

1 Introduction and Literature Review

1.1 Introduction

Tobacco dependence is still the leading cause of avoidable mortality in the world (Albert, 2005). 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 raise to 10 million people, including 7 million in the developing countries (Albert, 2005). Throughout the world, more than one billion of persons smoke every day, i.e. about one quarter of the adults (Anderson, 2006). Whereas tobacco dependence is on the decline in the developed countries, it keeps on increasing in the developing countries (Fakhfakh *et al.*, 2005). 82% of the smokers are living in the developing countries (Anderson, 2006). Tobacco is consumed in two forms: smoking tobacco through cigarette, pipe, narghile, cigar and smokeless tobacco consisting of snuff tobacco and chewing tobacco (Oloughlin *et al.*, 2009). In Sudan, tobacco is consumed smoked and smokeless for decades. If smoking tobacco is used throughout the country, smokeless tobacco (snuffed) represents the traditional consumption mode.

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 α 1,4 linkages of starch to glucose and maltose (Nater *et al.*, 2007). Serum α -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 (Noto *et al.*, 2005).

The noxious effects of tobacco dependence on human health are known, in fact, tobacco consumptions the cause of cardio vascular pathologies, pleura pneumonias and cancers (Gomina *et al.*, 2013). In addition, tobacco consumption modifies several biological parameters, including α -amylase (Weiner *et al.*, 2009). Smoking tobacco increases the value of α -amylase activity in serum and saliva. Saliva α -amylase concentrations are lower in tobacco snuffers, this associated with an increase in saliva flow (Rooban *et al.*, 2006). It is due to the fact that nicotine in this tobacco snuffing is absorbed primarily through the skin in the mouth (CDC, 2009), and compresses tooth periodontal membrane activate mechanoreceptors (Anderson and Hector, 1987), the activation of these mechanoreceptors induces salivary hypersecretion, which comes with a decline in salivary α -amylase activity through dilution effect (Larson *et al.*, 1961).

1.2 Literature Review

1.2.1 Tobacco

1.2.1.1 Tobacco Smoking and Smokeless Tobacco

Tobacco is a product processed from the dried leaves of plants in the genus *Nicotiana*. It can be used as a pesticide, and extracts form ingredients of some medicine, but is most commonly consumed as a drug (Geiss and Kotzias, 2007). Tobacco is a name for any plant of the genus *Nicotiana* of the Solanaceae family (nightshade family) and for the product manufactured from the leaf used in cigars and cigarettes, snuff, pipe tobacco, chewing tobacco, and flavored shisha (Geiss and Kotzias, 2007). The health effects of tobacco are the circumstances mechanisms, and factors of tobacco consumption on human health, epidemiological research has been focused primarily on cigarette tobacco smoking which has been studied more extensively than any other form of consumption (Mayo, 2009).

1.2.1.2 History of Tobacco

Tobacco is a plant grows natively in north and South America, it is believed the tobacco began growing in the America as about 6.000 B.C (Goodman and Jordan, 1993). During world war1, it was typical for tobacco products to be included in military nations. Following the war, cigarette smoking was advertised as part of care free life style, and become socially acceptable for woman (Cury *et al.*, 1998).

During the 1980, there were mainly low suits filed against the tobacco industry because of harmful effects of it is products (Goodman and Jordan, 1993). During the 80th and 90th, the tobacco industry starts marketing heavily in area outside USA, especially developing countries (Tigani, 1993). In the 1930 medical and military leaders grew concerned with the possibility that tobacco might be hazardous to human health (Cury *et al.*, 1998). Tobacco is an agriculture crop in Darfur state in western Sudan; it constitutes the backbone of local economy in this area (Tigani, 1993).

1.2.1.3 Types of Tobacco Consumer

1.2.1.3.1 Tobacco Smoking

Commercially manufactured cigarettes are seemingly simple objects consisting of mainly tobacco blend which may contain over 100 ingredients including tar and fla-vourants. Tar, a term used to describe the toxic chemicals found in cigarette is present in all cigarettes and tends to increase as the cigarette is burnt down. Tar in cigarette paralyzes the cilia in the lungs and

contribute to lung diseases such as emphysema, bronchitis, lung cancer (Onyesom *et al.*, 2012), smoking being a major risk factor for heart attacks, strokes, chronic obstructive pulmonary disease (COPD) and cancer. Children who grow up in a home where one or both of their parents smoke have twice risk of getting asthma and asthmatic bronchitis. They also have a higher risk of developing allergies (passive smoking). Smoking also causes peripheral vascular disease and Hypertension (Vainio, 1987). The effects depend on the number of years that a person smokes and on how much the person smokes. Starting smoking earlier in life and smoking cigarettes higher in tar increases the risk of these diseases (Vainio, 1987).

Smoking is a greater cause of death and disability than any single disease, says the world health organization (WHO), the United States centers for disease control and prevention describes tobacco use as (the single most important preventable risk to human health in developed countries and an important cause of premature death worldwide). Cigarettes sold in under developed countries tend to have higher tar content and, are less likely to be filtered, potentially increasing tobacco – related disease in these region (Nichter and Cartwrite, 1991). Cigarettes smoking contain over 4000 different chemicals, 400 of which are proven carcinogens, these carcinogens include aromatic amines, nitrosamines, oxidants such as oxygen free radicals and also high concentrations of toxic volatile aldehydes, such as acrolein, crotonaldehyde (a,b-unsaturated aldehydes) and acetaldehyde (saturated aldehyde), all of which presumably are major causes of damage to various molecules exposed to Cigarettes smoking (Weiner *et al.*, 2008).

