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Assessment of Serum Lipase Activity among Sudanese Males using Smoke and Smokeless Tobacco in Alkamlien City – Algazeria State

تقويم مستوي نشاط إنزيم اللايبيز في مصل دم السودانيين الذكور المدخنين ومستخدمي التبغ غير المدخن في مدينة الكاملين – ولاية الجزيرة

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قال اللَّه سبحانه وتعالى:

﴿نَبِّئْ عِبَادِي أَنِّي أَنَا الْغَفُورُ الرَّحِيمُ﴾

صدق اللَّه العظيم

الآية (49) من سورة الحِجر

DEDICATION

To Abdalla Osman (Faggad)

Who is my father, mother, best friend and everything: for his great love, kindness, patience, forgiveness and support all my life.

To my family and friends

For the true love, guiding, support and help all my life.

To my teachers

Ever teach me anything.

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Abstract

Tobacco use is one of the biggest public health threats the world has ever faced, killed millions of people yearly. Toombak is the smokeless tobacco type mainly used in Sudan particularly males which cause cancer and other medical conditions.

This comparative cross-sectional study, enrolled at period from September to December 2020, to assess serum lipase activity in 69 tobacco users (38 toombak users and 31 cigarette smokers) and 34 individuals who never used tobacco as control group, blood samples collected from all participants, serum was separated, and lipase activity was measured by Mindary BS 800 auto analyzer.

The lipase activity mean was 40.9±17.2, age mean was 41.8±12.4, body mass index mean 23.9± 4.8 and waist circumference mean 36.6±5.2 among all groups.

15.8% of toombak users showed higher lipase activity than the reference value, while 19.3% of cigarette smokers had higher values too.

This study result revealed that lipase activity was not significantly changed in all groups with (*P- value:* 0.595). Body mass index (*P- value:* 0.683) and waist circumference (*P- value:* 0.783) showed no difference between tobacco group and non-tobacco users as a control group.

The results also showed that there was no association between lipase activity and both body mass index ((*P- value:* 0.702, r = 0.038) and waist circumference (*P- value:* 0.468, r: 0.072) among all group, while a significant positive association was found between the lipase activity and frequency of using toombak (*P- value:* 0.025, r: 0.363) and duration of using toombak (*P- value:* 0.014, r: 0.397) among toombak users' group.

Age also was associated with lipase activity (*P- value:* 0.012, r: 0.246) among all groups and among toombak users group with *P-value:* 0.039, r: 0.337. There was no association between lipase activity and the frequency of smoking (*P- value:* 0.946, r: 0.013) and duration of smoking (*P- value:* 0.645, r: 0.086) among smokers' group.

In conclusion: Sudanese males using toombak and cigarette smoking had no difference in lipase activity when compared to non-tobacco users.

المستخلص

يعد تعاطي التبغ أحد أكبر التهديدات الصحية العامة التي واجهها العالم على الإطلاق ، حيث يودي بحياة ملايين الأشخاص سنويًا. التمباك هو نوع من أنواع التبغ الذي لا يُدَخَّن والذي يستخدم بشكل رئيسي في السودان وخاصة الذكور وهو يسبب السرطان وحالات طبية أخرى.

هذه الدراسة المقطعية المقارنة ، المسجلة في الفترة من سبتمبر إلى ديسمبر 2020 ، للوصول إلى نشاط إنزيم ليبيز مصل الدم في 69 من مستخدمي التبغ (38 من مستخدمي التمباك و 31 مدخنًا للسجائر) و 34 فردًا لم يستخدموا التبغ أبدًا كمجموعة ضابطة.

تم جمع مائة وثلاث عينات دم من جميع المشاركين ، وتم فصل مصل الدم ، وقياس نشاط الليبيز بواسطة محلل تلقائي Mindary BS 800.

كانت قيمة الوسط الحسابي لنشاط اللايبيز 17.2±40.9 , متوسط العمر 12.4±41.8 ,متوسط مؤشر كتلة الجسم 4.8 ±23.9 ومتوسط محيط الخصر 5.2±36.6 في كل المجموعات.

15% من مستخدمي التمباك أظهروا نتائج أعلى من المستوى الطبيعي و 19.3% من المدخنين لديهم مستويات عالية أيضاً.

أوضحت نتائج هذه الدراسة أن نشاط الليباز لم يتغير في جميع المجموعات بقيمة (*P- value*: 0.595).

كما أظهرت النتائج عدم وجود ارتباط بين نشاط الليباز وكلا من مؤشر كتلة الجسم (*P- value*:0.702 ، r: 0.038) ومحيط الخصر: (*P-value:* 0.468، r: 0.072) بين جميع المجموعات ، بينما كان هناك ارتباط إيجابي بين نشاط الليباز وتكرار استخدام التمباك (*P-*

value: 0.025، r: 0.363) ومدة استخدام التمباك (*P-value:* 0.014 ، r: 0.397) بين مجموعة مستخدمي التمباك.

كما ارتبط العمر بنشاط الليباز (*P-value:* 0.012، r: 0.246) بين كل المجموعات وفي المجموعة المستخدمة للتمباك أيضاً (*P-value:* 0.039, r: 0.337) ، ولم يكن هناك ارتباط بين نشاط الليباز وتكرار التدخين (*P-value:* 0.946 , r: 0.013) ومدة التدخين (*P-value:* 0.645, r: 0.086). بين مجموعة المدخنين.

مؤشر كتلة الجسم (*P-value:* 0.683) ومحيط الخصر (*P-value:* 0.783) لم يظهر أي فرق بين مجموعة التبغ ومستخدمي غير التبغ كمجموعة ضابطة.

في الختام: لم يكن لدى الذكور السودانيين الذين يستخدمون توماك وتدخين السجائر أي اختلاف في نشاط الليباز بالمقارنة مع غير مستخدمي التبغ.

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AMY: Amylase

BMI: Body mass index

CAT: Catalase

DNA: Deoxyribonuclease

EBV: Epstein–Barr virus

G6PD: Glucose 6-phosphate dehydrogenase

GGT: Gamma glutamyl transferase

HPV: Human papillomavirus

HSV: Herpes simplex virus

kDa: Kilo Dalton

LOY: Loss of chromosome Y

LPS: Lipase

MP : Maras Powder

NAB: N-nitrosoanabasine

NAT: N-nitrosoanatabine

NNAL : methylnitrosamino)-1-(3-pyridyl)-1-butanol

NNAL-O-Glucu: glucuronidated NNAL

NNK: 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone

NNN: N-nitrosonornicotine

OS: Oxidative stress

OSCC: Oral squamous cell carcinoma

OSCCs: Oral squamous cell carcinomas

PCR: Polymerase chain reaction

SLT: Smokeless tobacco

SOD: Superoxide dismutase

SPSS: Statistical package for the social sciences

TSNAs: Tobacco specific nitrosamines

UK: United of kingdom

USA: United States of America

WC: Waist Circumference

Chapter One

1. Introduction, rationale and objectives

1.1. Introduction:

Current tobacco use was the single most important risk factor for pancreatic diseases followed by obesity and heavy use of alcohol subsequently, since Tobacco and heavy use of alcohol had bigger effects on risk of acute pancreatitis and chronic pancreatitis than pancreatic cancer (Alsamarrai et al., 2014). Zaghrini and his team also liked between smoked nicotine specially heavy smoking and pancreatitis (Zaghrini *et al.*, 2018).

Toombak is smokeless tobacco type, widely use in Sudan specially among male, which is highly addictive, composed of a very high concentration of potent type of carcinogens known as TSNAs (tobacco specific nitrosamines) in compare with other smokeless tobacco as Swedish snus (Idres *et al*., 1998)**.**

The use of tobacco products in Sudan was increased significantly with age among males with a middle and lower school performance living in urban areas, so the prevention targeting adolescents, such as school-based tobacco control programs are recommended, so the prevention should start in primary school (Mohamed *et al.,* 2020).

It is recommended to measure lipase as the only diagnostic marker to eliminate unnecessary expenditures of measuring both lipase and amylase tests since lipase become a much more sensitive and specific biomarker in diagnosing pancreatitis (Ismail and Bhayana, 2017). As smoking is established as risk factor for pancreatitis, the relationship between smoking and pancreatitis is dose dependent and it is associated with progression of disease (Greer *et al*., 2015).

1.2 Rationale:

Tobacco use is a risk factor for six of eight leading causes of death killing more than 8 million people each year; however, it is the leading preventable cause of death in the world**.**

Although several studies showed the relationship between smoking and pancreatitis; to the best of our knowledge no published data concerning the toombak causality of pancreatitis among Sudanese population were found, that's why we attempt to do this aiming to assess the capability of toombak use to cause pancreatitis.

1.3. Objectives:

1.3.1. General objective:

To assess serum lipase activity among Sudanese males using smoke and smokeless tobacco type in Alkamlien City- Algazeira State.

1.3.2. Specific objectives:

1. To measure serum lipase activity in tobacco users (toombak users and cigarette smokers) and non-tobacco users.
2. To compare lipase activity means among all study groups.
3. To compare the body mass index and waist circumference means among tobacco among all groups.
4. To associate between lipase activity and the study variables (frequency and duration of use, body mass index (BMI), waist circumference and age).

Chapter Two

2. Literature review

2.1. Tobacco

Tobacco is product made from different types of ground plant leaves processed to many final forms, it can use in smoke or smokeless way, tobacco use is widely distributed around the world. Tobacco is highly addictive, and it is a major risk factor for cardiovascular and respiratory diseases, over 20 different types or subtypes of cancer, and other different health conditions. More than 8 million people die from using tobacco annually. Tobacco can also be deadly for non-smokers. Second-hand smoke exposure has also been implicated in adverse health outcomes, causing 1.2 million deaths annually (WHO, 2020).

2.1.1 Smokeless Tobacco

Smokeless tobacco refers to non-burned tobacco as dipping snuff, chewing or inhaling dry snuff. Smokeless tobacco contains such chemical compounds considered arisk factors for cancer, Smokeless tobacco (SLT) products are used by millions of people in over 130 countries around the world. Consumption of SLT has been estimated to cause different diseases leading to more than 0.65 million deaths yearly (Bhartiya *et al*., 2018) .

