ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Assessment of lipid profile among diabetic and non-diabetic subjects with and without khat chewing habit, in Adama, Ethiopia

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Abbreviation

CHOL: cholesterol

CVD: cardiovascular disease

HDLc: high density lipoprotein cholesterol

IDDM: insulin dependent diabetic mellitus

IHD: ischemic heart disease

KAP: Knowledge, attitude and practice

LDLc: low density lipoprotein cholesterol

MI: myocardial infraction

NIDDM: non-insulin dependent diabetic mellitus

PA: physical activity

TG: triglyceride

VLDL: very low density lipoprotein

WHO: world health organization

Abstract.

Background: The problem of dyslipidemia is high in patients with diabetes mellitus. There is known evidence that abnormalities in lipid metabolism are important risk factors for increased incidence of diabetes associated complications. The most important risk indicators for these complications are lipid profile abnormalities. Very few published papers have tried to draw a conclusion about khat chewing and it effect on lipid level. However, the influence of chewing khat on the lipid profile and dyslipidaemia is insufficiently investigated

Objective: This study aimed to assess lipid profile among diabetic and apparently healthy subjects with and without khat chewing habit.

Method: a comparative crossectional study was conducted in Adama. A total of 220(118 DM and 102 apparently healthy) study subject were included. Convenience sampling methods were used. Basic anthromotric and demographic data was collected using structured questionnaires. And Serum Lipid profile was measured using Cobas 311 automated clinical chemistry analyzer at Adama hospital. Data were analyzed statistically using spss version 20.0. Independent t test were used to compare the means of lipid profile among two groups and logistic regression is used to associate lipid profile parameter with different independent variables. P-value<0.05 at 95% confidence interval (CI) was considered as statistically significant.

Result: The results showed that the mean serum levels of TC =227.33mg/dl, HDL-C = 28.4mg/dl, TG =164.2mg/dl and LDL-C = 112.48mg/dl in khat chewers diabetic subject, while TC =178.4mg/dl, HDL-C = 35.96 mg/dl, TG =160.1 mg/dl and LDL-C = 109.02 mg/dl in non khat chewer diabetic subject. The difference for TC and HDL-C are statistically significance. The mean serum levels of TC, LDL-C, HDL and TG are (154.23 mg/dl), (86.33 mg/dl), (41.1 mg/dl) and (95.44 mg/dl) respectively in non-diabetic khat chewer while the TC, LDL-C, HDL and TG (149.3 mg/dl), (85.45 mg/dl), (43.28 mg/dl) and (90.5 mg/dl) in non-diabetic non khat chewer respectively. And the difference is statistically not significance:

Conclusion: Dyslipidemia is more prevalent in diabetics particularly in those with khat chewing habit. Thus, Khat has unfavorable side effects on the level of lipid profile.

Recommendation: Health education about the adverse effect of khat chewing on DM should be delivered to the community and healthy institution using available forum

1. Introduction

1.1. Back ground

Diabetes mellitus is a complex metabolic disorder characterized by impaired metabolism of carbohydrates, lipids, and proteins (1). It may lead to the development of pathological complications in many tissues if not properly controlled (2). Diabetes has multiple etiologies and is classified into 2 major types: insulin-dependent and non-insulin-dependent diabetes mellitus (IDDM and NIDDM, respectively) (3). Type 1 diabetes is caused by the immune system destroying the cells in the pancreas that make insulin. This causes diabetes by leaving the body without enough insulin to function normally (4). Type 2 diabetes causes are usually multifactorial There are a variety of risk factors for type 2 diabetes, any or all of which increase the chances of developing the condition. These include: Obesity, Living a sedentary lifestyle, Increasing age, Bad diet. Although both types exhibit hyperglycemia as their hallmark, IDDM, accounting for 5%–10% of diabetes diagnoses, NIDDM encompasses 90% of patients with diabetes (4).

Khat belongs to the family Celastraceae and is known with many other names such as qat and miraa (5). In terms of distribution, khat is cultivated widely in several countries in Africa and Yemen. The habit of chewing qat is significantly increasing among different categories of people (6). Leaves of khat are rich in several chemical compounds mainly alkaloids, glycosides, flavonoids, sterols, terpenoids, tannins, and amino acids. Among the chemical compounds found in khat, cathinone and cathine are the most important alkaloids due to their stimulating effects (7). The effects of cathinone and amphetamine in the body are relatively similar (8). The mechanism of khat action includes the activation and release of dopamine neurotransmitter from its storage (9).

Dyslipidemia is a family of lipoprotein metabolism disorders manifested by elevated total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), and reduced high density lipoprotein cholesterol (HDL-C) concentrations in the blood (10). From all risk factors for cardiovascular diseases (CVD), dyslipidaemia is one of the most crucial factors that evidently lead to CVD worldwide (11). There is a high percentage of mortality due to CVD. In the recent report of the World Health Organization, death due to CVD is remarkably high with an estimation of 17.7 million deaths annually (12). On the contrary, the presence of

dyslipidaemia during advanced age results in high risk of CVD with increasing age (13). It is well known that atherosclerotic process begins early in childhood and dyslipidemia plays a vital role in the progression of the disease (14). Increased lipid levels result in vessel wall reactions, including endothelial dysfunction, smooth muscle cells proliferation, lipid accumulation, foam cell formation, and, finally, necrosis and plaque development(15). Like other well-known risk factors such as diabetes, dyslipidemia is also associated with the development of atherosclerotic disease (16). Studies revealed that early onset of dyslipidemia is associated with the development of early atherosclerotic coronary and peripheral artery disease and increased incidence of cardiovascular disease in adulthood(17). Recently, dyslipidemia is increasingly prevalent in all age groups, and the incidence tends to be younger. In the last decade, Diseases caused by high total cholesterol (TC) increased global morbidity and mortality by 26.9% and 28.0%, respectively (17). There are a reasonable number of studies regarding the physiological and biochemical effects of qat in humans. However, the influence of chewing khat on the lipid profile and dyslipidaemia is insufficiently investigated. This study will aimed to assess lipid profile among diabetic and apparently healthy subjects with and without khat chewing habit.

1.3. Statement of the problem

There are many health hazards associated with the consumption of khat. It literally affects every human organ systems and induces adverse effects (18). It has various physiologic and metabolic effects associated with a decrease in appetite and body weight, possibly mediated via increasing the release of leptin from the stomach (19). The study showed that chewing khat significantly decreased subjective feelings of hunger and increased the sensation of fullness (20). its impact on elevated plasma leptin, esterified fatty acid production and resulting lipodystrophy, which leads to further problems on the cardiovascular system, urinary system, gastrointestinal tract, spermatogenesis, and impotence(21).

In the central nervous system it produces euphoria and mild excitement, which are later gradually replaced by mild dysphoria, anxiety, insomnia, and anorexia. The CNS effects of khat have been believed due to the chemical content cathinone, which is having closely similar structure of amphetamine, a known psycho stimulant (22). Hyperactivity and logorrhea also have been reported with khat consumption. In some case reports, the khat consumption has been found to induce schizophrenia form psychosis and paranoid psychosis secondary to other psychiatric

disorders (23). In the cardiovascular system, khat has been shown to induce acute myocardial infarction and coronary heart failure and ischemic conditions (24). One of the studies suggests the elevation of systolic blood pressure in khat consumers due to stimulant effect in β1 adrenoceptor in heart (24).

Various studies have reported that Khat becomes a serious public health issue in east Africa and Saudi Arabia due to its widespread use (25). Many unfavorable side effects have been associated with khat chewing. The WHO (2003, 2006) recorded that Khat consumption has created a major health problems by affecting numerous vital organs of the human body (26).