1.2.1.3.2 Smokeless Tobacco

The two main types of smokeless tobacco in the United States are chewing tobacco and snuff. Chewing tobacco comes in the form of loose leaf, plug, or twist. Snuff is finely ground tobacco that can be dry, moist, or in sachets (tea bag-like pouches). Although some forms of snuff can be used by sniffing or inhaling into the nose, most smokeless tobacco users place the product in their cheek or between their gum and cheek. Users then suck on the tobacco and spit out the tobacco juices, which is why smokeless tobacco is often referred to as spit or spitting tobacco. The nicotine in this tobacco is absorbed primarily through the skin in the mouth. Smokeless tobacco is a significant health risk and is not a safe substitute for smoking cigarettes. Smokeless tobacco contains 28 cancer-causing agents (carcinogens). It increases the risk of developing cancer of the oral cavity, is strongly associated with leukoplakia (a lesion of the soft tissue in the

mouth that consists of a white patch or plaque that cannot be scraped off) and recession of the gums (Guide, 2011). Using smokeless tobacco can lead to nicotine addiction and dependence and is not a safe alternative to smoking (CDC, 2009). Smokeless tobacco can stain the teeth, cause bad breath, tooth erosion, dental caries, tooth loss, a decreased sense of taste, alveolar bone destruction and gingival recession (Bharati *et al.*, 2013).

1.2.1.3.3 Other Forms of Tobacco Used

Cigar is the tightly rolled bundles of dried and fermented tobacco. Like other forms of tobacco use, cigar smoking poses a significant health risk depending on dosage (Symm *et al.*, 2005).

Hookahs Water pipe, narghile is a single or multi-stemmed water pipe for smoking. And has gained immense popularity, especially in the Middle East, and operates by water filtration and indirect heat. The water moisture induced by the hookah makes the smoke less irritating and may give a false sense of security and reduce concerns about true health effects. Additionally water in the hookah base is not changed after every session that renders the water contaminated to a greater degree and thus a possible source of dissolved carcinogens (Knishkowsky and Amitai, 2005).

1.2.2 Alpha-Amylase

Alpha-Amylase (AMY EC 3.2.1.1) 1, 4- α -D glucanohydrolase, a group of Hydrolyases that split complex carbohydrates constituted of α -D-glucose Unit linked through carbon atom 1 and 4 located on adjacent glucose residues. Both straight chain (linear) polyglucans such as amylose and branched polyglucans such as amylopectin and glycogen are hydrolyzed, although at different rates. The enzyme splits the chains at alternate α -1,4-hemiacetal (-C-O-C-) links forming maltose, glucose and residue of limit dextrin (Mayne, 1994). Alpha-Amylase is a small heterogeneous enzyme, with a molecular weight of 50-55 KDa, and it exists in different isoenzyme forms, salivary type (S-type) and pancreatic type (P-type). Several different cells and tissues synthesize the salivary type amylase, e.g. (salivary, lacrimal, sweat, lactating mammary) glands; genital tissues, lung; bronchogenic and ovarian tumors; leukocytes and thrombocytes cells (Merritt and Karn, 1977), but the pancreatic type (P-type) amylase is essentially tissue specific, and semen is being the only other source (Shimamura *et al.*, 1976).

1.2.2.1 Salivary Glands

Salivary glands are composed of specialized epithelial cells, and their structure can be divided into two specific regions: the acinar and the ductal regions. The acinar region is where fluid is generated and most of the protein synthesis and secretion takes place (Whelton, 1996). In human, the saliva is secreted by three pairs of major (large) glands, and by some minor (small) glands situated in the oral and pharyngeal membrane (Mader, 2001).

The major glands are: Parotid Glands are the largest of the saliva glands; they are situated at the side of the face, just below and in front of the ear. Each gland, weighs about 20 to 30 gm in adult & about 25% of the daily saliva secretion is contributed by them. This type of glands is made up of serous cells Predominately (Mader, 2001). These glands secrete thin and watery saliva. Submaxillary Glands are otherwise known as submandibular glands. These are located in submaxillary triangle medial to mandible. Each gland weighs 8 to 10 gm and about 70% of the daily secretion of saliva is contributed by the submaxillary glands (Mader, 2001). These are made up of both serous and mucus cells (Sembulingam, 2005), Sublingual Glands These are the smallest of the three major salivary glands, and are situated in mucosa at the floor of the mouth. Each gland weighs about 2 to 3 gm. It contributes only 5% of the saliva daily secretion (Sembulingam, 2005). These are made up of both serous and mucus cells. On the other hand, the minor salivary glands include: Lingual mucus glands, buccal glands and palatal glands this type of glands is made up of mucus cells mainly, they secrete thick and viscous saliva with more mucin. Labial glands (These are made up of both serous and mucus cells.), lingual serous glands this type of glands is made up of serous cells Predominately. These glands secrete thin and watery saliva (Sembulingam, 2005).