A list of 180 chemical compounds belonging to 22 chemical classes of different smokeless tobacco products, Fifty percent were expected to be mutagenic. Most of as NNN, NNK and all the compounds containing anthracenes and pyrenes (Bhartiya *et al*., 2018).

Smokeless tobacco extract may lead to many serious disorders as apoptosis oxidative stress and cause an imbalance between reactive oxygen species and antioxidants, such as Gamma Glutamyl (GGT) which may be used as a quick, easy

and precise marker for measuring (OS) in patients with chronic periodontitis and smokeless tobacco users. (Koregol *et al*., 2017).

Other study result suggest that heavy use of smokeless tobacco to a lesser extent cigar smoking may increase the risk of pancreatic cancer among nonsmokers of cigarette (Alguacil and Silverman, 2004).

PubMed, MEDLINE, and Google searched from January 1, 2020 to September 10, 2020 reported that tobacco use as smoking or chewing, has significant association with severe COVID-19 manifestation. It's known that tobacco concern with cardiovascular and respiratory diseases, diabetes and hypertension which make the treatment of such COVID-19 patients more challenging due to their rapid clinical deterioration (Gupta, 2020).

2.1.1.1 Toombak

Toombak is a smokeless tobacco snuff locally made in Sudan from tobacco plant species known as ''Nicotiana rustica'', the plant leaves dried and fermented in a powder form which can be stored. Upon use Toombak stock powder should mix with aqueous solution of sodium bicarbonate powder locally called ''Atron'' giving final alkaline moist product which is highly addictive and highly rich of Tobacco specific nitrose amines (TSNAs) types as N-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N-nitrosoanatabine (NAT) and N-nitrosoanabasine (NAB) in concentrations more than TSNAs content in Sweden snus (Idres *et al.,* 1991).

It was reported that toombak contained unusually high levels of tobacco-specific nitrosamines. NNK and NNAL were detected and estimated in urine of the toombak users. NNK and NNAL established as strong lung carcinogen believed to contribute to human lung cancer (Murphy *et al.,* 1994).

4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone(NNK) metabolized to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol(NNAL)and glucuronidated NNAL (NNAL-O-Glucu) which detected and measured in urine of smokers ,snuff dippers including toombak users which is highly carcinogenic tobacco products (Carmella, 2002).

Analysis of two types of toombak samples was conducted, and the identification of nicotine and carcinogenic N-nitrosamines (NNN and NNK) was confirmed in all cases of samples by using different confirmatory analytical procedures (Mohamed, 2020).

Tobacco specific nitrosamine (TSNAs) particularly 4(methylnitrosamino)-1-(3-pyridyl)–butanone (NNK), high choline and carnitine, volatile aldehydes; and benzyl alcohol were detected in toombak samples, 8 cations as iron and copper potent in Toombak due to high starting nicotine and rich nitrate reductase bacteria. High choline and carnitine can promote cardiovascular disease through their conversion to trimethylamine-N-oxide. Diphtheria and infective endocarditis are associated with cornyebacterium and facklamia respectively while staphylococcus can lead to numerous systemic opportunistic diseases (Sami *et al.*, 2020).

2.1.1.2 Toombak and other smokeless types of same tobacco species

In a comparative study between toombak and Swedish snuff as smokeless tobacco products, nicotine and TSNAs in various snus brands and saliva of snus dippers compared with concentrations of nicotine and TSNAs in various samples of toombak and saliva of toombak dippers, it was found that toombak contains concentrations of TSNAs 100-fold higher than those found in snus The level of NNN and NNK in the saliva of toombak dippers is also significantly higher than

those found in the saliva of snus dippers which play an important role in carcinogenicity of toombak (Idres *et al.*, 1998).

Another Smokeless dipping tobacco product called ‘’Naswar’’also belonging to ‘’Nicotina rustica’’ species of tobacco ground plant with different preparation process than Toombak preparation, which is used in Pakistan, Afghanistan, Iran and South Africa. Naswar use cause alteration in the lipid profile, antioxidant enzymes and comprise risk factors for the development of cardiovascular disease (Sajid and Bano, 2015).

Maras Powder (MP) is smokeless tobacco type widely used in the southeast region of Turkey. It is obtained from a tobacco species, Nicotiana rustica L and ash of oak or grapevine wood. Catalase (CAT), superoxide dismutase (SOD) and glucose 6-phosphate dehydrogenase (G6PD) were measured in this study showed that MP increases oxidative stress, which may cause many systemic disorders, including arteriosclerosis (Kilinc, 2004).

2.1.1.3 Dipping toombak and oral cancers

A molecular based study was done using PCR and direct DNA sequencing to analyse the prevalence of mutations in exon 2 of the P21wafi gene in oral squamous cell carcinomas (OSCCs) and non-malignant oral mucosal legions from Sudanese Toombak users, known In OSCCs cases from Norway, Sweden, USA and UK used for comparison. Mutations of P21wafi together with the previous P53 mutations found in OSCCs from Sudanese Toombak users may explain the high frequency of OSCCs in Sudan (Ibrahim *et al.*, 2002).

A study reported that Oral cancer, atypia and leukoplakia were high among males who were using toombak (Ahmed, 2007).

A case control study was enrolled at Khartoum teaching dental hospital (KTDH) confirmed that using toombak and cigarette smoking were found significantly associated with the development of Oral squamous cell carcinoma (OSCC) among both male and female users and there was a clinical association between the site of dipping close to the oral mucosa and the development of carcinoma in that site. The estimation of Toombak metabolites in urine may use to assess cancer risk arising from the users (Hassanin and Idris, 2017).

There is a strong epidemiological evidence that toombak is a major risk factor for both oral leukoplakia and oral squamous cell carcinoma (Patel, 2020).

2.1.1.4 Dipping toombak and oral infections

The prevalence of human papillomavirus (HPV), herpes simplex virus (HSV) and Epstein–Barr virus (EBV) DNA we investigated Using PCR/DNA sequencing in brush biopsies obtained from Sudanese toombak users, study findings illustrate that prevalence of HSV, HPV and EBV infections are common and may influence oral health and cancer development (Jalouli *et al.,* 2010).

Toombak using is affecting the quality and quantity of the oral normal flora because it has inhibitory effect on *viridians streptococci*, leading to rising of new colonizing species such as *Bacillus species* and *Aspergillus specie* especially aflatoxins producing *Aspergillus flavus*. These changes in the microenvironment may affect the immunity and health of the Toombak users and may lead to serious oral and systemic health problems (Abakar, 2020).

2.1.1.5 Toombak prevalence studies in Sudan

A house-to-house cross-sectional survey of a random population sample of 4,535 households was performed, 21,648 (92.6%) eligible individuals were questioned about tobacco use. Among children and adolescents (4–17 years) prevalence of

tobacco use was (2%, range 1–2%), but there was an abrupt increase up to 25% in late adolescence. Among the adult population aged 18 years and older the prevalence of toombak use (34%) and cigarette smoking (12%) among males were significantly higher than among females (2.5 and 0.9%, respectively). The prevalence of toombak use among the male population aged 18 years and older was significantly higher in the rural than in the urban areas (35% vs 24%), while cigarette smoking had a higher prevalence in urban areas (18% vs 12%). The highest rates of toombak use were found in rural areas among the male population ages 30 years and older (mean 46.6%, range 45–47%) (Idres *et al.,* 1998).

Toombak dipping prevalence attitude among male pharmacy students from different university studied, 55% of respondent was not using toombak. The common reason makes student prompt to use toombak is the strong influence of their friends (68.9%) and they always seek out groups of friends sharing the same Toombak dipping behavior. Toombak suggest changing the mouth pH affecting the salivary α amylase and its function. The toombak users was suffering from reduced appetite for food which lead to direct effect on health, and this need more investigation (Mahmoud *et al.,* 2015).

A cross sectional study was done in Khartoum state suggested that the prevalence of toombak use among Sudanese secondary school students is low and the more frequent users the male and older students' decision of using toombak was based upon experience with toombak use and the social image attached to its use. Easy access of toombak, friends and classmate's encouragement were among the factors which support intention to use toombak but only in the unadjusted analyses (Almahdi *et al.,* 2018).

The use of Toombak by school workers was related to poor awareness, a negative attitude towards their position in the control of Toombak and poor preventive

practice. Therefore, the use of toombak by school workers could decrease their motivation and their ability to prevent a major health problem caused using toombak and affects their role model actions. On the other hand, good awareness was correlated with school staff involvement with preventive practices in schools, which in turn empowers their positive attitude towards their role in charge of toombak (Almahdi, 2017).

2.1.2 Smoking cigarette

Cigarette smoking is type of tobacco that is burned, inhaled and absorbed in to blood stream. Cigarette is dried tobacco plant leaves which rolled in rice paper to perform round cylindrical shape called cigarette. When smoke, the tobacco contents as nicotine mixed with aerosol particles and gasses as carbon monoxide which penetrate in to lungs and then blood circulation (West *et al.*, 2007).

Cigarette smoke is a major cause of preventable morbidity and mortality, killed more than 3 million people per year around the world (Aurelio, 2005).

Cigarette smoke is containing more than 4,000 chemicals, including nicotine, carbon monoxide, acrolein, and oxidant compounds, exposure to it induces multiple pathological effects include oxidative stress which causing by the reactive oxygen species content of cigarette, plague, platelet activation, prothrombotic changes and cardiovascular disease (Csordas, 2013).

Reducing cigarette addiction by reducing the content of nicotine can potentially have a profound impact on the public health by either gradual or immediate nicotine reduction. This was resulting in significant smoking behavior that is compensatory which led to decreased level of nicotine and carbon monoxide in urine or plasma when smoking reduced nicotine cigarette (Hatsukami, 2015).