A high level of lipid is the most important modifiable risk factor for cardiovascular diseases. Its reduction decreases the risk of cardiovascular diseases in the population (27). According to the World Health Organization (WHO), about 17 million people die of CVDs annually. This rate is almost 2 times higher than the mortality rate for cancer, which is the second cause of death in the world (28). Data on the European population in 2012 showed that CVDs caused 38% and 35% of deaths among women and men < 75 years of age, respectively (29)

The study conducted in Poland in 2014 shows that cardiovascular diseases accounted for 45% of all deaths (40% among men, 50% among women)(30) thus being the first cause of death in the Polish population, preceding cancer. The average was 476 deaths a day. Similarly polish study in 2011 showed that the incidence of elevated low-density lipoprotein cholesterol (LDL-C) was approximately 58% for both sex. Improper levels of total cholesterol (TC) and LDL-C have been most frequently found in persons aged 40–59(31)

The polish study conducted in 2016 shows cardiovascular diseases are the first cause of death among men (26%), and the second cause of death among women (21% preceded by cancer – 41%(32). In addition to being the main cause of death, CVDs are also the main cause of morbidity, hospitalizations, invalidity and sickness absenteeism.

The prevalence of hypercholesterolemia in Jordan increased from 23.0% in 1994 to 44.3% in 2017, and the prevalence of hypertriglyceridaemia increased from 23.8% in 1994 to 41.9% in 2017 (33)

A number of studies show that dyslipidemia is an important modifiable risk factor for CVD. Therefore, early screening and effective control of lipid levels can reduce the morbidity and

mortality of CVD (34). The prevalence of dyslipidemia in the general population of China aged 18 and older has increased from 18.6% in 2002 to 40.4% in 2012 (35). Without timely and effective control, the rate of dyslipidemia will continue to rise, leading to a heavy burden of CVD. Therefore, it is important to identify the potential influencing factors of dyslipidemia, to manage this condition and reduce the burden of non-communicable disease (35)

Very few published papers have tried to draw a conclusion about khat chewing and it effect on DM. there is general belief that khat has no effect on body fat metabolism. But, there is lack of no baseline data in our setting argue these beliefs. This study aimed to assess lipid profile among diabetic and non-diabetic subjects with and without khat chewing habit

1.2. Significance of the study

This study will aimed to give the supportive information on whether khat can increase or decrease lipid level in diabetics and apparently healthy group. To increase the awareness of the community about the effect of khat by disseminating the result and giving healthy education in Adama medical college

2. Literature review

2.1. Khat chewing habit in diabetics and non-diabetic subject

Traditionally most people have the habit of chewing khat in the cheek for hours. In this way, it permits the mucosal absorption of 2 of the active alkaloids of khat, namely, cathinone (in fresh) and cathine (non-fresh), both of which are considered to be amphetamine-like substances. Traditionally, there are strong beliefs among khat chewers that khat has health benefits for treatment of diabetes mellitus and that it has anti-obesity effects due to the suppression of appetite. (36).

Khat and its active alkaloid have a plausible direct and indirect influence on monoamine release and uptake, a mechanism that has been known to affect food intake (37). A number of drugs have been investigated for treating obesity, either for the short term or the long term. These drugs act by modulating the release and uptake of monoamines mainly in the brain (38).

Study done on A total of 1540 Yemeni patients with type 2 DM for their knowledge; attitude and practice (KAP) study regarding khat chewing. They were asked if they thought khat chewing was beneficial for diabetes. Of the 1540 patients, (56.2%) thought that it was beneficial; only (12.3%) thought that it was harmful, and (31.5%) said that they did not know. Among the KC, 848/997 (85.05%) of them believed that khat chewing was beneficial for diabetes, only (3.2%) thought that it was harmful, and (11.73%) said that they did not know. Among the NKC, only 20/543 (3.68%) believed that it was good for diabetes, 157/543 (28.9%) believed that it was harmful, and 366/543 (67.4%) said that they did not know.(39)

cross sectional study conducted on total of 60 males, age 22- 45 years participant in department of Pharmacology, Faculty of Medicine, University of Malaya in 2009 shows that different khat extracts or cathinone produced changes in weight, fat mass, appetite, lipid biochemistry and hormones. These changes were more pronounced at high doses and long durations of exposed (40).The EDHS reports shows that the national prevalence of current khat chewing is 15.3 %(41)

According to study conducted at Department of Biochemistry, Faculty of Science, University of Tabuk, Yemen, from April 1 to September30, 2018 on 14000 healthy male university student Hypercholesterolemia, hypertriglyceridaemia, high LDLC level, and mixed hyperlipedemia were

lower in khat chewers than in non-khat chewers (16.6%, 58.3%, 16.3%, and 10.6% respectively, versus 20.8%, 64.9%, 18.2%, and 20.8%, respectively). However, the incidence of low HDL-C, were generally higher in khat chewers than that in non-khat chewers (83%, versus 75.3%, respectively).(42)

According to crossectional study done in 2009 in Department of Biochemistry, Faculty of Medicine and Health Sciences, Sana'a University on healthy student Show that the mean HDL-Cholesterol levels of khat chewers were significantly lowered (24.72±4.48 mg/dl) than the corresponding mean of non-khat chewers(HDL-c, 33.34±1.83 mg/dl) (control group)(42). In contrast the mean of triglyceride, LDL cholesterol and total Cholesterol levels of khat chewers were not significantly changes in khat chewers when compared with non-khat chewers (84.54±10.3 mg/dl, LDL-c, 73.6±28.3 mg/dl, Total cholesterol 110.3+ 29.4 mg/dl) and the corresponding mean of non-khat chewers (70.67+5.72 mg/dl, LDL-c, 76.4±29.1 mg/dl, Total cholesterol, 119.2 +31.7mg/dl) (control group) respectively (43).

The Experiment done on lipid profiles in rats shows that: Triglyceride levels (TG) were reduced significantly during khat feeding of both control and experimental group ($p<0.05$). After cessation of khat feeding, the TG level increased non-significantly to a level higher than that of the corresponding control. The total serum cholesterol and HDL-cholesterol were non-significantly increased. Similarly, the measured serum LDL- and VLDL-cholesterol levels were only slightly increased during and after the experimentation. According to a study by Toennes et al. and Widler et al., significant increases in systolic and diastolic blood pressures persist for between 3 and 4 hours after the onset of khat chewing [44]

In a study conducted in Kuwaiti on adults, 33.7% of men and 30.6% of women were reported to have hypercholesterolemia. Similarly another study conducted in Oman reported hypercholesterolemia prevalence of 33.6% [45]. On the other hand, a higher prevalence of hypercholesterolemia was reported from Saudi Arabia (54%) (46) While an Iranian meta-analysis reported a hypercholesterolemia prevalence of 41.6 %(47)

According to study conducted in Jordan on diabetic subject the Prevalence of hypercholesterolemia in females was 44.8% and 43.0% in males. Hypertriglyceridaemia was 36.5% in females significantly lower than in males (54.6%). Study participants showed no

statistical difference in prevalence of low HDL-C and high LDL-C between male and female participates. Prevalence of hypercholesterolemia and hypertriglyceridaemia was significantly higher among obese and overweight participants than normal BMI participants where as the prevalence of hypercholesterolemia and TG was 50.6% and 52.7%, respectively, in obese participants. Also, the highest prevalence of low HDL-C and high LDL-C was noticed among obese participants (48)

The study conducted on sample of 65,128 participants aged ≥35 years in Inner Mongolia during 2015–2017 shows that The prevalence of dyslipidemia was 31.8%, and the prevalence of elevated TC, LDL-C, TG, and low HDL-C was 5.2, 2.9, 16.5, and 15.0%, respectively. The age-standardized prevalence of dyslipidemia was 31.2%; the age-standardized prevalence of elevated TC, LDL-C, TG, and decreased HDL-C was 4.3, 2.4, 14.7, and 17.4%, respectively. The prevalence of dyslipidemia was significantly higher in men than in women ($P < 0.001$), but the prevalence of elevated TC and LDL-C is higher in women than in men.(49)

2.2. Factor affecting lipid profile

2.2.1 Physical Activity

Observational and experimental studies have shown that regular practice of physical activity (PA) induces desirable changes in plasma lipid levels, especially HDL increase and TG decrease, in addition to triggering beneficial effects on total cholesterol and its low-density and very-low-density fractions (LDL and VLDL, respectively). The effect of PA on HDL and TG levels seems to depend on neither weight nor diet changes (50). Despite the well-known benefits resulting from PA practice, there are controversies about which PA characteristic would be more important to improve lipid profile: exercise intensity, frequency, duration or a combination of frequency and intensity.