1.2.2.1.1 The saliva

The saliva is a glandular secretion; it is not one of the popular bodily fluids. It lacks the drama of blood, the sincerity of sweat and the emotional appeal of tears (Streckifus and Bigler, 2002). Saliva plays a critical role in the maintenance of oral and dental health. Whole saliva represents a mixture of oral fluids and includes secretions from both major and minor salivary glands (90% of saliva is produced by the major salivary gland) (Mader, 2001), approximately 10% produced by minor glands clustered in the oral mucosa) in addition to several constituents of non-salivary origin, such as gingival crevicular fluid (GCF), serum and blood derivatives from oral wounds, bacteria and bacteria secretions, viruses and fungi, desquamated epithelial cells, other cellular

components and food debris (Sreebny, 1989). Saliva can be collected with or without stimulation (Aristidis *et al.*, 2013). Stimulated saliva is collected by masticatory action (i.e., from a subject chewing on paraffin wax) or by gustatory stimulation (i.e., application of citric acid on the subject's tongue) (Aristidis *et al.*, 2013).

Unstimulated whole saliva is the mixture of the secretions which enter the mouth in the absence of exogenous gustatory, masticatory, or mechanical stimulation. The best two ways to collect whole saliva are the draining method, in which saliva is allowed to drip off the lower lip, and the spitting method, in which the subject expectorates saliva into a test tube (Aristidis *et al.*, 2013).

1.2.2.1.2 Saliva flow rate

Unstimulated saliva flow rates in healthy individuals are in the range of 0.3-0.5 ml/ min (Dawes, 1996). There is significant difference between genders in unstimulated flow rates (men have higher flow rates than women) (Dawes, 1996). The factors affecting unstimulated saliva flow rates are degree of hydration, body position, exposure to light, olfactory stimulation, and seasonal and diurnal factors. Less important factors are age, body weight, psychic effects, variety of disease state and several pharmacological agents. During sleep, flow from major glands virtually ceases (James *et al.*, 1988). Diminished salivary output can have deleterious effects on oral and systemic health (Atkinson and Wu, 1994). The reduction of the secretion of saliva is called hyposalivation, and occurs in: fever dehydration, obstruction of salivary ducts, paralysis of facial nerve, and in congenital absence of hypoplasia of salivary glands. Xerostomia (dry mouth) is the subjective feeling of oral dryness. The excessive secretion of saliva is known as hyper salivation or (sialorrhea), and it occurs in: Pregnancy, decay of tooth, neoplasm of mouth or tongue due to continuous irritation, disease of stomach and intestine, nausea and vomiting and some psychological conditions (Mader, 2001).

Salivary flow rate may be affected from several factors: Apart from perceived stress, and depression, other variables are age, alcohol consumption, exercise intensity, as well as cancer and radiation treatment (Allgrove *et al.*, 2008).

Recent observations indicate a relation between salivary amylase secretion and experience stressful condition. The enzyme concentration increases under both physical stress, such as treadmill exercise, running, bicycle exercise and cold exposure (Chatterton *et al.*, 1997). and psychological stress as well such as watching highly negative emotional pictures of mutilation or accidents, participating in collegiate level individually oriented athletic

competition, written examination, and Trier Social Stress Test (TSST) (Gordis *et al.*, 2006; Nater *et al.*, 2006).

When the concentration of catecholamine (epinephrine and nor- epinephrine) increases in the blood due to stress, the salivary amylase concentration also increases (Nater *et al.*, 2006).

1.2.2.1.3 Composition of Saliva

Composition of saliva depends on various factors such as, the flow rate, type of gland and diet. Saliva is 99.5% water and 0.5% solute (John, 1998). Among the solute are: Inorganic ions such as, Na⁺, K⁺, Cl⁻, HCO₃⁻, Ca²⁺, Mg²⁺, HPO₄²⁻, I⁻, SCN⁻ and F⁻ (John, 1998). Proteins such as enzymes, antibacterial substances, albumin, blood clotting factors, B2-microglobulin and immunoglobulins, amino acids, proline-rich proteins. And also the saliva contain small organic molecules such as cyclic AMP, and cyclic AMP binding proteins, urea, uric acid various lipids and corticosteroids (John, 1998). There are several ways by which serum constituents that are not part of the normal salivary constituents can reach saliva. Within the salivary glands; transfer mechanisms include intracellular and extracellular routes. The most common intracellular route is passive diffusion, although active transport has also been reported. Ultra filtration, which occurs through the tight junctions between the cells, is the most common extracellular route (Haeckel and Hanecke, 1996). Serum constituents are also found in whole saliva as a result of GCF out flow. Depending on the degree of inflammation in the gingival, GCF is either a serum transudate or, more commonly, inflammatory exudates that contain serum constituents (Kaufman and Lamster, 2000).

1.2.2.1.4 Major Functions of Saliva

Lubrication and fluid coating Mucins, proline-rich proteins and H₂O components of saliva provide physical protection against mechanical, thermal, chemical irritation Aids in speech and swallowing Cleaning. The H₂O component facilitates clearance of food and swallowing and bathing the teeth. Remineralization Super saturation of calcium and phosphate due to the presence of the salivary protein (statherin) facilitates remineralization of the teeth. Anionic proline-rich proteins bind calcium and phosphate and bring them to the apatite surface. Buffering Bicarbonate and, to a lesser extent, phosphate and macromolecules help neutralize plaque pH after meals, thereby reducing time available for demineralization. Antimicrobial action Specific (sIgA) and non specific (lactoferrin, lysozyme and salivary peroxidase) help to control antimicrobial mechanisms, Digestion and taste Digestive enzymes found in saliva include

amylase, protease, lipase and nuclease, water of saliva functions as a solvent to facilitate taste (Frederick and Rosen, 2001).