2.2. Pancreas

The pancreas is the second large organ located in the curve made by the duodenum outside the gastrointestinal tract play an important role in the digestive process. compose of endocrine and exocrine tissues, the endocrine tissue produces insulin, glucagon, gastrin, and somatostatin hormones while the exocrine tissue contains acinar cells secrete fluid rich of digestive enzymes reach the duodenum through the common bile duct, which is highly alkaline to neutralize the gastric fluid produce from stomach and contain different type of digestive enzymes which are responsible of proteins, carbohydrates, and fats digestion and metabolism. Many diseases affect the pancreas function rather than trauma, diabetes meletus, cystic fibrosis, pancreatic cancer, acute pancreatitis and chronic pancreatitis. (Bishop *et al.,* 2010). Cigarette smoking has been identified as a cause of pancreas cancer in most epidemiological studies of pancreatic carcinoma, most studies have been based only on male subjects or have combined men and women in statistical analyses. Smoking is a cause of pancreatic cancer in women and that the risk is higher than in men for categories of amount and duration of smoking (Muscat *et al.,* 1997).

Also an evidence candidates for carcinogenicity of smokeless Tobacco-specific N-nitrosamines causing cancer of pancreas gland and other organs (Boffetta *et al.,* 2005)**.**

2.2.1 Pancreatitis:

Pancreatitis is inflammation of the pancreas caused by auto digestion of the pancreatic cells as a result of reflux of bile or duodenal contents into the pancreatic duct. Acute edema with large amounts of fluid accumulating in the retroperitoneal space and an associated decrease in effective circulating blood volume will lead to

cellular infiltration and eventually necrosis. Pancreatitis classified to acute which is rapid episode with reversible cell damage and chronic with recurrent episode and irreversible damage of pancreatic cells which can also be acute or chronic. Pancreatitis occur in midlife the Painful episodes usually reaching a maximum within minutes or hours, lasting for several days or weeks, and commonly accompanied by nausea and vomiting. The predisposing factors include alcohol abuse, hyperlipoproteinemia and hyperparathyroidism. Both acute and chronic pancreatitis compromise the exocrine activity of pancreas which diminish the digestion process leading general malabsorption syndrome particularly the fats malabsorption which cause steatorrhea and impair the metabolism of electrolytes, water, vitamins (particularly fat-soluble vitamins A, D, E, and K), minerals and vitamin B12. Amylase and Lipase are the most common enzymes use to diagnose the pancreatitis (Bishop *et al.*, 2010).

Chronic pancreatitis is a progressive fibro inflammatory disease that exists in large-duct (often with intraductal calculi) or small-duct form. In many patients this disease results from a complex mix of environmental (eg, alcohol, cigarettes, and occupational chemicals) and Pain in the form of recurrent attacks of pancreatitis or constant and disabling pain is usually the main symptom. (Braganza *et al.*, 2011).

The current tobacco use is the first of risk factors associated with significant increases in acute pancreatitis, chronic pancreatitis, and pancreatic cancer, Prevention strategies should consider Vegetables and fruit consumption are associated with reduced risk for pancreatic diseases (Alsamarrai *et al.*, 2014). Nicotinic anti-inflammatory pathway has been demonstrated an essential regulator of inflammation during experimental pancreatitis in mice, the study showed for the first time, that pharmacologic stimulation of the peripheral part of the nicotinic anti-inflammatory pathway receptors attenuates inflammation in vivo. The

nicotinic anti-inflammatory pathway may be a future target for the treatment of pancreatitis (van Westerloo *et al.*, 2006)**.**

2.2.2 Smoking cigarette and pancreatitis

An experimental study was conducted in two groups of Albino rats treated with nicotine [(S)-3-(1-Methyl-2-Pyrroli-dinyl) pyridine] serum lipase was measured, and histological procedure was done on rats' pancreatic tissue, histological changes were observed in the pancreatic acinar cells of nicotine- treated animals, Serum level of lipase enzyme demonstrated a significant decrease (Hamed *et al*., 2017).

PubMed and Embase databases were collected up to April 13th, 2019 for eligible studies This meta-analysis studies and nested case-control studies within cohorts that investigated the association between tobacco smoking and the risk of pancreatitis found an increased risk of chronic pancreatitis, acute pancreatitis and chronic/acute pancreatitis combined among smokers and there is a lower risk among former smokers than among current smokers. There was relationship between increasing number of cigarettes per day smoked and pack-years of smoking and pancreatitis risk. A positive association was observed between current smoking and non-gallstone- related pancreatitis, but not with gallstone-related pancreatitis (Aune *et al.,* 2019).

Smoke tobacco also association with loss of chromosome Y (LOY) in blood cells which is associated with high risk of nonhematological tumors has been demonstrated. The data also suggest that smoking has a transient and dose-dependent mutagenic effect on LOY status. The finding that smoking induces LOY thus links a preventable risk factor with the most common acquired human mutation (Dumanski *et al.*, 2015).

2.3. Lipase enzyme

Lipase (LPS) is pancreatic enzyme also known as triacylglycerol acylhydrolase. LPS is a single chain glycoprotein with a molecular weight of 48 kDa. S gene resides on chromosome 10. For maximum activity and greatest specificity bile salts and cofactor secreted by the pancreas called colipase are required. LPS is a small molecule readily filtered through the glomerulus and It is totally reabsorbed by the renal tubules, there for normally not detected in urine. LPS role is to hydrolyze glycerol esters of long-chain fatty acids to intermediate diglyceride then monoglyceride and eventually to glycerol and fatty acid. The most serum lipase derived from pancreas while some of it secreted from gastric and intestinal mucosa. LPS is the most sensitive and specific enzyme used to diagnose the acute pancreatitis. Upon attack, serum LPS activity rises within 4 to 8 hours reach the peaks at about 24 hours and retain to the normal within 8 to 14 days, the increase in lipase activity is not necessarily proportional to the attack severity. Serum lipase activity can increase also in pancreatic carcinoma, cholecystitis and renal insufficiency. LPS activity measured by different methods; used both triglyceride and non-triglyceride substrates, the methods assay lipase include titrimetric, turbidimetric, spectrophotometric, fluorometric, and immunological techniques. The upper reference limit is 38 U/L at 37 "C. The activity in serum is stable at room temperature for 1 week for 3 weeks in the refrigerator and for several years if frozen (Burtis and Bruns, 2014).

Serum lipase level was demonstrated which was slightly but significantly higher in patients with chronic dyspepsia without any explainable cause than in those with chronic dyspepsia due to a known cause. Hence, mild functional pancreatic disorder may underlie the cause of dyspepsia in these (Okada *et al.,* 2009)**.** The diagnosis of acute pancreatitis is based on clinical signs, biochemical tests and

imaging. Normal serum amylase has been reported in acute pancreatitis, but normal lipase is extremely rare, however the diagnosis of acute pancreatitis should be entertained even with normal serum amylase and lipase levels (Shah *et al.*, 2010).

Although serum pancreatic enzymes as amylase and/or lipase levels below the normal serum range are highly diagnostic for chronic pancreatitis patients (Oh *et al.*, 2017), but serum lipase alone is the preferred test for diagnosing acute pancreatitis, since it is more sensitive than serum amylase and remains elevated longer, Concurrent use of amylase and lipase testing to diagnose acute pancreatitis is an unnecessary expense for the hospital and can negatively influence the patient care as it can lead to further tests and prolonged hospitalization (Akhtar *et al.*, 2017).

Chapter Three

3. Materials and methods

3.1 Study Design:

This is a comparative cross-sectional study.

3.2 Study area and duration:

This study was conducted in Alkamlien city- Algzeira State, Sudan in the period from September to December 2020.

3.3 Ethical considerations

This study was approved by scientific committee of Clinical Chemistry Department, College of Medical Laboratory Science in Sudan University of Science and Technology, an informed consent was obtained from each participant (Appendix I). The data required was collected through an interview and administrated questionnaire designed to obtain all the main information needed in the study (Appendix II).

3.4 Study population

A total of 103 male participants were enrolled in this study; 31 of them were cigarettes smokers, 38 were toombak users and 34 apparently healthy non-tobacco users were served as control group. The age range was matched between all groups (20 - 65 years old).

Inclusion criteria

Toombak users and cigarette smokers as case group and non-tobacco users as control group, all groups were apparently healthy individuals.

Exclusion criteria:

Alcohol abusers, individuals using other type of smokeless tobacco, individuals with known pancreatic diseases were excluded from the study.

3.5 Samples

About 4mls of venous blood were collected from each participant under aseptic condition and placed in plane vacutainer, after clotting the samples centrifuged for 5mins at 3000 RPM to obtain serum which stored at -20c till the time of analysis.

3.6 Method of lipase activity assessment (Enzymatic colorimetric assay)

Principle: The determination of lipase is based on the cleavage of apecific chromogenic lipase substrate1,2-o-dilaurylrac-glycero-3-glutaric acid-(6-methyl-resorufin) ester emulsified in stabilized micro-particles. In the presence of specific activators of pancreatic lipase and colipase, calcium ions and bile acids, the substrate is converted to 1,2-o-dilauryl-rac-glycerol and glutaric acids-6-methylresorufinester which decomposes spontaneously to glutaric acid and methylresorufin. The increase of absorbance at 570nm, due to methylresorufin formation, is proportional to the activity of lipase in sample.

3.7 Calculation of BMI

The body weight in kilograms divided by the height in meters squared

BMI = Kg/ $m^{2.}$

3.8 Quality control

One level of control and calibrator provided in the reagent kit by manufacturer was analyzed with sample run by Mindary 380 auto analyzer to assure the accuracy and precision of the results.

3.9 Data analysis

The data was analyzed by SPSS program version 23, ANOVA test was used for comparison of means and a Pearson's correlation test was used to study the association between serum lipase activity and study variables. P- value < 0.05 was consider significant.

Chapter Four

4. Results

This study aimed to assess lipase enzyme activity in tobacco and anon-tobacco user groups, the statistical analysis was done by using SPSS computer program and the results obtained were as follow:

Table 4.1 Shows demographic data; the means and standard deviations of the study variables among all groups.

Table 4.2 Shows Age categories percent among toombak users and cigarette smokers group.

Table 4.3: BMI and WC percent among all groups.

Table 4.4 shows the high lipase results among all groups.

Table 4.5 Shows no difference between the means of lipase activity with *P-value* 0.595, BMI with *P-value* 0.683 and WC with *P-value* 0.783 among all groups.

Figure 4.1 shows significant weak positive correlation between lipase activity and the frequency of using toombak per day among toombak users' group with *P-value:* 0.025, r: 0.363.