A crossectional study conducted on 15,105 active and retired individuals, aged 35 to 74 years, from teaching and research institutions in six Brazilian capitals in 2010 shows that both moderate and vigorous PA and PA practice ≥ 150 min/week were significantly associated with higher HDL and lower TG levels. Vigorous PA was associated with lower LDL only on univariate analysis. After adjustments, moderate and vigorous PA increased mean HDL level by 0.89 mg/dL and 1.71 mg/dL, respectively, and reduced TG geometric mean by 0.98 mg/dL and 0.93

mg/dL, respectively. PA practice ≥ 150 min/week increased mean HDL level by 1.05 mg/dL, and decreased TG geometric mean by 0.98 mg/dL (50).

2.3.2 Effect of Sedentary Work

Sedentary work refers to that type of work that involves sitting or spending most of the working hours in an office. It is believed to be a factor in obesity and other disorders. Individuals who expend less than 2,000 calories per week through exercise have a higher risk of heart disease than active person. Having a sedentary lifestyle leads to being overweight, and this can lead to diabetes or elevated blood pressure, both of which are risk factors for coronary heart disease

The fasting serum TC, TG, HDL-C, LDL-C and VLDL levels of 80 apparently healthy Nigerian male and female workers, living sedentary lifestyles, aged between 20 and 60 years old were analyzed. The mean ± SD for all the groups showed a statistically significant increase ($p<0.05$) in TC, TG, LDL-C and VLDL when compared with the control subjects, while the HDL-C showed a significant decrease ($p<0.05$) when compared with the control. Test of difference in mean ± SD (gender difference) showed a statistically significant increase ($p<0.05$) in TG and LDL-C while a non-significant increase ($p>0.05$) was observed in TC, HDL-C and VLDL of females in comparison to their male counterparts. A statistically significant increase ($p<0.05$) was observed in the lipid profile of sedentary workers not undergoing exercise (51)

2.3.3 Frequency and duration of alcohol intake

Alcohol consumption has been found to be associated with increased serum levels of TG and high density lipoproteins (HDL). The increase in HDL cholesterol has been estimated to account for half of the beneficial effects of alcohol consumption on cardiovascular events. Alcohol has narrow therapeutic range and only the moderate drinking has beneficial effects on cardiovascular health. Prolonged excessive drinking causes various structural and functional abnormalities of heart (52).

Some Study has demonstrated that definitive lipid profile changes in patients of alcohol dependence, with some correlation to the liver dysfunction. Alcohol causes alteration in various parameters of lipid metabolism including those which predispose to CHD. Low to moderate alcohol use over prolonged periods has been linked to have protective influence for development

of coronary heart disease (CHD), through increase in high density lipoprotein cholesterol (HDL-C) levels (53)

2.3.4 Waist circumference

The National Institutes of Health has determined that abdominal fat is an independent predictor for morbidity, and that waist circumference (WC) is a clinically-acceptable measure of abdominal fat. Studies have been conducted that examined either the effect of WC on the risk factors for diabetes, the incidence of diabetes, risk factors of CVD, or the incidence of CVD, and usually focused on obese individuals or diabetic individuals, or both(54)

2.3.5 Body mass index (BMI)

is frequently used to categorize individuals as underweight, normal, overweight and obese. It has been extensively described that BMI is a strong predictor of heart diseases and T2DM (55). Several data have reported that obesity is related to Higher BMI associated at all ages with a higher plasma triglyceride level, lower HDL cholesterol level, and higher total and non HDL cholesterol levels, which contributes to the development of the metabolic syndrome

2.3.6 Pregnancy

Not only demands more metabolic fuels but also causes an alteration in hormonal levels, which may cause few changes in lipid profile during pregnancy

Previous study showed that total cholesterol, triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein-cholesterol (VLDL-C) increases in the last two trimesters. The increase is even greater in the third trimester when compared to the second. However, high-density lipoprotein cholesterol (HDL-C) levels are decreased in the third trimester when compared to that of the second (56)

2.3.7 Sleep habits:

It has been increasingly recognized that sleep habits, along with other lifestyle habits, such as eating, exercising, smoking, and drinking, are potential risk factors for diabetes mellitus, obesity, hypertension, and cardiovascular disease (CVD)(57)

According to study conducted at National Health and Nutrition Survey in November 2003 by the Japanese Ministry of Health, Labour and Welfare on residents in the districts selected randomly from all over Japan, the percentages of subjects among men who slept <6 h and ≥8 h per night were 23.3% and 13.6%, respectively. Among women, the corresponding percentages were 31.2% and 8.2%, respectively. The number of subjects with shorter sleep duration was greater for women than for men, and the number of subjects with longer sleep duration was smaller for women than for men ($P < 0.001$). The mean (standard deviation [SD]) serum triglyceride level was 153.9 (112.2) mg/dL for men and 123.2 (86.5) mg/dL for women, and was thus significantly higher among men ($P < 0.001$). The mean (SD) serum HDL cholesterol level was 56.3 (15.0) mg/dL for men and 64.8 (15.6) mg/dL for women, and was thus significantly lower among men ($P < 0.001$). The mean (SD) serum LDL cholesterol level was 113.2 (31.5) mg/dL for men and 118.6 (32.0) mg/dL for women, and was thus significantly higher among women ($P < 0.001$). The prevalence of a high triglyceride level was 36.5% among men and 24.0% among women, and was thus significantly higher among men ($P < 0.001$). The prevalence of a low HDL cholesterol level was 12.1% among men and 3.4% among women, and was thus significantly higher among men ($P < 0.001$). The prevalence of a high LDL cholesterol level was 17.6% among men and 23.9% among women, and was thus significantly higher among women ($P < 0.001$) (58).

The mechanism by which khat reduces plasma lipids has not been fully investigated. Very few published papers have tried to draw a conclusion about khat chewing and it effect on DM. there is general belief that khat has no effect on body fat metabolism. But, there is lack of no baseline data in our setting argue these beliefs. This study aimed to assess lipid profile among diabetic and non-diabetic subjects with and without khat chewing habit

3. Objectives

3.1. General objectives

- To assess lipid profile among diabetic and apparently healthy subject with and without khat chewing habit in Adama, Ethiopia, from January to April 2020 GC

3.2 Specific objectives

- To assess lipid profile among diabetic subject with and without khat chewing habit in Adama, Ethiopia from January to April 2020 GC
- To assess lipid profile among apparently healthy subject with and without khat chewing habit in Adama, Ethiopia from January to April 2020 GC
- To evaluate the associated risk factor among khat chewer diabetic and apparently healthy participant in Adama, Ethiopia, from January to April 2020 GC

4. Hypothesis

Alternative hypothesis (H1): khat chewing has effects on lipid profile level

Null hypothesis (Ho): khat chewing has no effect on the level of lipid profile

5. Methods and Materials

5.1 Study area and period

The studies were conducted from January to April 2020 in Adama medical college. Adama town is 98km from Addis Ababa to the East on the main road to harar. the City Forms a Special Zone of Oromiya and surrounded by east Shewa Zone. It is located at 8.54°N 39.27°E at an altitude of 1712 meters (59). The city sits between the base of an incline to the west, and the Great Rift Valley to the east. Its annual average temperature and rainfall is 20.5oC and 809mm respectively. Adama city have population size of 228,623 based on figures from the Central Statistical Agency in 2005 and the population is amulti-ethnic group and mixed cultures (60). In Adama town, nine government, and eighteen private health facilities are available. From government health Facility, one Teaching referral Hospital and eight are Health center. There is also one regional Laboratory where different referred sample are tested with in the region. The Adama hospital has a total of 184 beds and its admission rate was 173 patients per weeks and its average outpatient flow was 856, its annual outpatient flow is 226000 (60).