Salivary amylase activity is of short duration because, on swallowing is Inactivated by the acidity of the gastric contents (Lehrnerl *et al.*, 1976). Saliva can be considered as one of the chief defense systems against aqueous soluble components of tobacco (Zappacosta *et al.*, 2002). As formerly reported, some components of the saliva could neutralize the toxic effect of aldehydes, oxidants, and carcinogens. Saliva contains non-enzymatic and enzymatic antioxidant systems, chiefly superoxide dismutase (SOD), glutathione peroxidase, uric acid, catalase, and glutathione (GSH) (Moore *et al.*, 1994).

1.2.2.2 The Pancreas

The pancreas is a large gland that is involved in the digestive process, but located outside of the GI system. It is composed of both endocrine and exocrine tissue. The endocrine functions of the pancreas include production of insulin and glucagon; both hormones are involved in carbohydrate metabolism. Exocrine function involves the production of many enzymes used in the digestive process (Bishop *et al.*, 2010).

1.2.2.2.1 Physiology of Pancreatic Function

As a digestive gland, the pancreas is only second in size to the liver, weighing about 70–105 g. It is located behind the peritoneal cavity across the upper abdomen at about the level of the first and second lumbar vertebrae, about 1–2 inches above the umbilicus. The pancreas composed of two morphologically and functionally different tissues: endocrine tissue and exocrine tissue. The endocrine (hormone-releasing) component is by far the smaller of the two and consists of the islets of Langerhans, which are well-delineated, spherical or ovoid clusters composed of at least four different cell types. The islet cells secrete at least four hormones into the blood: insulin, glucagon, gastrin, and Somatostatin. The larger, exocrine pancreatic component (enzyme-secreting) secretes about 1.5–2 L/day of fluid, which is rich in digestive enzymes, into ducts that ultimately empty into the duodenum. This digestive fluid is produced by pancreatic acinar cells (grape like clusters), which line the pancreas and are connected by small ducts. Normal, protein-rich, pancreatic fluid is clear, colorless, and watery, with an alkaline pH that can reach up to 8.3. This alkalinity is caused by the high concentration of sodium the pancreas is a large gland that is involved in the digestive process, but located outside of the GI system. It is composed of both endocrine and exocrine tissue (Bishop *et al.*, 2010).

Pancreatic amylase performs the major digestive action on starch once the polysaccharides reach the intestine. Amylase is small enough to pass through the glomerular of the kidney, so it is the only plasma enzyme normally found in urine (Noble, 2000).

1.2.2.2.2 Disease of Pancreas

The disease causes the small and large ducts and the acini to dilate and convert into small cysts filled with mucus, eventually resulting in the prevention of pancreatic secretions reaching the duodenum or, depending on the age of the patient, a plug that blocks the lumen of the bowel, leading to obstruction. As the disease progresses, there is increased destruction and fibrous scarring of the pancreas and a corresponding decrease in function. Cystic fibrosis is transmitted as an autosomal recessive disorder with a high degree of penetrance. The cystic fibrosis gene known as CFTR occurs on chromosome 7, and more than 900 mutations causing this disorder have been identified; however, some occur more commonly than others. Pancreatic carcinoma is the fourth most frequent form of fatal cancer and causes about 27,000 deaths each year in the United States, which represents about 5% of all deaths from malignant neoplasm, the diseases slightly more common in males than females. The 5-year survival rates less than 5% and more than 90% of patients die within 1 year of diagnosis. Most pancreatic tumors arises adenocarcinomas of the ductal epithelium. Because the pancreas has a rich supply of nerves, pain is a prominent feature of the disease. Islet cell tumors of the pancreas affect the endocrine capability of the pancreas. If the tumor occurs in beta cells, hyperinsulinism is often seen, resulting in low blood glucose levels, sometimes followed by hypoglycemic shock. Pancreatic cell tumors, which over produce gastrin, are called gastrinomas; they cause Zollinger-Ellison syndrome and can be duodenal in origin. These tumors are associated with watery diarrhea, recurring peptic ulcer, and significant gastric hypersecretion and hyperacidity. Pancreatic cell glucagon-secreting tumors are rare; the hypersecretion of glucagon is associated with diabetes mellitus. Pancreatitis, or inflammation of the pancreas, is ultimately caused by auto digestion of the pancreas as a result of reflux of bile or duodenal contents into the pancreatic duct. Pathologic changes can include acute edema, with large amounts of fluid accumulating in the retroperitoneal space and an associated decrease in effective circulating blood volume; cellular infiltration, leading to necrosis of the acinar cells, with hemorrhage as a possible result of necrotic blood vessels; and intrahepatic and extrahepatic pancreatic fat necrosis. Pancreatitis is generally classified as acute (no permanent damage to the pancreas), chronic (irreversible injury), or

relapsing/recurrent, which can also be acute or chronic. It commonly occurs in mid life. Painful episodes can occur intermittently, usually reaching a maximum within minutes or hours, lasting for several days or weeks, and frequently accompanied by nausea and vomiting. Pancreatitis is often associated with alcohol abuse or biliary tract diseases such as gall stones, but patients with hyperlipoproteinemia and those with hyperparathyroidism are also at a significantly increased risk for this disease (Bishop *et al.*, 2010). Other etiologic factors associated with acute pancreatitis include mumps, obstruction caused by biliary tract disease, gallstones, pancreatic tumors, tissue injury, atherosclerotic disease, shock, pregnancy, hypercalcemia, hereditary pancreatitis, immunologic factors associated with post renal transplantation, and hypersensitivity (Bishop *et al.*, 2010).