Figure 4.2 shows significant weak positive correlation between lipase activity and duration of using toombak per year among toombak users' group with *P- value:* 0.014, r: 0.397.

Figure 4.3 shows no correlation between lipase activity and frequency of smoking cigarette per day among smokers' group with *P-value:* 0.946, r: 0.013.

Figure 4.4 shows no correlation between lipase activity and duration of cigarette smoking per year among smokers' group with *P-value:* 0.645, r: 0.086.

Figure 4.5 shows no correlation between lipase activity and Body Mass Index (BMI) among all groups with *P- value:* 0.702, r 0.038.

Figure 4.6 shows no correlation between lipase activity and waist circumference among all groups with *P-value:* 0.468, r 0.072.

Figure 4.7 shows significant weak positive correlation between lipase activity and age among all groups with *P- value:* 0.012, r 0.246.

Figure 4.8 Shows significant weak positive correlation between lipase activity and age among toombak users group with *P-value:* 0.039, r: 0.337.

Figure 4.9 Shows no correlation between lipase activity and age among cigarette smokers with *P-value:* 0.300, r: 0.192.

Tobacco use/smoking/toombak/No		Lipase Activity U/L	Body Mass Index	Age	Waist Circumference/inch
Not tobacco user N 34 (33%)	Mean	38.6	23.7	42.0	36.3
	Std. Deviation	15.3	4.5	11.8	4.3
Toombak user N 38 (39.6%)	Mean	42.8	23.6	43.7	36.6
	Std. Deviation	18.0	4.8	13.3	4.4
Cigarette smoker N 31 (30.1%)	Mean	41.1	24.6	39.33	37.2
	Std. Deviation	18.5	5.3	12.1	7.1
Total N 103 (100%)	Mean	40.9	23.9	41.8	36.6
	Std. Deviation	17.2	4.8	12.4	5.2

Table 4.1: Demographic data; lipase activity, BMI, waist circumference and age means and standard deviation among all groups.

Age categories	Toombak users (percent)	Cigarette smokers (percent)
20-30 y	23.7%	32.3%
31-40 y	15.8%	19.4%
41-50 y	26.3%	25.8%
51-65 y	34.2%	22.6%

Table 4.2: Age categories percent among toombak users and cigarette smokers groups.

BMI among all groups (percent)	
Normal Overweight Obese	64.1% 26.2% 9.7/5
Waist circumference among all groups (percent)	
< 40 inch ≥ 40 inch	74.8% 25.2%

Table 4.3: BMI and WC percent among all groups.

Lipase activity results > 60 U/L	
Toombak users group	15.8%
High results with Frequency > 15 times/day	100%
High results with duration > 30 year	50%
High results with age > 50y	66.7%
Cigarette smokers group	19.3%
Non-tobacco users	5.9%
Total high results	13.6

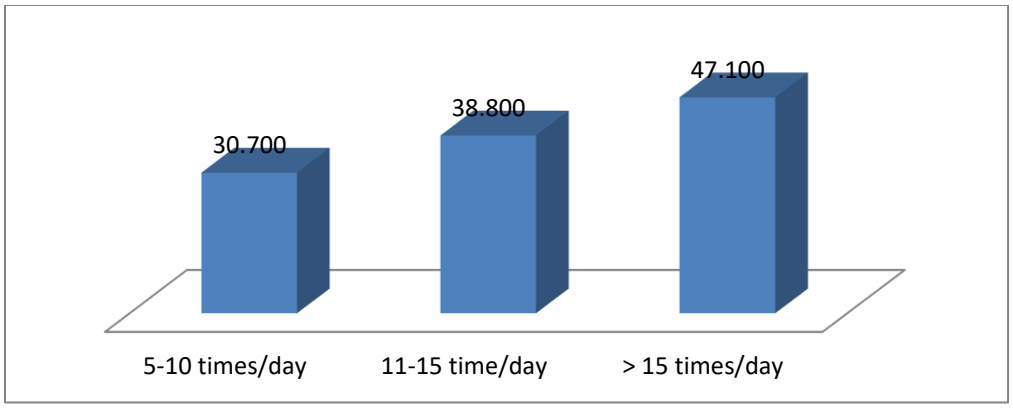
Table 4.4: High lipase activity results among all groups.

	Toombak users group	Cigarette smokers group	Non-tobacco users group
Lipase activity mean U/L	42.8	41.1	38.6
P- value 0.595			
BMI mean	23.6	24.6	23.7
P-value 0.683			
WC mean inch	36.6	37.2	36.3
P-value 0.783			

Table 4.5: Comparison between lipase activity, BMI and WC means among all groups.

One way ANOVA test

P-value considered significant when < 0.05



Frequency of using toombak/day categories and lipase activity means.

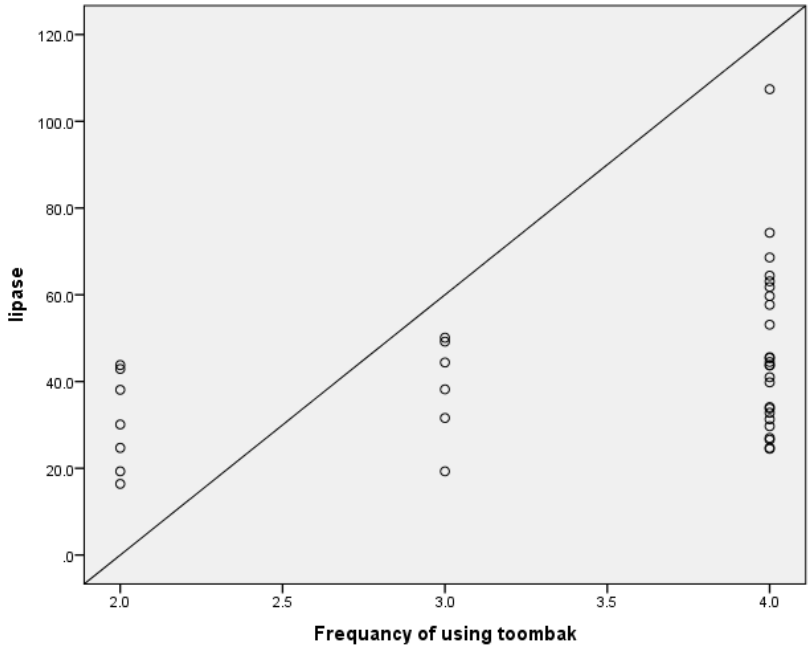
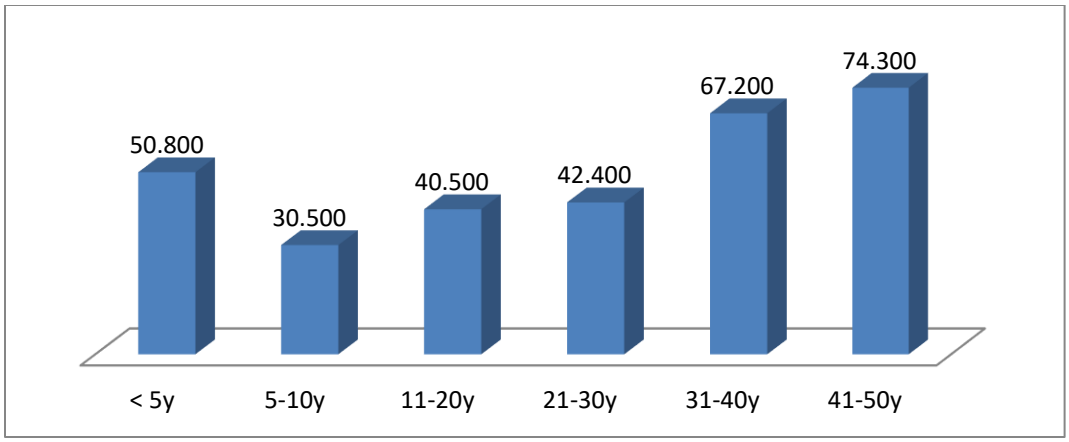


Figure 4.1 Correlation between the Lipase activity means and frequency of using toombak/day among toombak users' group with *P- value:* 0.025, r 0.363.

A scatter plot

P-value: is significance of correlation when <_0.05.

r: is coefficient and strength of correlation.



Duration of using toombak/year categories and lipase activity means.

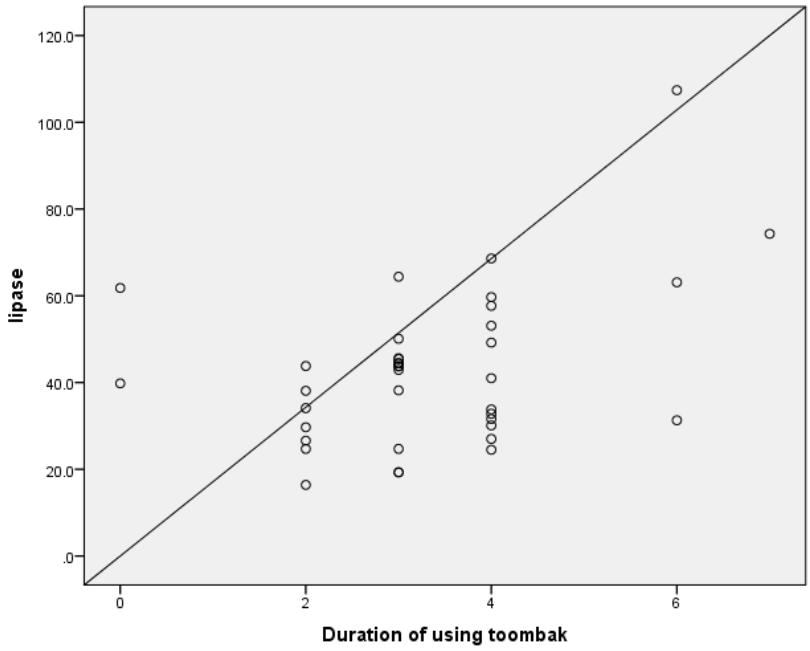
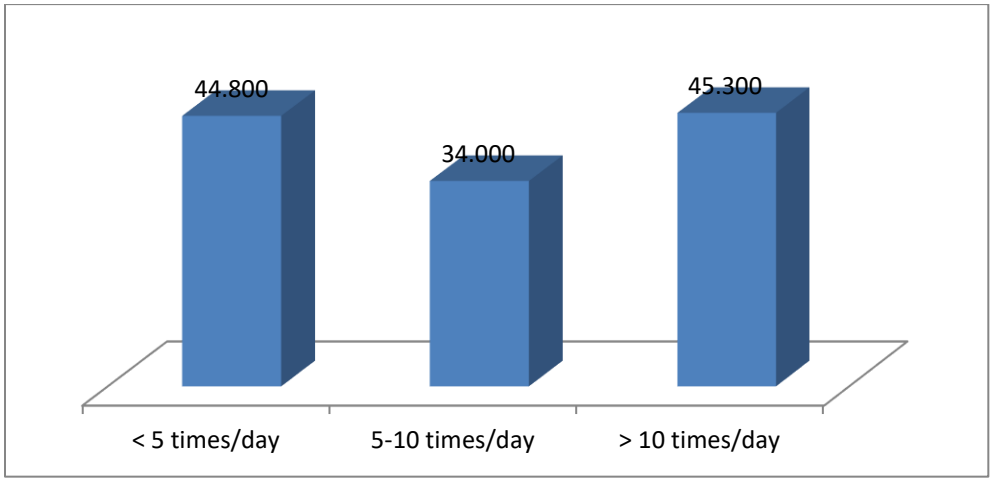


Figure 4.2 Correlation between lipase enzyme activity means and duration of using toombak per year among the toombak users' group with *p-value*: 0.014, r: 0.397.