5.2 Study design

Hospital based comparative cross sectional study was conducted in Adama hospital.

5.3 population

5.3.1. Source population

All diabetic patients who have follow-up in Adama hospital and apparently healthy individual both hospital worker and the guardian were taken as a source population.

5.3.2. Study population

Diabetic patients who visited the diabetic clinic for follow-up and are volunteered to give sample and apparently healthy subject who are aged greater than 18 years and volunteers to give sample were used as study population.

5.4. Eligibility criteria’s

5.4.1. Inclusion criteria’s

DM subject who are follow-up treatment for at least six months and able to provide consent and communicate with data collectors in Adama hospital during the study period and apparently healthy subject(hospital worker and guardians) who are on fasting state were included

5.4.2. Exclusion criteria

Those diabetic patients who did not give consent as well as who were critically ill and unable to communicate and apparently healthy subject who are used breakfast was excluded from the study

5.5 Variables

5.5.1 Dependent variable

- Level of lipid profile

5.5.2 Independent variable

- ✓ Age
- ✓ Sex
- ✓ BMI
- ✓ Occupation
- ✓ Waist circumference
- ✓ Alcohol intake
- ✓ Physical exercise
- ✓ Smoking cigarette
- ✓ Fruit and vegetable intake
- ✓ Chewing khat
- ✓ Sleeping habit
- Contraceptive pills
- Pregnancy
- DM
- Khat

5.6. Measurement and Data collection

5.6.1 Sample size determination

The sample size is calculated using single population proportion formulae considering the report by the EDHS which is found a national prevalence of current khat chewing of 15.3% (41)

$$n = \frac{(z_{\alpha/2})^2 \times pq}{d^2}$$

N=1.96^2(0.153*0.85)/0.05^2=199.8by adding 10% non-respondent rate 19.96=19.96+199.8=**220**

So 118 from diabetic group and 102 from non-diabetic individual is in included under study participant

5.6.2 Sampling method

Convenience sampling technique were used, during data collection in hospital

5.6.3 Data collection procedure

Blood were collected, serum separated by centrifugation at 3000rpm for 5 minutes. All blood lipid analyses were done on the days of blood collection using analyser. Total cholesterol (TC), triglyceride (TG), High density lipoprotein cholesterol (HDL-C) and Low density lipoprotein cholesterol (LDL-C) were assayed using automated analyzer (cobas 311). Waist circumferences and BMI were measured at the time of data collection. Other information was collected using a pre-tested structured questionnaire on socio demographic characters and on other associated factors. The data were collected by two medical laboratory technologists who were trained about the objectives of the study and the data collection procedures.

5.6.4 Laboratory principle and procedure for determining lipid profile

LDL Cholesterol

Test principle

Homogeneous enzymatic colorimetric assay

Cholesterol esters and free cholesterol in LDL are measured on the basis of a cholesterol enzymatic method using cholesterol esterase and cholesteroloxidase in the presence of surfactants which selectively solubilize only LDL. The enzyme reactions to the lipoproteins other than LDL are inhibited by surfactants and a sugar compound. Cholesterol in HDL, VLDL and chylomicron is not determined**.** In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-aminoantipyrine and EMSE to form a red purple dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically. (61)

TG (triglyceride)

Triglycerides are hydrolyzed by lipoprotein lipase (LPL) to glycerol and fatty acids. Glycerol is the phosphorylated to glycerol-3-phosphate by ATP in a reaction catalyzed by glycerol kinase (GK). The oxidation of glycerol-3-phosphate is catalyzed by glycerol phosphate oxidase (GPO) to form dihydroxiacetone phosphate and hydrogen peroxide H2O2). In the presence of

peroxidase (POD), hydrogen peroxide affects the oxidative coupling of 4-chlorophenol and 4-aminophenazone to form a red-colored dye. The increase in absorbance is directly proportional to the concentration of triglycerides in the sample (61)

Total cholesterol (TC)

Test principle:

Enzymatic colorimetric method

Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol oxidase then catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminoantipyrine to form a red quinone-imine dye. The color intensity of the dye formed is directly proportional to the cholesterol concentration. It is determined by measuring the increase in absorbance.(61)

HDL Cholesterol

Homogeneous enzymatic colorimetric test

Non-HDL lipoproteins such as LDL, VLDL and chylomicron are combined with polyanions and a detergent forming a water-soluble complex. In this complex the enzymatic reaction of Cholesterol esterase and cholesterol oxidase towards Non-HDL lipoproteins is blocked. Finally only HDL-particles can react with Cholesterol esterase and cholesterol oxidase. The concentration of HDL-cholesterol is determined enzymatically by Cholesterol esterase and cholesterol oxidase. Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by Cholesterol esterase. In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-amino-antipyrine and EMSE) to form a dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically (61)

5.7. Data quality assurance

The data collection instruments were pre-tested on study population to ascertain the contents and clarity of the questionnaire. The questioners were designed based on one-to one interview. Initially the data were assessed to understand its quality challenges. Data were checked on daily basis for its completeness

5.8. Data interpretation and Analysis

The data was analyzed statistically using the software package of the Statistical Package for the Social Sciences version 20.0. The data were entered, coded, and analyzed accordingly. The data were analyzed descriptively for mean, standard deviation, frequencies, and percentage values for qualitative (e.g. demographic variables) and quantitative variables (TC, TG, HDL, and levels LDL). Independent t test were used to compare the means of lipid profile among two groups and Logistic regressions were used to associate independent variable with lipid profile level. .P-value <0.05 at 95% confidence interval (CI) were considered as statistically significant

5.9 Ethical consideration

Ethical clearance was obtained from AAU College of healthy science department of medical laboratory science and Adama hospital head office, research coordinator and laboratory head. All the study participants were informed about the purpose of the study, their right to refuse and assured confidentiality and informed verbal consent were obtained prior to the interview. The instruments and procedures were not causing any harm to the study subjects, the community, the data collectors and supervisors, which were involved in the study. The benefit that the study participant gain from this study are, information regarding side effect of khat during face to face interviewee and their level of lipid profile where checked. I was communicated with diabetic center staff and told them the abnormal result to give care as soon as possible

5.10. Dissemination of Result

The study result will be presented to Department of Medical Laboratory Sciences, School of Allied Health Science, College of Health Science, and AAU as MSc thesis. In addition, the result will also submitted to Oromiya regional health office, Adama Hospital medical college, and presented to any concerned body that is interested to know the magnitude of health effect of khat on diabetic patient. Based on the finding, the necessary action will take with the concerned body

5.11. Operational Definitions

Apparently healthy: an individual who have no any history of diabetes and other problems like liver, renal and CVD which effect lipid profile level

Khat chewer: was defined as an individual who chews khat for 4 h or more daily or even occasionally for the past 1 year