Several constituents of tobacco smoke have been linked to development of pancreatic and pancreatic carcinoma (EL Gareem *et al.*, 1991). In addition to nicotine, tobacco smoke also contains other cytotoxic agent such as acetaldehyde which may affect exocrine pancreatic function by inhibiting cholecystokinin (CCK) which responsible for release of α -amylase from pancreatic acini (EL Gareem *et al.*, 1991).

1.3 Rationale

Cigarette smoking and other tobacco use imposes public health problems globally. According to the WHO, in 2001; there were 1.1 billion smokers, 800 million of whom inhabit developing countries. This equates to approximately one third of the world's entire population over 15 years of age and represents an enormous global health problem. Tobacco use is directly related to a variety of medical problems including cancer, low birth weight, pulmonary and cardiovascular diseases. It affects the oral cavity first, so it is evident that smoking has many negative influences on oral cavity too. Researcher found that cigarette smoking and other tobacco affect serum and salivary α -amylase activity. Some studies have demonstrated increase activity of α -amylase in tobacco consumption compared to normal non-tobacco consumption (Gomina *et al.*, 2013; Reddy *et al.*, 1980; Onyesoma *et al.*, 2012). To the best of our knowledge, in Sudan, there are no studies on the impact of tobacco dependence on biological parameters in general, and on α -amylase activity in particular. Accordingly the present study conducted to evaluate serum and salivary α -Amylase activity in tobacco and non-tobacco consumers in Khartoum State.

1.4 Objectives

1.4.1 General Objective

- To evaluate serum and saliva α -amylase activity among tobacco consumers in Khartoum State.

1.4.2 Specific Objectives

- To compare serum and saliva α -amylase activity between tobacco (test) and non-tobacco consumers (control) individuals.
- To measure the relationship between different routes of tobacco consumption and α -amylase activity.
- To correlate between duration and Serum and saliva α -amylase activity.

2 Materials and Methods

2.1 Materials

2.1.1 Study Design

This is a descriptive cross-sectional study, carried out during the period from February 2014 to July 2014.

2.1.2 Study Area

This study was conducted in Khartoum state.

2.1.3 Study Population

Sudanese males on tobacco consumption were enrolled in this study as case group and volunteer non-tobacco consumers as control group.

2.1.4 Inclusion Criteria

Selected criteria are males on two routes of tobacco consumption (smokers, snuffers and /or both routes).

2.1.5 Exclusion Criteria

Other routes of tobacco consumption, females, and GI diseases mainly pancreatic disease had been excluded.

2.1.6 Sampling

The 4 ml of blood samples were collected in the morning through superficial venous puncture on dry tubes and 3 ml of Saliva were also collected in the morning from tobacco consumers and non-tobacco consumers.

2.1.7 Sample Size

Seventy six males on tobacco consumption classified as test group and thirty volunteers non-tobacco consumption were considered as control group.

2.1.8 Processing Biological Samples Collected

The blood samples collected were centrifuged at 3000 RPM for five minutes to get serum, which was used on the same day to determine α -amylase activity. Saliva samples collected were also centrifuged at 3000 RPM for five minutes, and supernatant fluid was used to determine α -amylase activity after diluted 100 times with distill water.

2.1.9 Data collection

A questionnaire was administered to each selected subject, blood sample was drawn and saliva collected from each subject.

2.1.10 Ethical Consideration

The objectives of the study clearly and explained to all individuals participating in the study, and verbal inform consent was obtained.

2.2 Methods

2.2.1 Measurement of α -amylase activity

Principle

Alpha-Amylase enzyme catalyzes the hydrolysis of 2-chloro-4-nitrophenyl-malto-triosed (CNP-G3) to 2-chloro- 4-nitrophenol (CNP). The catalytic concentration is determined from the rate of 2-chloro-4-nitrophenol formation, measured at 405 nm.

Procedure

1. The reagent and the samples were allowed to come to reaction temperature, instrument (spectrophotometer) was adjusted by adjusted temperature (37°C), and selected suitable wavelength (405nm).
2. Centrifuged saliva samples were brought to room temperature (30°C) and diluted by distilled water (1:100) before assay.
3. The instrument was adjusted to zero by used DW. 0.5ml from working reagent was pipette into a tube then 10 μ l from sample was added, mixed well, stopwatch Started and sample was transferred into cuvette (1cm light path, 0.5ml) and inserted into the spectrophotometer.
4. Initial absorbance was recorded at 1 minute first then intervals thereafter for 3minutes.
5. The difference between consecutive absorbance and the average absorbance difference per minute ($\Delta A/\text{min}$) was calculated.

Calculation

The α -amylase activity in the sample is calculated using the following general formula

$$\Delta A/\text{min} \times \frac{(V_t \times 106)}{(C \times I \times V_s)} = \text{IU/L} \quad - \quad \text{appendix}$$

Concentration of salivary α -amylase multiply by 100 (df)

2.2.2 Statistical analysis

Data was analyzed using the SPSS computer program and Excel. The means and standard deviation of serum and saliva α - amylase were detected and t-test and one ANOVA test was used for comparison (P-value of < 0.05 is considered to be significant). Linear regression analysis was used to assess correlation between duration and serum and saliva α - amylase activity.

