya, M.Okuno; Toxicology letters., **66**, 257-261 (1993).
- [13] N.Jose, F.L.Marriana; Fluoride., **34**, 55-60 (2001).
- [14] M.Somogyi; Clin.Chem., **6**, 23-34 (1960).
- [15] D.Lowry, N.J.Rosebrough, A.L.Farr, R.J.Randall; J.Biol.Chem., **193**, 265-275 (1951).
- [16] S.Sengupta, M.K.Chattopadhyay; J.Pharm. Pharmacol., **45**, 80-92 (1993).
- [17] R.M.Nagler, I.Klein, A.Z.Reznick; Harefuah., **140**, 614-618,677 (2001).
- [18] F.P.Brooks; New Eng.J.Med., **286**, 300-303 (1972).
- [19] W.B.Salt, S.Schenker; Amy.Its Clin.Sig.Med., **55**, 269-289 (1978).
- [20] P.Chowdhury, R.Do, K.Inoue, H.Kaji, P.L.Rayford; Int.J.Pancreatology., **18**, 256-264 (1995).