A scatter plot

P-value: is significance of correlation when <_0.05.

r: is coefficient and strength of correlation.



Frequency of smoking/day categories and lipase activity means.

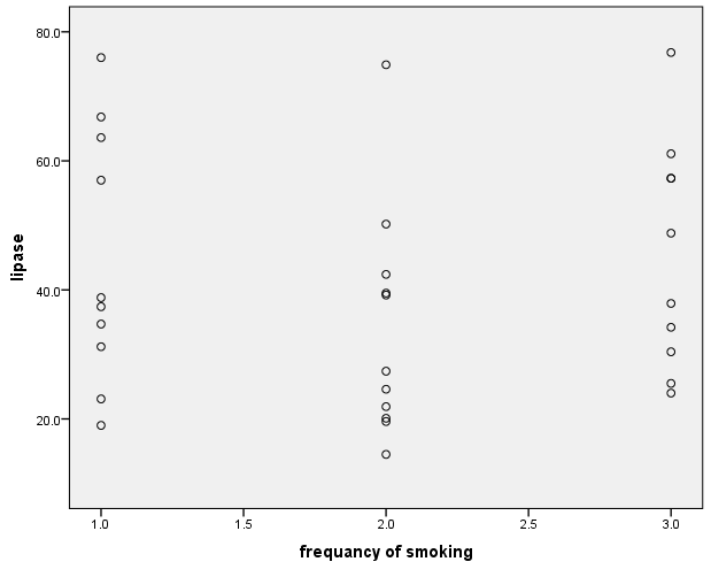
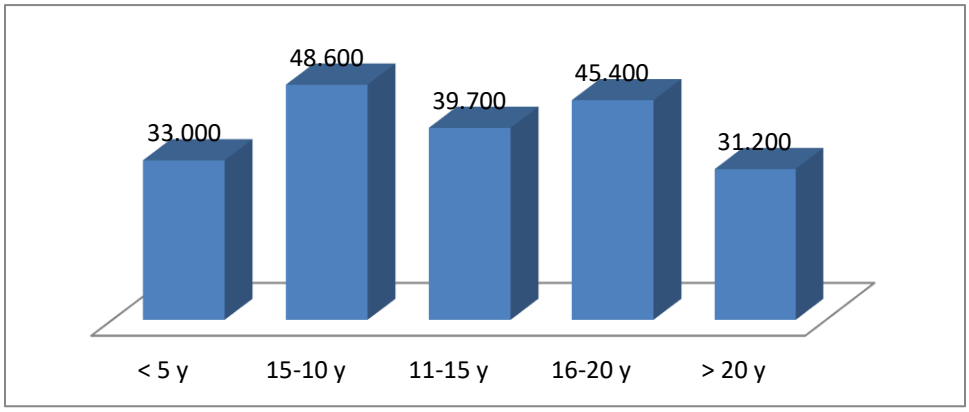


Figure 4.3 Correlation between lipase enzyme activity and frequency of smoking cigarette per day among smokers' groups with *P- value:* 0.946, r: 0.013.

A scatter plot

P-value: is significance of correlation when <_0.05.

r: is coefficient and strength of correlation.



Duration of smoking/year categories and lipase activity means.

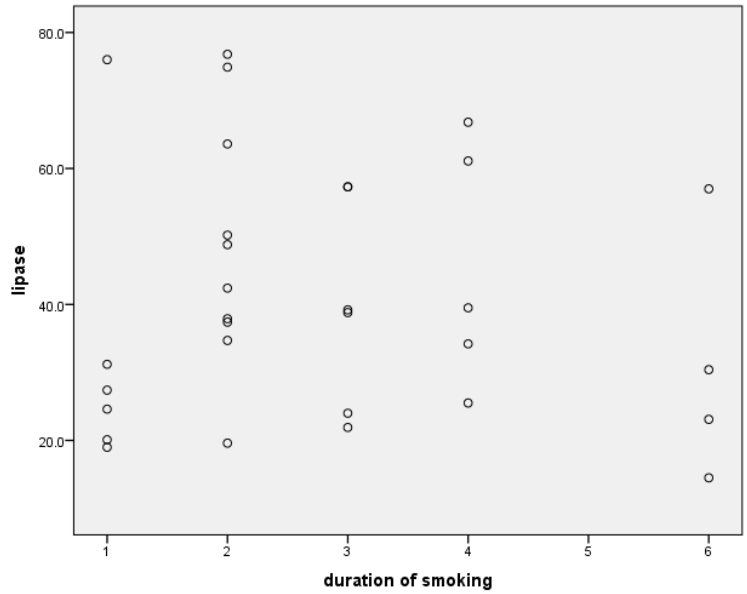


Figure 4.4 Correlation between lipase activity and duration of cigarette smoking per year among smokers' group with *P-value:* 0.645, r: 0.086.

A scatter plot

P-value: is significance of correlation when < 0.05.

r: is coefficient and strength of correlation.

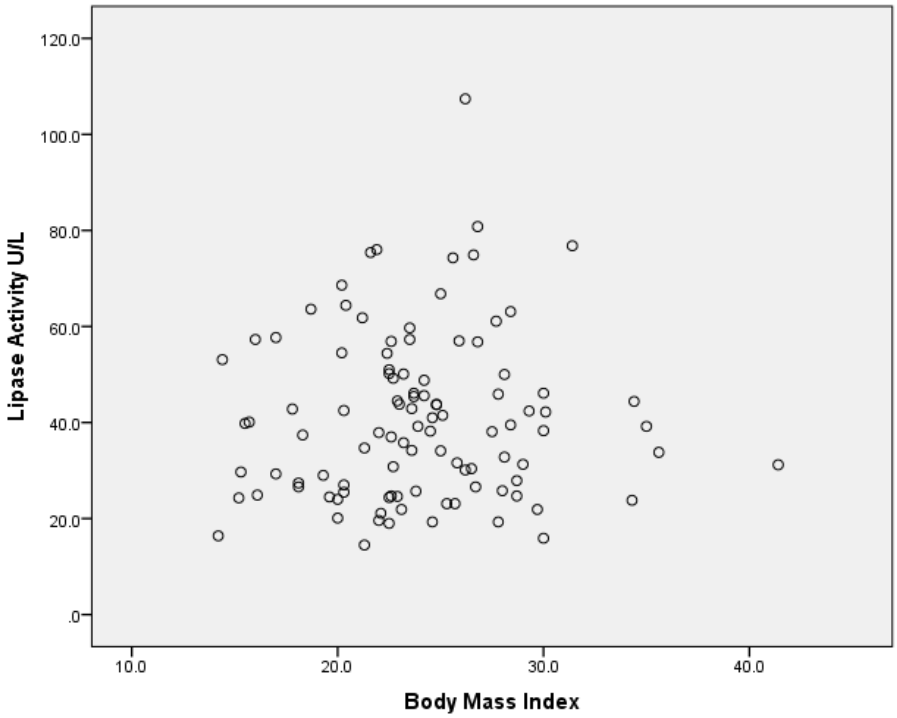


Figure 4.5 Correlation between lipase enzyme activity and Body Mass Index (BMI) among all groups with *P- value:* 0.702, r: 0.038.r: is coefficient and strength of correlation.

A scatter plot

P-value: is significance of correlation when <_0.05.

r: is coefficient and strength of correlation.