3 Results

The study included 106 healthy men, 76 of them were tobacco consumption (30 Smokers, 25 Snuffers, 21 both (Smokers and Snuffers) and 30 were non tobacco consumption were enrolled in this study. The serum α -amylase activity was estimated and data analyzed statistically using computer SPSS program and Excel as in below

Fig (3.1), show distribution of tobacco consumption (Smokers, Snuffers, Both (Smokers and Snuffers)) in age group, show as percentage (%).

Table (3-1), show insignificant difference between the means of serum and saliva α -amylase activity of tobacco consumers compared to non-tobacco consumers. Result expressed as ($M \pm SD$) With (P -value = 0.46, 0.6) respectively.

Fig (3.2) show insignificant difference between the mean of serum α -amylase activity in smoker, snuffer, both (Smoker and Snuffer), and control group. Result expressed as ($M \pm SD$) With (P -value = 0.8).

Fig (3.3) show a significant difference between the mean of serum α -amylase activity in smoker, snuffer, both (Smoker and Snuffer), and control group. Result expressed as ($M \pm SD$) With (P -value = 0.003).

Table (3-2) show comparison means of serum α -amylase activity between tobacco consumers groups and control, and between tobacco consumers groups, smokers versus control, snuffers versus control, both (smoker and snuffer) versus control, smokers versus snuffers, smokers versus Both (Smoker and Snuffer), snuffers versus both (smoker and snuffer). Result expressed as ($M \pm SD$) With (P -value = 0.8, 0.4, 0.4, 0.5, 0.5, 0.9) respectively.

Table (3-2) show comparison means of saliva α -amylase activity between tobacco consumers groups and control, and between tobacco consumers groups, smokers versus control, snuffers versus control, Both (smoker and snuffer) versus control, smokers versus snuffers, smokers versus Both (Smoker and Snuffer), snuffers versus Both (smoker and snuffer). Result expressed as ($M \pm SD$) With (P -value = 0.001, 0.1, 0.6, 0.00, 0.009, 0.4) respectively.

Table (3-3) show insignificant weak positive correlation between the serum and saliva α -amylase activity and duration of tobacco consumption groups (in year), smokers ($r = 0.32$, P -value = 0.08, 0.1), snuffers ($r = 0.168$, P -value = 0.4, 0.07), both (smoker and snuffer) ($r = 0.05$, P -value = 0.8, 0.5) respectively.

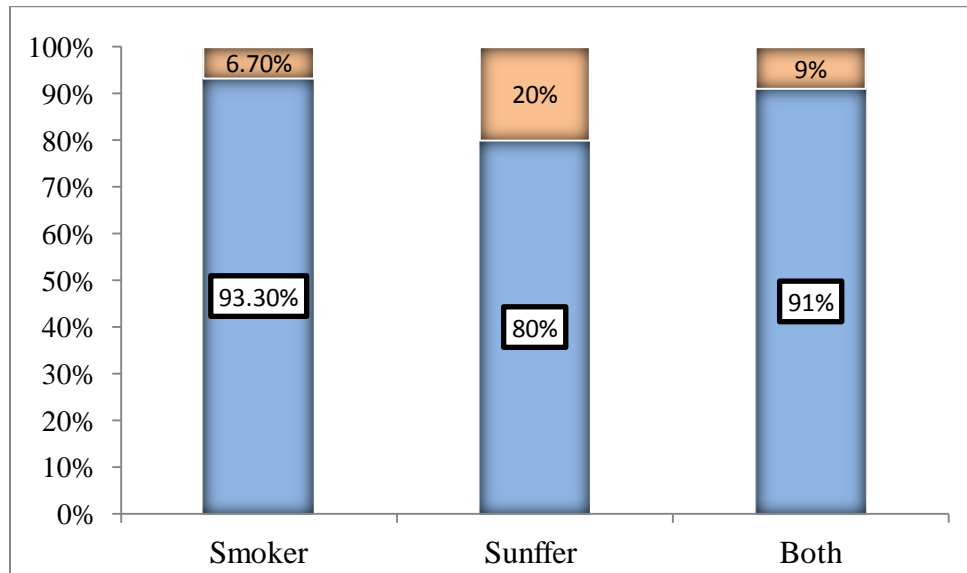


Fig (3.1) Distribution of tobacco consumption (Smoker, Snuffer, Both (Smoker and Snuffer))
in age group

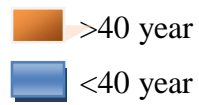


Table (3-1) Comparison means of serum and saliva α -amylase activity between tobacco consumers and non-tobacco consumers.