Non-khat chewer: was defined as an individual who does not chew khat daily or occasionally for the past 1 year

DM: patient who have diabetic follow up for at least six month

6. Result

6.1. Sociodemographic, anthropometric and biochemical characteristics of the participants

A total of 220 participants (diabetic patient and non-diabetic individual) composed of 41 Khat chewers and 77 non- chewers from DM and 50 khat and 52 non khat chewer from non-diabetic group are participated in this study. From the 216 study subjects involved in the study, 44% of them (n=95) were men, and the rest were women. The mean age was 44±10.1 for diabetic participant and 42±7.2 for non-diabetic participant. But the mean age of both study groups is 43±8 and majority of study participant are lies between ages 35-54. 78.6% (n=173) of them were in urban. In this study the majority of chewer participants 54.1(%) chew khat everyday, 27(%) chew khat once a week, 8.2% chew khat 2-3 days per week and the remaining percent chew khat occasionally. The majority 85.8(%), of participants responded that they have been chewing khat for more than 2years. Concerning occupational status of participants, 82.9(%) were government employed, 8.8% were merchant, 5.1% were private employer, 0.9% were farmer and the remaining were on other occupation.

Table1: socio demographic factor of study participant at Adama medical college in 2020

		DMKC	DMNKC	Healthy KC	Healthy NKC
Sex	Male	23	29	26	19
	Female	18	48	24	33
Occupation	Gov't employe	24	55	50	52
	Private	8	4	0	0
	Merchant	18	12	0	0
	Farmer	0	2	0	0
	Others	1	4	0	0
Age group	25-34	7	10	10	7
	35-44	14	37	22	23
	45-54	11	23	16	17

	55-64	7	3	4	3
	>64	2	4	0	0

Table2: the mean blood pressure of study participant at Adama medical college in 2020

Value	Systolic				Diastolic			
	DMKC	DMNKC	APHKC	APHNKC	DMKC	DMNKC	APHKC	APHNKC
Mean	110.3	110.9	107.38	107.82	76.83	77.78	77.87	77.76
SD	8.74	9.44	6.7	6.5	8.65	5.05	3.82	3.87

Note, DMKC=diabetic khat chewer, DMNKC= diabetic non khat chewer, APHKC= apparently healthy khat chewer, APHNKC= apparently healthy non khat chewer

The systolic pressure and diastolic pressure of both diabetic khat chewer and diabetic non- khat chewer are (systolic, 110.3vs110.99) and (diastolic, 76.83vs77.78). The difference between khat chewer and non khat chewer is statistically not significance. Similarly The systolic pressure and diastolic pressure of both apparently healthy khat chewer and apparently healthy non- khat chewer are (systolic, 107.38vs107.82) and (diastolic, 77.87vs77.76). The difference between khat chewer and non khat chewer is statistically not significance

6.2. Prevalence of dyslipidemia and mean concentration of lipid profile among study subject

Table 3: the mean of lipid profile among study participant for both DM and apparently healthy at Adama medical college in 2020

Variable	Diabetic subject		P val	Apparently healthy subject		Pvalue
	KC mean + SD	NKC mean + SD		KC mean + SD	NKC mean + SD	
TC(mg/dl)	227.33±130	178.41±97	0.021	154.23±37	149.3±37.5	0.55
TG(mg/dl)	164.2±37	160.17±36	0.96	95.44±37	90.5±40	0.56
HDL-C(mg/dl)	28.4±12.8	35.96±15.2	0.006	41.69±9.9	43.28±8.2	0.225
LDL-C(mg/dl)	112.48±27.3	109.02±26	0.54	86.33±26.3	85.45±25.4	0.94

The mean serum levels of TC (227.33 mg/dl) is significantly increased in khat chewers than non khat chewer TC (178.41 mg/dl) and mean serum levels of HDL-C (28.4 mg/dl), is significantly decreased in khat chewer than non-khat chewer HDL-C (35.96 mg/dl) (P<0.05), respectively in DM group and the difference is statistically significance. In contrast, the mean serum level of TG and LDL-C was relatively similar 164.2 mg/dl, 160.1 mg/dl and 112.48 mg/dl, 109.02 mg/dl in khat chewers and non-khat chewers respectively and there was no significant difference.

The mean serum levels of TC, LDL-C, HDL and TG are (154.23 mg/dl), (86.33 mg/dl), (41.69 mg/dl) and (95.44 mg/dl) respectively in apparently healthy khat chewer while the TC, LDL-C, HDL and TG (149.3 mg/dl), (85.45 mg/dl), (43.28 mg/dl) and (90.5 mg/dl) in apparently healthy non khat chewer respectively. The difference is not significant

Table 4: comparison of lipid profile among DM and apparently healthy at Adama medical college in 2020

Groups	LDL	HDL	CHOL	TG
DM subject	109.95±26.6	33.34±14.169	202.5±112.7	162.1±36.6
Apparently healthy	85.9±25.6	42.5±9.15	151.8±37.1	152.1±37.1
p-value	<.05 (0.000)	<.05(0.000)	<.05(0.000)	>0.05(0.056)

The mean of lipid profile among diabetic patient is higher than that of apparently healthy participant and the difference is statistically significance (Pvalue for LDL, HDL, CHOL is < 0.05), while TG for DM is slightly higher than that of apparently healthy subject and the difference is statistically not significance

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Table 5: prevalence of dyslipidemia for both DM and apparently healthy at Adama medical college in 2020

Variable	Diabetic subject		Pvalue	Apparently healthy		Pvalue
	Khat chewer	Non khat chewer		Khat chewer	Non khat chewer	
Hypercholesterolemia	20(50%)	22(29%)	0.025	4(8%)	6(12%)	0.5
Hypertriglyceridaemia	27(65.3%)	48(63%)	0.84	7(14%)	7(13.6%)	0.76
Low HDL-C	30(72.5%)	30(39.5%)	0.001	14(28%)	6(12%)	0.046
High LDL-C	7(20%)	17(24.6%)	0.538	2(4%)	4(8%)	0.64

The prevalence of dyslipidaemia in khat chewers and non-khat chewers among DM study subject and non diabetic subject.. Hypertriglyceridaemia were slightly higher in khat chewers than in non-khat chewers (65.3%, versus 63%,) and the difference is statically not significance The prevalence of Hypercholesterolaemia and LHDL is higher in khat chewer than non khat chewer and the prevalence is statically significance ($p<0.05$). But the overall occurrence of dyslipidemia was higher in khat chewers than that in non-khat chewers by approximately 12.8% (51.875% versus 39%) among diabetic subjects.

Hypercholesterolaemia, hypertriglyceridaemia, high LDLC level, were lower in khat chewers than in non-khat chewers (8%, 14%, 4%, respectively, versus 12%, 13.6%, and 8%, respectively) among healthy group. The difference of prevalence is statically not significance. But the prevalence of LHDL-C is higher in khat chewer (28%) than non khat chewer (12%), it is statically significance ($p<0.05$). but the overall occurrence of dyslipidemia was higher in khat chewers than that in non-khat chewers by approximately 1.6% (10.4% versus 8.8%) and the difference is statically not significance

Table 6: correlation of lipid profile with different independent variable of study participant at Adama medical college in 2020

Variable	LDL	HDL	TC	TG
	P value	P value	P value	P value
Chewing habit	0.987	0.006	0.04	0.682
Frequency of chewing khat	0.26	0.76	0.009	0.105
Duration of chewing	0.354	0.39	0.1	0.314
Frequency of drinking alcohol	0.479	0.92	0.018	0.226

Duration of drinking alcohol	0.86	0.867	0.087	0.619
Using contraceptive method	0.98	0.679	0.698	0.272
Pregnancy	0.86	0.195	0.823	0.819
Sleep duration	0.704	0.965	0.675	0.107
Frequency of eating fat food	0.000	0.033	0.817	0.404
Freq. of eating fruit $ vegetbl	0.287	0.345	0.617	0.316
Frequency of smoking cigar	0.155	0.701	0.222	0.271
Physical exercise	0.009	0.527	0.1	0.016

From these we observed that chewing khat, frequency of khat chewing and frequency of drinking alcohol have significance association with total cholesterol level ($p<0.05$). Similarly khat chewing habit and frequency of eating fat containing food have significance association with HDL-C ($P<0.05$). Again as we see from the blow table frequency of eating fat containing food and physical exercise have significant association with LDL-C ($P<0.05$). Only physical exercise has significant association with TG value.