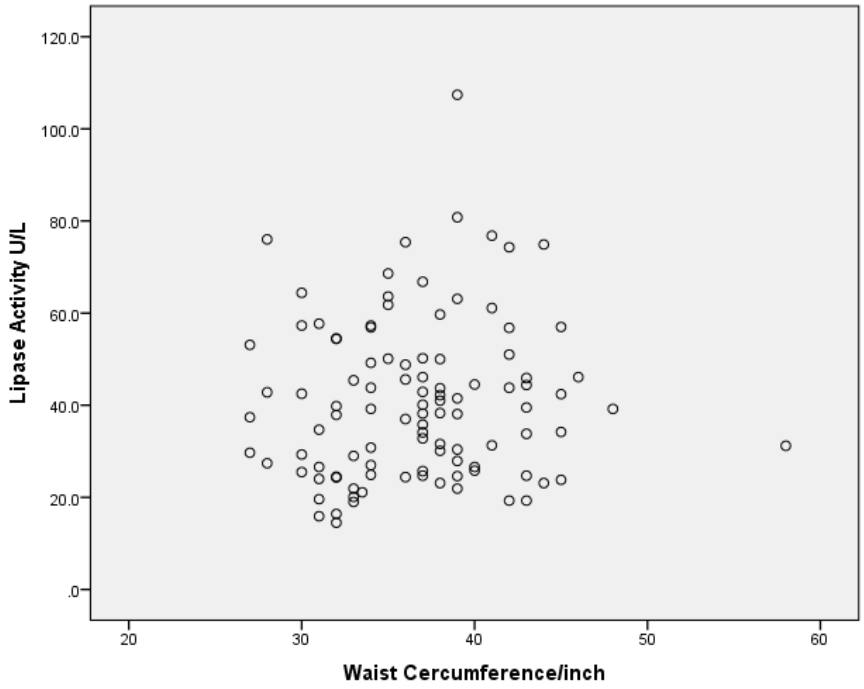
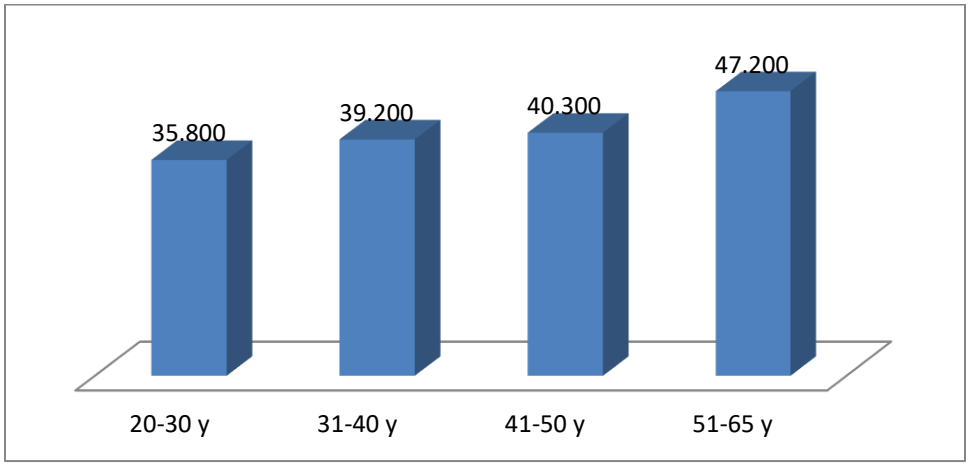


Figure 4.6 Correlation between lipase enzyme activity and waist circumference among all groups with *P-value* 0.468, r 0.072.

A scatter plot

P-value: is significance of correlation when <_0.05.

r: is coefficient and strength of correlation.



Age categories and lipase activity means.

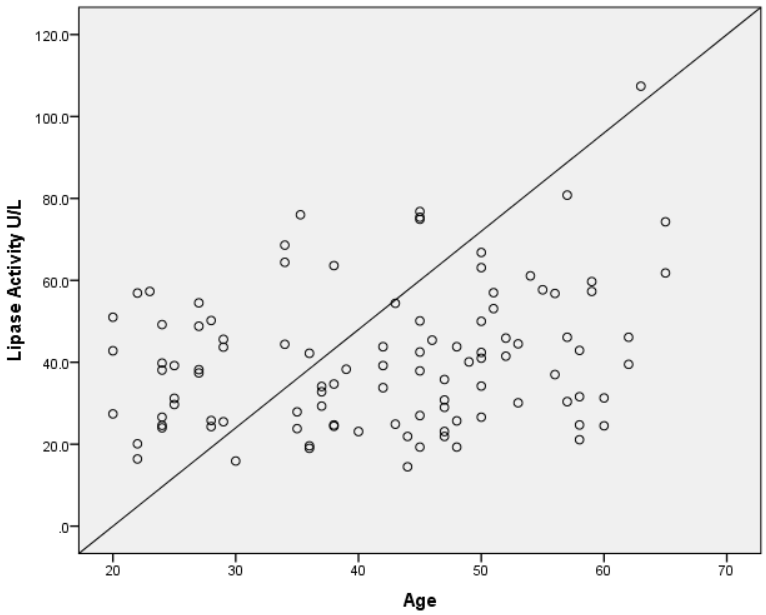


Figure 4.7 Correlation between lipase enzyme activity means and age among all groups with *P-value* 0.012, r 0.246

A scatter plot

P-value: is significance of correlation when <_0.05.

r: is coefficient and strength of correlation.

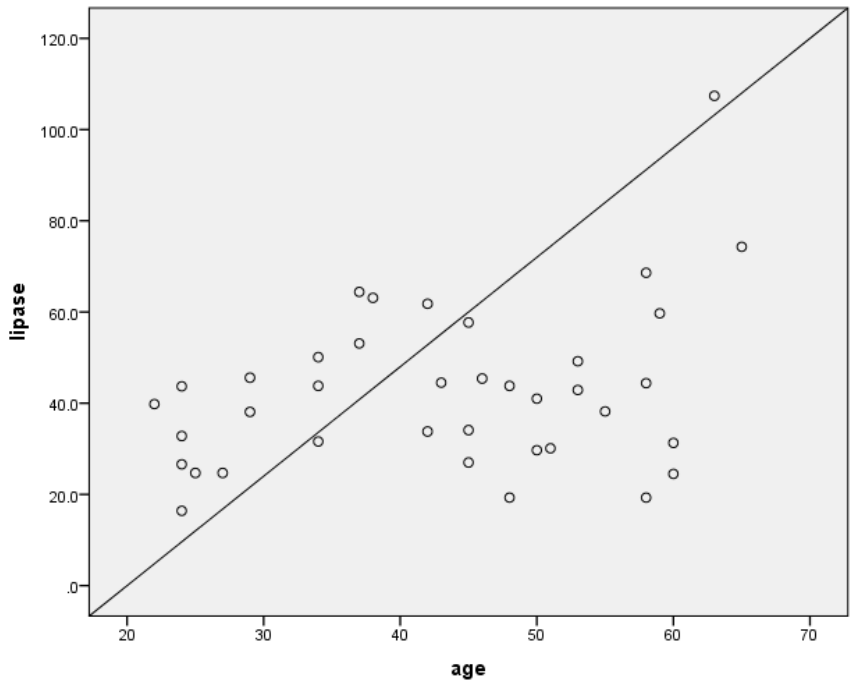


Figure 4.8 Correlation between lipase enzyme activity means and age among toombak users group with *P-value* 0.039, r 0.377

A scatter plot

P-value: is significance of correlation when <_0.05.

r: is coefficient and strength of correlation.

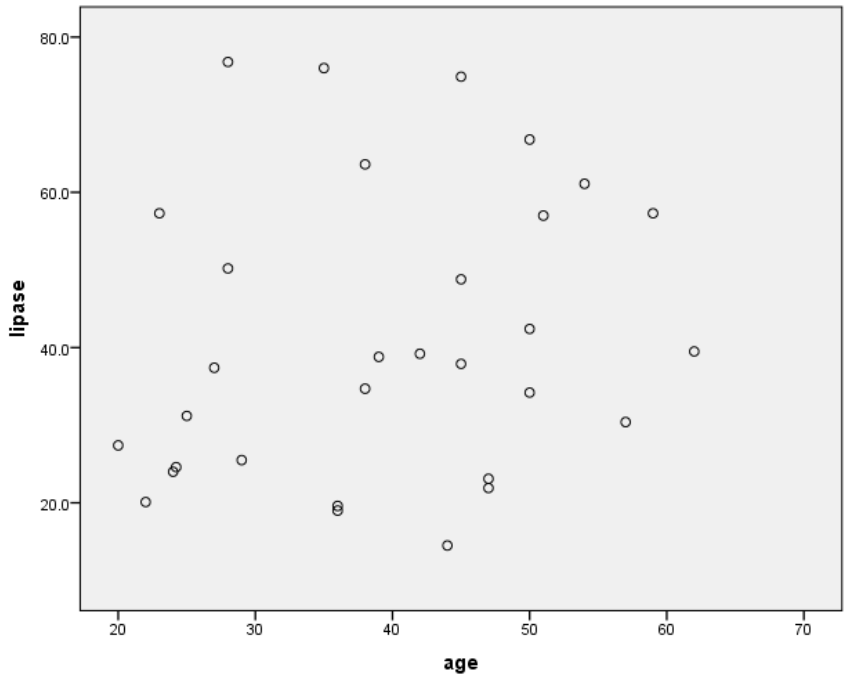


Figure 4.9 Correlation between lipase enzyme activity means and age among cigarette smokers group with *P-value:* 0.300, r: 0.192

A scatter plot

P-value: is significance of correlation when <_0.05.

r: is coefficient and strength of correlation.

Chapter Five

5. Discussion, conclusion and recommendations

5.1. Discussion:

In the current comparative cross-sectional study which aimed to assess lipase activity among tobacco and non-tobacco user groups, statistical analysis showed that 13.6% of lipase activity results were higher than the reference value among the research groups. 5.9% of non-tobacco user results were higher than the reference value compare to 19.3% of smoker's results and 15.8% of toombak user's results. All the toombak users with high results were using toombak with the highest frequency which was more than 15 times per day, 66.7% of them were elder than 50 years old, and 50% of them were using toombak for more than 30 years ago, this indicate the effect of long term and high frequency of use on the lipase activity induce its elevation.

65.5% of toombak users were from 41-65 years old compare to 39.5% were from 20-40 years old, which mean the elder people were more likely used toombak, although 23.7% of toombak users were from 20-30 years old compare to 15.8% toombak users with age of 31-40 years old, that means the younger also more likely used toombak. In the smokers group the more likely age category smoking cigarette was the younger age 20-30 years old with 32.3%.

In this study, twenties were more likely to use toombak and smoke cigarette this agreed with the study conducted among pharmacy students from different university in Khartoum found that 68.9% of the student were using toombak because of the strong influence of their friends and they always seek out groups of friends sharing the same behavior (Mahmoud *et al.,* 2015).The age range of 31-40 years old was the lesser range used toombak and smoking in this study, this findings need additional studies regarding tobacco use among all ages.

Statistical analysis also shows no difference between the means of lipase activity with *P-value* 0.595, BMI with *P-value* 0.683 and WC with *P-value* 0.783 among all groups. Although the frequency of using toombak and the duration of use showed a significant weak positive correlation with lipase enzyme activity among toombak users group with *P- value:* 0.025, r: 0.363 for frequency and *P- value:* 0.014 , r 0.397 for duration of use which indicate the association of frequency and duration with increase the lipase enzyme activity, this study results also showed no association between frequency of smoking per day with *P-value:* 0.946, r: 0.013 and duration of smoking per year with *P-value:* 0.645, r: 0.086 among smokers' group a results which disagreed with what reported by Greer and his team as they highlighted that the smoking is one of pancreatitis risk factors which is dose dependent and affect the progression of the disease (Greer *et al.,* 2015). The good explanation of this study results that it was obtained from Sudanese population were using only cigar with no use of alcohol or other tobacco type.
Although it is reported that body mass index and waist circumference indicate the body weight standardization and obesity, this study found no association between BMI *P- value:* 0.702, r 0.038 and waist circumference *P-value:* 0.468, r 0.072 with lipase activity among all groups.

As per this study, aging may affect the activity of lipase because there was a significant weak positive correlation between the age and the lipase activity among all groups with *P-value:* 0.012, r: 0.246 and among toombak users with *P-value:* 0.039, r: 0.337 while no association was found between lipase activity and age among smokers *P-value:* 0.300, r: 0.192 which mean lipase activity increase by aging with effect of toombak use which well explained by the association of lipase activity with frequency and duration of using toombak over atime. This result

disagreed with Yamamoto and his team study results which found that lipase activity decreased by aging in rats (yamamoto *et al*., 2014) , this disagreement of results may be due to difference between human and rat age and biological mechanism and the effect of using toombak.

5.2. Conclusion

This study result revealed no association between lipase activity among toombak dippers and cigarette smokers when compared to non-tobacco users. Lipase activity is associated with frequency and duration of using toombak among toombak users but not associated with the frequency and duration of cigarette smoking among cigarette smokers.

5.3. Recommendations:

1. Further studies on a relation between toombak and pancreatic and other organs function needed since the lipase activity affected by the frequency and duration of using toombak.
2. Additional studies needed to enroll on large populations of toombak users to estimate the effect of using toombak on health as general because its use is widely frequent in Sudan and it may involve in many medical conditions, also prevalence studies of using tobacco generally in Sudan is required specially among younger and the elder age range too among both male and female population.
3. The associations between cigarette smoking and lipase activity or pancreatic function need further investigational studies in Sudan because much research in other population reported the positive association between them.
4. A health program is recommended to be designed for awareness of dangers and effects of toombak using, cigarette smoking and other tobacco types and encourage the tobacco use cessation.

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Appendices

Sudan University of Science and Technology
Collage of Graduate Studies
Collage of Medical Laboratory science
Clinical Chemistry Department
Researcher Name : Dalal Abdalla Osman

جامعة السودان للعلوم والتكنولوجيا
كلية الدراسات العليا
كلية علوم المختبرات الطبية
قسم الكيمياء السريرية
اسم الباحث: دلال عبدالله عثمان

Informed Consent for Participation in Research — *استمارة موافقة على المشاركة في بحث*

THIS RESEARCH WILL SUBMETT IN A PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER'S DEGREE. **هذا البحث سيقدم ك بحث تكميلي لنيل درجة الماجستير**

Research Title: Assessment of serum Lipase activity Among Sudanese males using smoke and smokeless tobacco in Alkamlein City-Algazeira state

عنوان البحث: تقويم مستوى نشاط انزيم اللايبيز في مصل دم السودانيين الذكور المدخنين ومستخدمي التبغ غير المخن في مدينة الكاملين-ولاية الجزيرة

Introduction about the Research: using toombak and smoking cigarette are types of tobacco widely use in Sudan especially among males which are evidently causes many diseases. This research will conduct to study the capability of toombak and smoking cigarette leading pancreatitis. This questionnaire will provide important information needed for the research

مقدمة عن البحث: استخدام التمباك وتدخين السيجار هما من أنواع التبغ تستخدم بصورة واسعة في السودان خصوصا عند الرجال ولقد ثبت أن استخدامهما يسبب العديد من الأمراض. هذا البحث يقوم بدراسة احتمالية تسبب استخدام التمباك وتدخين السيجار لمرض إلتهاب البنكرياس. هذا الاستبيان يوفر معلومات مهمة مطلوبة للبحث .

Research purpose: To assess the toombak use and smoking cigarette capability leading pancreatitis by measuring lipase enzyme level among toombak users, cigarette smokers and nontobacco users group.

الغرض من البحث: دراسة استعمال التمباك وتدخين السيجار ومقدرتهما على التسبب بالتهاب البنكرياس بواسطة قياس مستوي انزيم لايبيز في الدم لمستخدمي التمباك ومدخني السيجار مجموعة غير مستخدمة للتبغ.

The participation Risks and Discomfort: There is no known risks for participation in this study and participant will not suffer from anything rather than the little pain will feel from the venipuncture while collecting the blood sample and usually there is no complications following this process.

مخاطر المشاركة في الدراسة: لا توجد مخاطر معلومة للمشاركة في الدراسة ولن يعاني المشارك إلا من الألم القليل أثناء أخذ عينة الدم من الوريد وعادة لا توجد مضاعفات لهذه العملية.

The anticipated benefits: This study will provide significant information particularly about using toombak and smoking cigarette and their possibility to cause pancreatitis. The study participant will get a result copy of the blood test will be done without payment which is useful information about his/her pancreatic health status.

فوائد المشاركة: ستوفر الدراسة معلومات ذات قيمة عن استخدام التمباك وتدخين السيجار وعن احتمالية تسببهما لمرض إلتهاب البنكرياس. سيتحصل المشارك في الدراسة على نسخة من نتيجة فحص الدم الذي سيتم بدون مقابل مالي وهي معلومات مفيدة عن حالة البنكرياس الصحية لدى المشارك.

The confidentiality and privacy: privacy and confidentiality of all participants' personal information will be maintained only the researcher and the research work team can reach the record that identifying the participants and if the results of the study will publish, the study participant identity will remain confidential.

خصوصية وسرية المعلومات: سيتم المحافظة على خصوصية وسرية كل المعلومات الشخصية للمشاركين، يسمح للباحث وفريق عمل البحث فقط بالوصول للسجلات التعريفية بالمشتركين وسيتم المحافظة على سرية هويتهم حتى في حالة نشر نتائج الدراسة.

Compensation for participation: There will be no payment or medical compensation anticipated for the participation in this study.

التعويض عن المشاركة: لن يكون هناك اي تعويض مادي او طبي متوقع مقابل المشاركة في هذه الدراسة.

The contact with the researcher: The participants have a right to contact the researcher for answers to related questions about the research or to inform him in case of withdrawing using this phone number 0129105115.

التواصل مع الباحث: يحق للمشاركين التواصل مع الباحث للإجابة على أسئلتهم المتعلقة بالبحث او إعلامه في حالة الانسحاب عن طريق الإتصال بالهاتف0129105115

Voluntary participation: Participant will voluntarily participate, willing to collect blood sample from him and provide information needs for the study by filling the given questionnaire. He/she can withdraw from the study at any time with no loss of benefit.

التطوع بالمشاركة: سيوافق المشارك على المشاركة طواعية مع موافقته على أخذ عينة من الدم **و**إعطاء المعلومات المطلوبة للدراسة بواسطة ملأ الاستبيان المصاحب ومن حقه الانسحاب من المشاركة في أي وقت من غير فقدان الفائدة.

I have read and understood all aspects of research, and I willingly agree to participate in the research entitled above with my signature.

لقد قرأت وفهمت كل جوانب البحث وعليه أوافق على المشاركة بالبحث المعنون أعلاه وعلى ذلك أوقع

اسم المشترك ..... الإمضاء ..... التاريخ ..... توقيع الباحث .....

Participant name: ..... Signature .....

Researcher signature : ..... Date .....

Sudan University of Science and Technology

Collage of Graduate Studies

Collage of Medical Laboratory Science

Clinical Chemistry Department

Researcher Name: DALAL ABDALLA OSMAN

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا

كلية علوم المختبرات الطبية

قسم الكيمياء السريرية

اسم الباحث: دلال عبدالله عثمان

Questionnaire استبيان

THIS RESAECH WILL SUBMITT IN A PARTIAL FULFILLMENT OF REQUAIRMENTS FOR THE AWARD OF MASTER'S DEGREE

بحث تكميلي لنيل درجة الماجستير

Research Title: Assessment of serum Lipase activity Among Sudanese males using smoke and smokeless tobacco in Alkamlein City-Algazeira state

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Location: …….. الموقع: .....

Participant code number: ☐ **رقم المشارك:** ☐

Height ☐ cm Weight ☐ Kg **الطول** ☐ سم **الوزن** ☐ كج

Waist Circumference ☐ cm محيط الخصر ☐ سم

Age: ☐ **العمر:** ☐

Gender: Male ☐ Female ☐ **الجنس:** ذكر ☐ أنثى ☐

Duration of using Toombak / year**:** **فترة استخدام التمباك / السنة:**

Less than 5 y ☐ 5-10 y ☐ 11-20 y ☐ 21-30 y ☐ أقل من 5 سنوات ☐ 5-10سنة ☐ 11-20 سنة ☐

31-40 y ☐ 41-50 y ☐ more than 50 y ☐ 21-30سنة ☐ 31-40سنة ☐ 41-50سنة ☐ أكثر من 50سنة ☐

Frequency of using Toombak per day: **عدد مرات إستخدام التمباك في اليوم:**

Less than 5 times ☐ 5-10 times ☐ أقل من 5 مرات ☐ 5-10 مرات ☐

11-15 times ☐ more than 15 times ☐ 11-15 مرة ☐ أكثر من 15 مرة ☐

Are you smoking cigarette? YES ☐ NO ☐ **هل تدخن السيجار؟** نعم ☐ لا ☐

Duration of smoking cigarette / year: **فترة تدخين السيجار / السنة:**

Less than 5y ☐ 5- 10y ☐ 11-15 y ☐ أقل من 5 س ☐ من 5-10 س ☐ من 11- 15 س ☐

16-20 y ☐ More than 20 y ☐ 16-20 س ☐ أكثر من 20 سنة ☐

Frequency of smoking cigarette per day: **عدد مرات تدخين السيجار في اليوم:**

Less than 5 times ☐ 5-10 times ☐ More than 10 times ☐ أقل من 5 مرات ☐ 5-10 مرات ☐ أكثر من 10 مرات ☐

Participant Name: ……..... اسم المشارك: .....