	Tobacco consumers (n= 76)	Non-tobacco consumers (n= 30)	<i>P</i> -value
Serum α -amylase (IU/L)	22.7 ± 8.7	21.39 ± 7.8	0.47
Saliva α -amylase (10^4 IU/L)	2.16 ± 1.57	2.0 ± 1.3	0.6

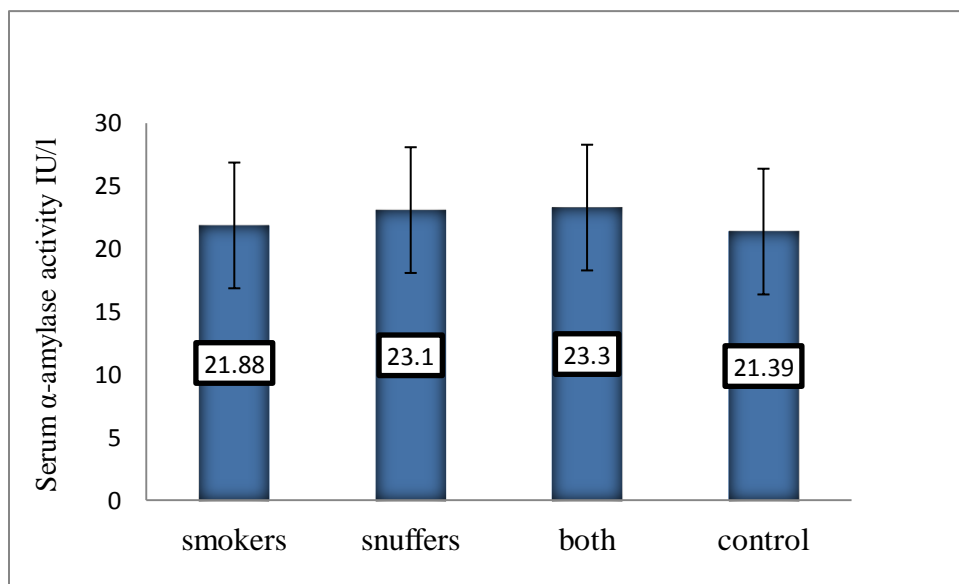


Fig (3.2) Mean ($M \pm SD$) of serum α -amylase activity in smoker, snuffer, both (Smoker and Snuffer), and control group

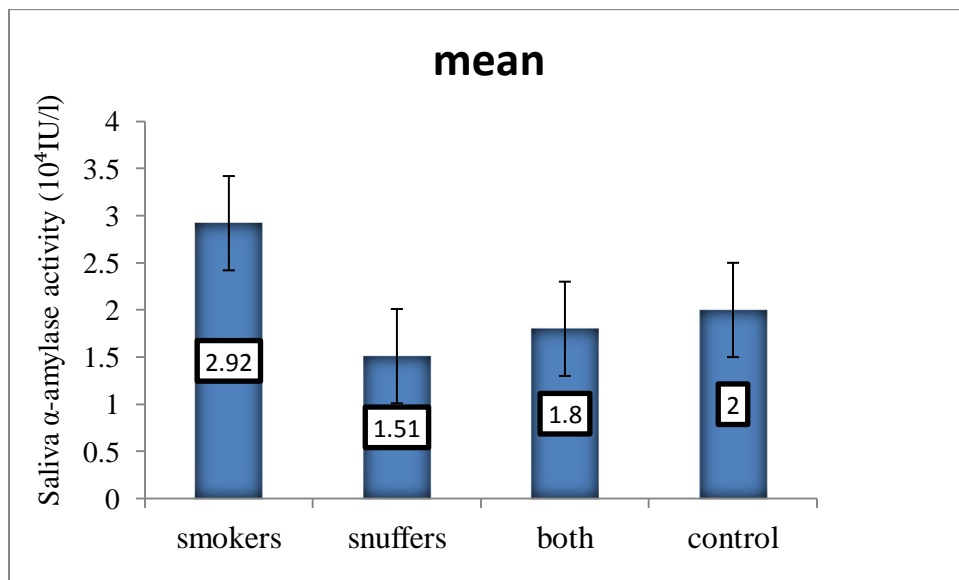


Fig (3.3) Mean ($M \pm SD$) of saliva α -amylase activity in smoker, snuffer, both (Smoker and Snuffer), and control group

Table (3-2) Comparison means of serum and saliva α -amylase activity between tobacco consumers groups and control, and between tobacco consumers groups.

	Serum α -amylase (IU/L)	<i>P</i> -value	Saliva α -amylase (10^4 IU/L)	<i>P</i> -value
Smokers (n=30)	21.88 ± 9.3	0.8	2.92 ± 1.75	0.01
Control (n=30)	21.39 ± 7.8		2.0 ± 1.33	
Snuffers (n=25)	23.1 ± 10.3	0.4	1.51 ± 1.26	0.1
Control (n=30)	21.39 ± 7.8		2.0 ± 1.33	
Both (n=21)	23.39 ± 5.7	0.4	1.8 ± 1.16	0.6
Control (n=30)	21.39 ± 7.8		2.0 ± 1.33	
Smokers (n=30)	21.88 ± 9.3	0.5	2.92 ± 1.75	0.00
Snuffers (n=25)	23.1 ± 10.3		1.51 ± 1.26	
Smokers (n=30)	21.88 ± 9.3	0.5	2.92 ± 1.75	0.009
Both (n=21)	23.39 ± 5.7		1.8 ± 1.16	
Snuffers (n=25)	23.1 ± 10.3	0.9	1.51 ± 1.26	0.4
Both (n=21)	23.39 ± 5.7		1.8 ± 1.16	

-Both (Smoker and Snuffer)

Table (3-3) Correlation between the serum and saliva α -amylase activity and duration of tobacco consumption groups (in year).