TG value and HDL-C have negative correlation with their associated variable, while TC and LDL-C have positive correlation with their associated variable except LDL-C have negative correlation with physical exercise

Graphical representation

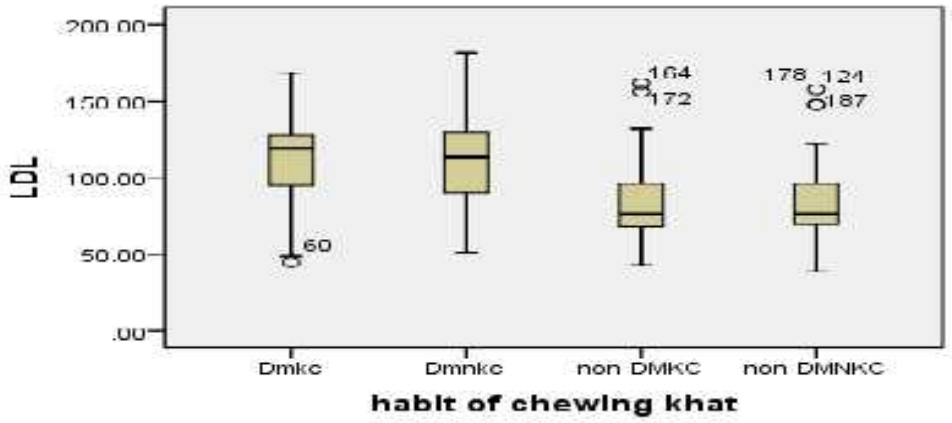

Figure: 1 LDL value between khat chewer and non-khat chewer for both non-diabetic and diabetics group

From this we observed that the mean of non-khat chewer and khat chewer for both group is relatively similar (p value = 0.54 for DMKC VS DMNKC and P = 0.94 non-DM KC VS non-DM NKC). But the mean among the group (DM and non-DM) is difference and the difference is statically significance (p=0.000).

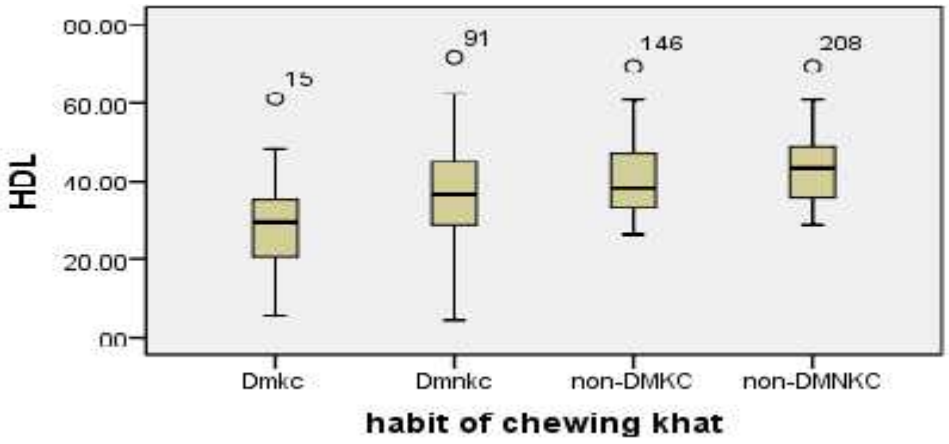

Figure: 2 HDL value between khat chewer and non-khat chewer for both non-diabetic and diabetics group

As we observed from the above figure the mean of khat chewer is more than non-khat chewer for diabetics group and the difference is statically significance (p=0.001). Similarly there is little difference between khat chewer and non khat chewer in non-diabetic group and the difference is statically not significance (P = 0.225). But the mean among the group (DM and non-DM) is difference and the difference is statically significance (p=0.000).

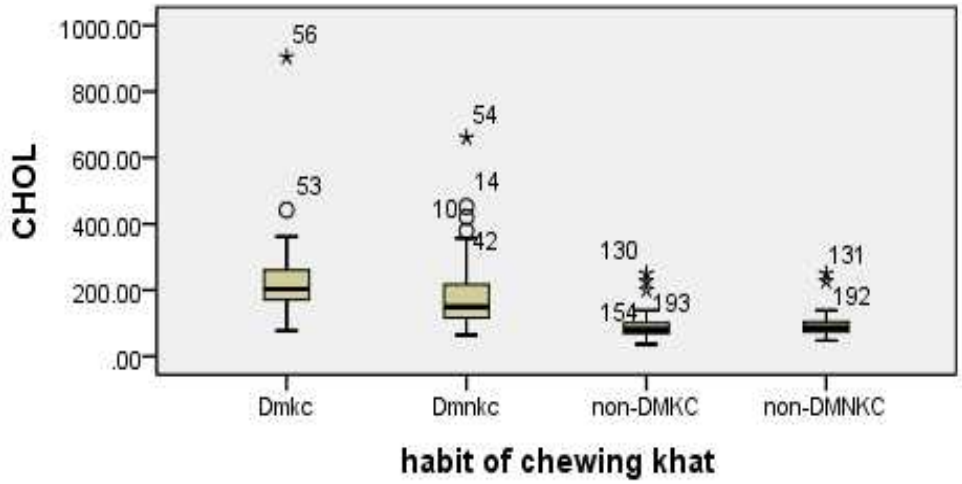

Figure 3: the level of cholesterol between khat chewer and non-khat chewer for both diabetics and healthy participant

Here the above graph shows the mean of cholesterol among khat chewer and non khat chewer are relatively similar for non-DM group (P=0.55) and difference for diabetic group and the difference is statically significance (0.004). Again the mean among non-DM and DM is difference and the difference is statically significance (p=0.000)

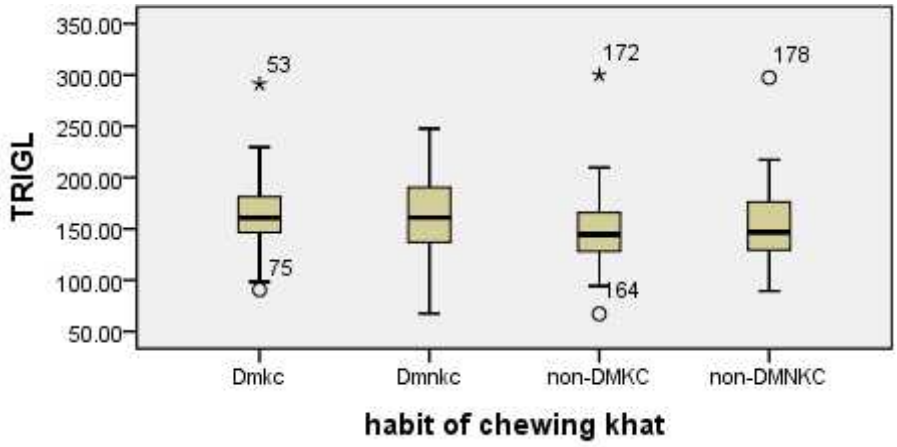

Figure: 4 the level of triglyceride between khat chewer and non-khat chewer for both diabetics and non-diabetic participant

In similar fashion to the above result there is mean difference between khat chewer and non khat chewer in both group and the difference is statically not significance. Again the mean difference among the group (DM VS non-DM is statically not significance

7. Discussion

Khat chewing has been a common habit for major people in Ethiopian society in the recent decades, which in turn has led to health problems such as body fat imbalance. In addition to euphoria and increased alertness obtained by khat chewing (22)

In the present study, we have evaluated the pattern of lipid profile parameters in diabetic subjects and healthy participant and its correlation with khat chewing habit .There were more females (56%) than male (44%) in this study. The current study revealed a high prevalence of lipid abnormalities in diabetes mellitus patient as most of them had two and more than two abnormal lipid profile parameters. This result is in the line with the previous studies which reported that dyslipidemia is a common association in diabetic patients (16). The most common dyslipidemia a significantly detected in this study was hypertriglyceridaemia (63.8%) in DM group.