Signature …… Date …… التوقيع ..... التاريخ.....

Sudan University of Science and Technology

Collage of Graduate Studies

Collage of Medical Laboratory Science

Clinical Chemistry Department

Researcher Name: DALAL ABDALLA OSMAN

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا

كلية علوم المختبرات الطبية

قسم الكيمياء السريرية

اسم الباحث: دلال عبدالله عثمان

Questionnaire استبيان

THIS RESEARCH WILL SUBMITT IN A PARTIAL FULFILLMENT OF REQUAIRMENTS FOR THE AWARD OF MASTER'S DEGREE

بحث تكميلي لنيل درجة الماجستير

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Location: ……. ..... الموقع:

Participant code number: ☐ **رقم المشارك:** ☐

Sex: Male ☐ Female ☐ **الجنس:** ذكر ☐ أنثى ☐

Height ☐ cm **الطول** ☐ سم

Weight ☐ Kg **الوزن** ☐ كج

Waist Circumference ☐ cm محيط الخصر ☐ سم

Age ☐ **العمر** ☐

Participant Name: …….. .....اسم المشار

Signature …… Date …… التوقيع ..... التاريخ .....

.

LIP

mindray

Generic Name : Lipase Kit (Enzymatic Colorimetric Assay Method)
Abbreviated name :LIP

Order Information

Cat. No.	Package size
LIP0202	R1 1×35 mL + R2 1×9 mL
LIP0203	R1 1×40 mL + R2 1×10 mL
LIP0204	R1 2×40 mL + R2 2×10 mL
LIP0302	R1 1×35 mL + R2 1×9 mL + Calibrator 1×3 mL
LIP0303	R1 1×40 mL + R2 1×10 mL + Calibrator 1×3 mL
LIP0304	R1 2×40 mL + R2 2×10 mL+ Calibrator 1×3 mL
LIP0102	R1 1×35 mL + R2 1×9 mL + Calibrator 1×3 mL + Quality control 1×5 mL
LIP0103	R1 1×40 mL + R2 1×10 mL + Calibrator 1×3 mL + Quality control 1×5 mL
LIP0104	R1 2×40 mL + R2 2×10 mL + Calibrator 1×3 mL + Quality control 1×5 mL

Intended use

In vitro test for the quantitative determination of LIP concentration in serum or plasma on photometric systems.

Summary[1-3]

Lipases are glycoproteins with a molecular weight of 47000 daltons. They are defined as triglyceride hydrolases which catalyse the cleavage of triglycerides to diglycerides with subsequent formation of monoglycerides and fatty acids. Lipase enzymes are produced in the pancreas and also secreted in small amounts by the salivary glands as well as by gastric, pulmo-nary and intestinal mucosa. Determination of lipase is used for di-agnosis and treatment of diseases of the pancreas such as acute and chronic pancreatitis and obstruction of the pancreatic duct.

Method

Enzymatic Colorimetric Assay Method

Reaction Principle

The method for the determination of lipase is based on the cleavage of specific chromogenic lipase substrate 1,2-O-dilaurylrac-glycero-3-glutaric acid-(6'methyl-resorufin)-ester emulsified in stabilized micro-particles. In the presence of specific activators of pancreatic lipase as colipase, calcium ions and bile acids, the substrate is con-verted to 1,2-O-dilauryl-rac-glycerol and glutaric acid-6'-methylresorufinester which decomposes spontaneously to glutaric acid and methylresorufin. The increase of absorbance at 570 nm, due to methylresorufin formation, is proportional to the activity of lipase in the sample.

LIP

mindray

Reagents

Components and concentrations

R1	Tris	40 mmol/L
	Desoxycholate	1.8 mmol/L
	Taurodesoxycholate	7.2 mmol/L
	Colipase	>1mg/L
R2:	Tartrate buffer,	15 mmol/L
	Calcium chloride	0.13 mmol/L
	Lipase Substrate	≥ 0.7 mmol/L
Standards		Activity see label
Quality control		Activity see label

Warnings and precautions

1.For in vitro diagnostic use only.
2.Take the necessary precautions for the use of laboratory reagents.
3.Preservative contained. Do not swallow. Avoid contact with skin and mucous membranes.
4.Disposal of all waste material should be in accordance with local guidelines.
5.Material safety data sheet is available for professional user on request.
6.All human material should be considered potentially infectious.
7.The reagent must be used together with the calibrator bearing the same lot number.

Reagent Preparation

Reagent are ready to use.

CalibratorPreparation

Carefully open the bottle, avoiding the loss of lyophilizate, and pipette in exactly 3.0 mL of distilled/deionized water. Carefully close the bottle and dissolve the contents completely by occasional gentle swirling within 30 minutes. Avoid the formation of foam.

Quality control Preparation

Carefully open the bottle, avoiding the loss of lyophilizate, and pipette in exactly 5.0 mL of distilled/deionized water. Carefully close the bottle and dissolve the contents completely by occasional gentle swirling within 30 minutes. Avoid the formation of foam.

Storage and stability

Up to expiration date indicated on the label, when stored unopened at 2-8 ℃ and protected from light.
Once opened, the reagents are stable for 28 days when refrigerated on the analyzer or refrigerator.
Contamination of the reagents must be avoided.

Do not freeze the reagents.

Once dissolved, the calibrator and control are stable for 30 days at -20℃(Only frozen once).

Reagent blank absorbency

The absorbance of LIP reagent blank at 570 nm should be <0.6 A.

Materials required but not provided

1.NaCl solution 9 g/L.

2.General laboratory equipments.

Specimen collection and preparation

1.Human serum and plasma is suitable for samples. Whole blood, hemolysis is not recommended for use as a sample. Freshly drawn serum is the preferred specimen.

2.Use the suitable tubes or collection containers and follow the instruction of the manufacturer; avoid effect of the materials of the tubes or other collection containers.

3.Centrifuge samples containing precipitate before performing the assay.

4.Samples Stability:

3 days at 15-25℃

7 days at 2-8℃

2 months at -20℃ (Only frozen once)

Assay procedure

	Blank	Sample
Reagent 1	200 µL	200 µL
Dist. water	2 µL	—
Sample	—	2 µL
Mix, incubate at 37℃ for 3 min, then add:		
Reagent 2	40 µL	40 µL
Mix thoroughly, incubate 37℃ for 2 min, and then read the absorbance change value within 2 min.		
ΔA/min = [ΔA/min sample]- [ΔA/min blank]		

Calibration

1.It is recommended to use the calibrator in kits and 9 g/L NaCl for tow-point calibration

2.Traceability of the calibrator: The calibrator is traceable to manufacturer's selected measurement..

3.Calibration frequency:

After reagent lot changed.

As required following quality control procedures.

Quality control

At least one level of control material should be analyzed with each batch of

samples. In addition, these controls should be run with each new calibration, with each new reagent cartridge, and after specific maintenance or troubleshooting procedures as detailed in the appropriate system manual. We recommend using the control in kits to verify the performance of the measurement procedure; other suitable control material can be used in addition.

Each laboratory should establish its own internal quality control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

Calculation

The analyzer calculates the LIP concentration of each sample automatically after calibration.

Or: C sample = (ΔA sample/ΔA calibration)×C calibration

Reference Intervals [4]

Each laboratory should establish its own reference intervals based upon its patient population. The reference intervals measured at 37℃ listed below were taken from literature:

Sample Type	S.I. Units
Serum/plasma	≤ 60 U/L

Performance Characteristics

Representative performance data obtained from Mindray system (Mindray BS series analyzers / Mindray LIP Reagent) is given below. Results may vary if a different instrument, an individual laboratory or a manual procedure is used.

Limitations-interference

The following substances were tested for interference with this methodology. Criterion: Recovery within ±10 % of initial value.

Substance	Level Tested	Observed Effect
Ascorbic acid	30 mg/dL	NSI*
Bilirubin	40 mg/dL	NSI
Lipemia	500 mg/dL	NSI
Hemoglobin	150 mg/dL	NSI

* NSI: No Significant Interference (within ±10%)

Linearity Range

The Mindray System provides the following analytical ranges:

Sample Type	S.I. Units
Serum/plasma	5～250 U/L

If the value of sample exceeds 250 U/L, the sample should be diluted with 9 g/L NaCl solution (e.g. 1+ 9) and the result should be multiplied by 10.

Analytic Sensitivity/Limit of Detection

The lowest measurable LIP concentration that can be distinguished from zero is 5 U/L with 99.7% confidence.

Precision

Precision performance using the CLSI Approved Guideline EP5-A2 to assay serum control appears in the table below[5]. U: U/L

Type of Imprecision	Level 1			Level 2		
	Mean	SD	CV %	Mean	SD	CV %
Within-run	53.97	1.217	2.255	87.37	1.799	2.059
Between-run		0.644	1.194		1.206	1.380
Between-day		1.747	3.236		1.800	2.060
Within-device		2.224	4.121		2.816	3.223

Method Comparison

A comparison between Mindray System (Mindray BS series analyzers /Mindray LIP Reagent) (y) and Hitachi/ Roche System (Hitachi /Roche (LIP) (x) using 40 samples gave following correlation (U/L): $y = 1.013x + 2.6513$ $R^2 = 0.9996$

Details of the comparison experiments are available on request.

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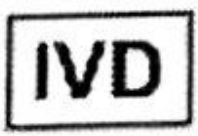
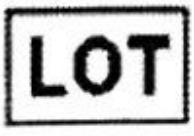
Graphical symbols

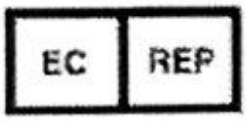


In Vitro Diagnostic medical device



Batch Code


European Conformity



Authorized representative in the European Community


Use By


Consult Instructions for use


Biological Risks


Temperature Limit


Manufacturer

Control

Catalogue number

Manufacturer: Shenzhen Mindray Bio-Medical Electronics Co., Ltd.
Address:Mindray building, Keji 12th Road South, Hi-tech Industrial Park,Nanshan, Shenzhen, 518057 P.R.China
E-mail Address: service@mindray.com
Website: www.mindray.com
Tel:+86-755-81888998
Fax:+86-755-26582680
EC-Representative: Shanghai International Holding Corp. GmbH(Europe)
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