	Both (n=21)		Smokers (n=30)		Snuffers (n=25)	
	Serum α -amylase	Saliva α -Amylase	Serum α -amylase	Saliva α -Amylase	Serum α -amylase	Saliva α -Amylase
r	0.05	0.12	0.32	0.3	0.17	0.37
<i>P</i> -value	0.8	0.5	0.08	0.1	0.4	0.07

-Both (Smoker and Snuffer)

4 Discussions, Conclusion, and Recommendation

4.1 Discussion

Tobacco dependence is still the leading cause of avoidable mortality in the world. Tobacco consumptions cause cardio vascular pathologies, pleura pneumonia, cancers, pancreatitis and periodontitis, so it is evident that smoking has many negative influences on oral cavity too. In addition, tobacco consumption modifies several biological parameters, including α -amylase researchers found that, smoking tobacco may increase the value of α -amylase activity in serum and saliva. Accordingly the present study aims to evaluate the effect of tobacco consumption (smoker, snuffer and both (smoker and snuffer)) on serum and saliva α -amylase activity in Khartoum state.

The results of distribution showed that, the percentage of tobacco consumption increased among young adult males (less than 40 years old) compared with elderly adult males (more than 40 years old), this observation found in all Tobacco consumptions routes (smokers, snuffers and both (smoker and snuffer)), several reports have indicated that smoking and smokeless tobacco use almost always begins by the time kids graduate from high school before age of 18, also tobacco use among aged youth or adults must include a focus on reducing experimentation and regular tobacco use among teenagers and pre-teens (SAMHSA, 2012).

In addition cigarette smoking and other tobacco use imposes public health problems globally. According to the WHO, in 2001; there were 1.1 billion smokers, 800 million of whom inhabit developing countries. This equates to approximately one third of the world's entire population over 15 years of age and represents an enormous global health problem (Neha and Rakesh, 2014).

The results of the present study provided experimental evidence that the mean activity of saliva α -amylase significant increase in smokers when compared with tobacco non-consumers (P -value = 0.01). Our finding agreed with some reports done by Onyesom and Gomina, whom stated that, saliva α -amylase activity significant increase in smokers than in tobacco non-consumers due to increase secretion of α - amylase from salivary gland by affect of cigarette (Onyesom *et al.*, 2012; Gomina *et al.*, 2013). There is discrepancy between this observation and previous studies done by Nagler and Weiner, whom reported that unsaturated aldehydes contained in cigarette smoke inhibition saliva α -amylase activity (Nagler *et al.*, 2000; Weiner *et al.*, 2009).

The results showed significant decrease in saliva α -amylase activity in snuffers when compared with smokers, (P -value = 0.00). Our finding agreed with report done by Gomina, who stated that, saliva α -amylase activity, was significant decreased in snuffer than in smokers (Gomina *et al.*, 2013), because this decrease is probably associated with an increase in saliva flow due to the fact that nicotine in this tobacco snuffing is absorbed primarily through the skin in the mouth and compresses tooth periodontal membrane and thus, activation mechanoreceptors which induces salivary hypersecretion, which comes with a decline in salivary α -amylase activity through dilution effect.

The results showed significant decrease in saliva α -amylase activity in both (smokers and snuffers) when compared with smokers, (P -value = 0.009), this due to effect of tobacco snuffing (decrease saliva α - amylase).

Serum and saliva α -amylase activity increases in smokers as soon as duration of tobacco consumption becomes more significant, with a correlation, which is however, weak and insignificant. This result agrees with the study done one by EL Garen, Gomina and Onyesom (EL Garen *et al.*, 1991; Gomina *et al.*, 2013; Onyesom *et al.*, 2012), whom observed insignificant weak correlations between serum and saliva α -amylase activity and duration of tobacco consumption.

Serum and saliva α -amylase activity increases in snuffers as soon as duration of tobacco consumption becomes more significant, with a correlation, which is however, weak and insignificant, there is discrepancy between this observation and previous study done by Gomina, who observed decline with insignificant weak correlations between serum and salivary α -amylase activity and duration of tobacco consumption (Gomina *et al.*, 2013).

4.2 Conclusion

The results conclude that:

- 1- High percentage of tobacco consumption was found among young adult (less than 40 year).
- 2- The activity of saliva α -amylase increase in smoker subjects, followed by both and snuffers.
- 3- Saliva α -amylase activity is decrease in snuffers.
- 4- There is positive weak correlation between the activity of serum and saliva α -amylase with duration of tobacco consumers.

4.3 Recommendations

From the results of this study, it is recommended that:

- 1- Health education programs to improve people awareness about serious health effect of tobacco consumption especially among young adult which account for high percentage of tobacco consumption in this study and community.
- 2- Measuring other parameters such as Ca^{++} , mg^{++} , IgA, Lactoferrin, Lysozyme, Peroxidase, Mucin, ALP and Cortisol.
- 3- Further studies to differentiate between Macroamylasimia and amylasimia by measuring IgG.
- 4- Further proteomic studies to detect different type of proteins.
- 5- Further studies should be done to identify the components of local snuff which help to understand the toxic effect of snuff on α -amylase activity.

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Questionnaire

Sudan University of Science and Technology

College of Graduate Studies

Evaluation of Serum and Saliva Alpha-Amylase Activity among Habitual Adults Tobacco

Consumers

Name: No:

Age /Years: Sex:

Residence: Education Status:

Any Diseases:

Any Drugs used:

Types of Tobacco:

Smoking: Yeas: No:

Tobacco: Yeas: No:

Both (Smoking and Tobacco): Yeas: No:

Duration of Consumption:

NO of Cigarette /day:

Times of tobacco /day:

Laboratory investigation:

Serum α -amylase:IU/L

Saliva α -amylase:IU/L