The current study shows that the mean HDL-Cholesterol levels of apparently healthy khat chewers were not significantly lowered (41.6±9.9 mg/dl) than the corresponding mean of apparently healthy non-khat chewers (HDL-c, 43.28±8.2 mg/dl) This is not consistent with study done in Sana'a University on healthy student that the mean HDL-Cholesterol levels of khat chewers were significantly lowered (24.72±4.48 mg/dl) than the corresponding mean of non-khat chewers (HDL-c, 33.34±1.83 mg/dl) (control group) (43) this inconsistent result may be due to other factors like duration of khat chewing, amount of khat chewed per days and etc

The levels of TC and HDL-C showed significant difference between the two studied groups especially among diabetic subject, and it is shown that HDL-C is lower in qat chewers (28.4mg/dl) than in non-qat chewers(35.96mg/dl), while TC is higher in khat chewer(229.6 mg/dl) than non-khat chewer(178.12 mg/dl) all among diabetic subject. This finding is consistent with that of the other related studies.

The contributory effect of khat in reducing the level of lipid could be attributed to the amphetamine-like effect of cathinone, which stimulates beta adrenergic receptors and enhances lipolysis (37) additionally; the presence of tannins in khat leaves could lower the intestinal absorption of some lipid.

Our current study done on apparently healthy participant showed that Hypercholesterolaemia, hypertriglyceridaemia, high LDLC level, were non-significantly lower in khat chewers than in non-khat chewers (8%, 14%, 4% versus 12%, 13.6%, and 6%, respectively). But the prevalence of LHDL-C is significantly higher in khat chewer (28%) than non khat chewer (12%). This finding is consistent with that of Yemen, Hypercholesterolemia, hypertriglyceridaemia and high LDLC level, were lower in khat chewers than in non-khat chewers (16.6%, 58.3% and 16.3%, respectively, versus 20.8%, 64.9% and 18.2%, respectively). But the incidence of low HDL-C is generally higher in khat chewers than that in non-khat chewers (83% versus 75.3% respectively).(42)

This study shows that the mean serum levels of TC, LDL-C, HDL and TG are (154.23 mg/dl), (86.33 mg/dl), (41.69 mg/dl) and (95.44 mg/dl) respectively in apparently healthy khat chewer while the TC, LDL-C, HDL and TG (149.3 mg/dl), (85.45 mg/dl), (43.28 mg/dl) and (90.5 mg/dl) in apparently healthy non khat chewer respectively and The difference is not significant This is consistent with the study done on healthy subject in Sana'a University that the mean of triglyceride, LDL cholesterol and total Cholesterol levels of khat chewers were not significantly changes in khat chewers when compared with non-khat chewers (84.54±10.3 mg/dl, LDL-c, 73.6±28.3 mg/dl, Total cholesterol 110.3+ 29.4 mg/dl) and the corresponding mean of non-khat chewers (70.67+5.72 mg/dl, LDL-c, 76.4±29.1 mg/dl, Total cholesterol, 119.2 +31.7mg/dl) respectively(43)

Our current results showed that dyslipidaemia (hypercholesterolemia and low HDL) was observed more in qat chewers (51.79%) that in non-qat chewers (39.5%) among diabetic subject this high prevalence was associated with the increasing incidence of low values of HDL-C by 72.5% and 39.5% in both khat chewers and non-khat chewers, respectively. This result is inconsistent with the study done on rat that shows plasma cholesterol decreased in rats after treatment with qat. (44)

Our study demonstrates high prevalence of low HDL-C in qat chewer (28%) among healthy participant. This figure is lower than those in Jordanians. That reported by Abujbara et al. that 59.5% low HDL-C levels among healthy participant. Prevalence of hypertriglyceridaemia among qat chewers was 12%, among healthy participant is much lower than that in Iranian (28.0%) and Jordanian (41.9%) studies. (47, 48)

The mean of lipid profile among diabetic patient is higher than that of non-diabetic participant for both khat chewer and non-khat chewer and the difference is statically significance. Similarly prevalence dyslipidemia (hypercholesterolemia and low HDL) is higher among diabetic patient than non-diabetic participant for both khat chewer and non-khat chewer and the difference is statically significance

Chewing khat, frequency of khat chewing, frequency of eating fat containing food, frequency of drinking alcohol and physical exercise have significance association with lipid profile parameter in DM patient but there is no significance association with non-diabetic group. However other independent variables have no significant association with lipid profile either with diabetic patient or healthy group. Thus, improving the level of physical activity decreasing of consuming fat containing food decreasing chewing khat and drinking alcohol among khat and non-qat chewers can be one of the most important strategies to prevent dyslipidaemia

8. Conclusion and Recommendations

8.1. Conclusion

The current study demonstrated that dyslipidemia is highly prevalent in diabetics particularly in those with khat chewing habit. Thus, Khat have unfavorable side effects on the level of lipid profile and Khat consumption has created a major health problems by affecting numerous healthy character like BMI blood pressure, leads to other complication.

8.2. Recommendations

- Healthcare providers should counsel that khat chewing have many healthy problems and this problems cause mortality
- Supporting scientific research on khat in different institutions and Universities to explore the different effects of khat on public health should be appreciated.
- Health education about the adverse effect of khat chewing on DM should be delivered to the community and health institutions using available forum.

9. Strength and Limitations of the study

9.1. Limitations of the study

The study had some limitations.

- Due to convenience sampling method is used this study not includes equal number of khat chewer and non khat chewer among Dm study subjects
- The data cannot address all age in equal number

9.2. Strength of the study

- for non-diabetic participant equal number of khat chewer and non khat chewer where included

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8. ANNEXS

Annex I: - Participant information sheet

Information sheet English version

Project Title: -study of the effect of khat on Lipid Profiles among diabetic patient and healthy individual who have the habit of khat chewing and non at diabetic clinic Adama medical college Adama, Ethiopia.

Principal Investigator: Jemal Hussein (BSc, MSc candidate)

Name of the Organization: - Adama medical college Adama, Ethiopia.

Introduction-: My name is Jemal Hussein .I am MSc student in Addis Ababa university college of Health science department of Medical Laboratory science. You are invited to participa te as a study participant in a research on study of lipid profile. Please take as much time as you need to read or listen in the information sheet.

- **Purpose of the Research Project: -** The purpose of this study is To find out the impact of khat chewing on lipid profile levels in diabetes mellitus patient and healthy individual
- **Procedures and the expected participation: -** If you are willing to participate, you need to understand the purpose of the study and give your consent. Not only this but also the required volume of fasting blood specimen will be collected by experienced laboratory technologist in clinical chemistry department. Then, you are requested to give your consent to the sample collector. After consent, a sample will be taken from your arm. Moreover, there will be a face-to-face interview for additional questions.

Potential risks and Discomforts

There might be some minimal risk and discomfort when we take venous blood. However, during collection of blood specimens from you, appropriate precaution will be taken and sample will be collected by experienced laboratory technologist. If anything happened, appropriate medical care will be provided to you.

Confidentiality

We respect your privacy and confidentiality in this study. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study. Results will be coded by unique number. Without your permission your personal information will not be disclosed to anybody.

Potential benefits or incentives to subjects and/or to the society

You will not receive any payment for your participation in this research study as compensation. However, the result of the study will be used by clinicians and is beneficial in the management of diabetic complication by managing glycemic control. Hence, you are indirectly benefiting other patients and the society in this respect.

Participation and Withdrawal from the Study

The participation is voluntary and you have the right not to participate in this study. You may withdraw at any time and place without consequences of any kind. You may also reject to give any sample. You can ask any questions regarding to this study and you have a right to get a laboratory diagnosis result free.

Contact information

If you have any questions about this study you can contact the following research team for further information.

Jemal Hussein phone # 0919633767 Email: - jemswatt67@gmail.com

ANEXX II

Informed consent

This is a study to be conducted with the objectives of find out the impact of khat chewing on lipid profile levels in diabetes mellitus patient

As the study is directly related to diabetic patient, and healthy volunteer you are one of the participant who are selected to participate in this study, therefore you are kindly requested to participate in this study and provide the information required from you. Your participation in this study is completely on voluntary basis and you have the right to refuse from participating. Your responses will be kept confidential and there will be no way of linking your individual responses to the final results of the study findings.

We would like to inform you that the responses that you provide to the questions are very essential, not only, for the successful accomplishment of the study, but also for producing relevant information which will be helpful to improve the service utilization

Questioner

1. Code number of the subject: _____ Date_____
2. Address: A. Rural B. Urban
3. Age: _____
4. Sex A. Male B. Female
5. Occupation _____
6. Do you have a habit of khat chewing? A. Yes B. No
7. If yes, how often? A. Every day B. 2-3 days per week C. Once a week D. Occasionally
8. For how long have you been chewing khat? A) 6 months B) 1 year C) 2 years D) > 2 years
9. Do you have a habit of drinking alcohol? A. Yes B. No
10. If yes, how often? A. Every day B. 2-3 days per week C. Once a week D. Occasionally

11. For how long have you been drinking alcohol? A) 6 months B) 1 year C) 2 years D) > 2 years
12. Have you history of hypertension? A. yes B. no
13. Have you ever smoke cigarette? A, Yes B, No
14. If yes how often? A. daily B. occasionally C. once per week D. 2-3 days per week
15. For how long have you been smoke cigarette? A) 6 months B) 1 year C) 2 years D) > 2 years
16. Do you have a habit of eating fat? A. Yes B. No
17. If yes, how often?

    A. every day B. Once a week

    C. 2-3 days per week D. Occasionally
18. Have you history of liver infection previously? A. yes B. no
19. Do you have a habit of doing physical exercise?
20. If yes how often? ____/day/week/month

21. Have you have the habit of eating fresh fruit/leaves A YES B. NO
22. 17. If yes, how often did you consume? A. daily B 2-3 days per week C. Once a week D. Occasionally
23. How long did you sleep per day A. < 6hrs B. >8hrs

24 Are you pregnant? A. YES B. NO

25. Have you have the habit of using family planning method (contraceptive pills) A. YES B.no

5.6.4 Laboratory principle and procedure for determining lipid profile

LDL Cholesterol

Test principle

Homogeneous enzymatic colorimetric assay

Cholesterol esters and free cholesterol in LDL are measured on the basis of a cholesterol enzymatic method using cholesterol esterase and cholesterol oxidase in the presence of surfactants which selectively solubilize only LDL. The enzyme reactions to the lipoproteins

other than LDL are inhibited by surfactants and a sugar compound. Cholesterol in HDL, VLDL and chylomicron is not determined**. (47**)

LDL-C esters + H_2O___cholesterol esterase___→ cholesterol + free fatty acid

LDL-Cholesterol + O_2___ Cholesterol oxidase ___→ Cholestenone + H_2O_2

2 H_2O_2 + 4-aminoantipyrine + EMSE + H_2O + H^+_peroxidase→red purple pigment + 5H_2O

In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-aminoantipyrine and EMSE to form a red purple dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically.

Reagents-working solutions

R1 = 150µL and R2 = 50 µl

Sample volumes = 2µl

(Roche/Hitachi cobas c 311 methods, Lot#37297201) was used for this assay.

Procedure: 2 µl of sample was mixed with **150**µl R1 and **50**µlR2of working reagent

TG (triglyceride)

Test principle

Enz**y**matic colorimetric test

Triglyceride + 3 H_2O __LPL_ →glycerol + 3 RCOOH

Glycerol + ATP __GK_ →Glycerol -3-phosphate +ADP

Glycerol -3-phosphate + O_2 __GPO →dihydroxiacetone phosphate + H_2O_2

H_2O_2 + 4-aminophenasone + 4-chloro phenol_peroxidase_→4-phenasone + H_2O +HCI

Reagents - working solutions

R1 = 120µL

Sample volumes = 2µl

(Roche/Hitachi Cobas c 311 methods) was used for this assay.

Procedure: 2 µl of sample was mixed with **120**µl R1 of working reagent

Total cholesterol (TC)

Test principle:

Enzymatic colorimetric method.

Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol oxidase then catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminoantipyrine to form a red quinone-imine dye (**47)**.

Cholesterol esters + H_2O __CE_→ Cholesterol + RCOOH

Cholesterol + O_2 __CHOD_→ Cholest-4-en-3-one + H_2O_2

$2H_2O_2$ + 4-AAP + phenol __POD_→ quinone-imine dye + 4 H_2O

The color intensity of the dye formed is directly proportional to the cholesterol concentration. It is determined by measuring the increase in absorbance.

Reagents - working solutions

R1 = 47µL

Sample volumes 2µl

(Roche/Hitachi cobas c 311 methods) was used for this assay.

Procedure: 2 µl of sample was mixed with **47**µl R1 of working reagent

HDL Cholesterol

Homogeneous enzymatic colorimetric test.

Non-HDL lipoproteins such as LDL, VLDL and chylomicron are combined with polyanions and a detergent forming a water-soluble complex. In this complex the enzymatic reaction of Cholesterol esterase and cholesterol oxidase towards Non-HDL lipoproteins is blocked. Finally

only HDL-particles can react with Cholesterol esterase and cholesterol oxidase. The concentration of HDL-cholesterol is determined enzymatically by Cholesterol esterase and cholesterol oxidase. Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by Cholesterol esterase (**47**)

$$\text{HDL-cholesterol esters} + H_2O \xrightarrow{\text{CHER}} \text{HDL-cholesterol} + \text{RCOOH}$$

$$\text{HDL-cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenone} + H_2O_2$$

In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-amino-antipyrine and EMSE) to form a dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically

$$2\ H_2O_2 + \text{4-amino-antipyrine} + \text{EMSE} + H^+ + H_2O \xrightarrow{\text{Peroxidase}} \text{Colored pigment} + 5\ H_2O$$

Reagents - working solutions

R1 = 120 µl and R2 = 40 µl

Sample volumes 2.4µl

(Roche/Hitachi cobas c 311 methods, Lot#39304001) was used for this assay.

Procedure: 2.4 µl of sample was mixed with **120**µl R1 and **40**µlR2of working reagent

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Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: **JemalHusen (B.Sc.)**

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: **Samuel Kinde (MSc, PhD candidate)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: **GobenaDedafo (BSc, MSc)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia