ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF BIOCHEMISTRY

Assessment of Urine Cotinine Levels in Active and Passive Smokers, and Studies of Cardiovascular Risk Factors in Ogolcho Town, Oromia, Ethiopia

Gobena Dedefo Dekebo

Advisor(s): Frank Ashall, B.A. (Oxon), M.D., D.Phil.

A Thesis submitted to Addis Ababa University School of Graduate Studies, Department of Biochemistryin partial fulfillment of the requirements for the Degree of Master Science in Medical Biochemistry.

Addis Ababa, Ethiopia, June, 2015

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Approved by the examining board

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Table of Contents

List of Figures

List of Tables

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Abstract

Introduction: Tobacco use is a global escalating public health problem, especially in low-income countries, one-third of the adult males. Non-communicable diseases (NCDs) are a growing problem in Africa and it is partially caused by tobacco use. There are few studies of smoking practices and other cardiovascular risk factors in Ethiopia and it is unclear how prevalent many of these problems are, because their prevalence varies in different areas of the country. A more recently survey of smoking prevalence in 187 countries showed that global smoking prevalence fell from 41% in 1980 to 31% in 2012, and that Ethiopia ranks in the bottom five countries with the lowest smoking prevalence (below 10%), but there is evidence that smoking prevalence is increasing in Ethiopia.

Objective: To assess active tobacco and passive smoking, for further screening of major cardiovascular risk factors (serum cholesterol levels, hypertension, fasting blood glucose, Body Mass Index and waist circumference) among residents of Ogolcho town, Oromia; and to examine carbon monoxide levels in homes in Ogolcho town.

Methods: Across-sectional study was conducted on tobacco users, passive smokers and non-smokers in a rural population of Ethiopia in Ogolcho, from May 2014– May 2015.Subjects were interviewed, using a standardized WHO questionnaire, about their lifestyle habits and substance use. Blood specimens from individuals over 18 years old were taken for serum lipid profile and blood glucose levels. Urine was screened for cotinine (a nicotine metabolite) in active and passive smokers, using a One Step cotinine test that detects urine cotinine levels above 200 ng/mL.Anthropometric indices (BMI, waist circumference) and blood pressure were measured. Frequencies ofthe different variables were analyzed statistically.

Results: Of 95 total participants, 33% (31) were active smokers, 56.3% (54) were passive smokers, 8.6% (8) were neither active nor passive smokers, and 2% (2) were purely shisha smokers. Over 80% of active and passive smokers chewed khat regularly, whereas about a half of smokers and non-smokers used alcohol. All active smokers smoked either in their house or in public places, where exposure of many nonsmokers, especially children and women, occurs. A high percentage of smokers smoked at home (41%), and passive smokers that were strongly positive for urine cotinine included two children aged 4 and 5. Of 85 passive and active smokers, 67 % (57) were positive for urine cotinine. Almost all (90%) active smokers, and 54% of passive smokers, were positive for urine cotinine. Cardiovascular risk factors other than smoking were common in the population studied. Active smokers had 25.8% prevalence of abdominal obesity, 48.4% were underweight, 3.2% overweight, 54.8% had elevated triglycerides, 42% were hypertensive, 48.4% had low HDL and 3.2% high fasting glucose. 10.3% of passive smokers had abdominal obesity, 17% were underweight, 24% overweight, 55% hypertensive, 38 % had elevated triglycerides and 24% had low HDL. Prevalence of CVD risk factors among non-smokers were: 12.5% elevated blood pressure, 50% abdominal obesity, 12.5% overweight and 37.5% overweigh. Non- smoker lipid profile prevalence was: elevated total cholesterol in 37.5% of subjects and decreased HDL level prevalence in 25% of non-smokers. Carbon monoxide levels were generally safe in homes, except within centimeter distances of charcoal stoves.

Conclusion: This cross-sectional analysis indicates that khat and alcohol consumption, or both combined, are common among smokers and non-smokers in Ogolcho, and the prevalence of passive smoking is high. Urine cotinine testing was an effective way of studying passive and active exposure to cigarette smoke. The prevalence of CVD risk factors was high in the studied population, strongly supporting the importance of screening, prevention and treatment of smoking and other cardiovascular risk factors in Ethiopia.

Chapter 1: Introduction

1.1. Global Epidemiology of Tobacco Use and Cigarette Smoking

Smoking is a global escalating public health problem because of increased consumption in low-income countries (WHO, 2008; Fisher S *et al.*, 2008; Slama K, 2008) and is practiced by approximately one-third of the world‘s adult males (WHO, 2011). As a result, a disproportionate share of the global tobacco burden falls on developing countries, where 84% of the world‘s 1.3 billion current smokers reside (Jha P *et al*., 2000). Tobacco causes the deaths of six million people per year globally. This number is expected to exceed ten million deaths yearly by 2020, with over 80% of these deaths occurring in developing countries. Tobacco related deaths currently rank 2nd in middle-income and 7th in low-income countries as a major cause of death, and constitute the most prevalent preventable cause of death globally, causing one out of every 10 deaths from all causes. In Sub-Saharan Africa smoking caused just 100,000 deaths in 1990 and is projected to increase significantly unless strong tobacco prevention measures are taken (Murray CJ *et al*, 1997).

Tobacco is responsible for 1.4 million cancer deaths yearly. Lung, oral, and nasopharyngeal cancers are some of the major cancers caused by tobacco consumption. In addition to health problems, use of tobacco adds a burden to national economies by increasing costs in health expenditure and other indirect costs related to illness due to tobacco borne diseases. Tobacco-related diseases cause hundreds of billions of dollars of economic damage worldwide each year. Smoking related diseases causes more deaths each year than all deaths from human immunodeficiency virus (HIV), illegal drug use, alcohol use, motor vehicle injuries, suicides, and murders combined (WHO, 2011).

Cigarette smoke contains harmful chemicals with hazardous adverse effects on almost every organ in the body of smokers as well as of nonsmokers exposed to environmental tobacco smoke (ETS). Up to one-half of all tobacco users can be expected to die from a tobacco-related disease and adult smokers lose an average 13 to 15 years of life-expectancy because of their smoking behaviors (Peeters A et al, 2003). The economic burden of tobacco use is estimated to be $197 billion per year, which includes $96 billion in health care costs and an additional $97 billion in productivity losses (CDC, 2008).

Despite the growing problem of global tobacco use, accurate information on the prevalence and patterns in African countries is lacking. Descriptive statistics show the highest cigarette use in African countries occurs in Madagascar (prevalence of 27.3%), and the lowest smoking rates occur in sub-Saharan Africa, including Ethiopia (prevalence of less than 10%). Studies of men generally show highest cigarette use among urban, less educated, and lower economic status workers. Results for women show much lower prevalence than men but similar social patterns of use. The range of smoking prevalence in men across Africa from 8.0% to 27.3% demonstrates considerable diversity. The two west central African nations, Nigeria (prevalence 8%) and Ghana (prevalence 8.3%), have a low cigarette smoking prevalence, as does Ethiopia (8.3%) in 2008 (Mpabulungi L *et al*., 2008).

1.2. Epidemiology of Cigarette Smoking in Ethiopia

There is paucity of studies of smoking practices and other preventable medicine parameters in Ethiopia and it is unclear just how prevalent many of these problems are, in particular because their prevalence may vary significantly in different areas of the country. For example, in 2008 the World Health Organization (WHO) estimated that about 7% of adult males and 0.9% of

adult females smoke in Ethiopia (WHO, 2008), although the subsequent WHO report for 2011 did not include Ethiopia due to there being insufficient data (WHO, 2011). A more recently published survey of tobacco smoking prevalence in 187 countries showed that, although the total number of smokers globally has increased due to population increase, global smoking prevalence has fallen from about 41% in 1980 to about 31% in 2012, and that Ethiopia ranks in the bottom five countries with the lowest smoking prevalence, with a smoking prevalence below 10% (Ng *et al.*, 2014). The WHO estimated that 2% of all deaths in Ethiopia were attributable to tobacco use in 2004, including 3% of deaths due to non-communicable diseases (NCDs). Tobacco use accounted for 25% of deaths due to lung, trachea and bronchus cancer, 8% of deaths due to respiratory diseases, including 15 % of death due to COPD (WHO, 2012).

However, in one study of a rural town in Eastern Ethiopia, about 28% of adults interviewed said that they smoked cigarettes daily; this prevalence is higher than that in many countries (Reda AA *et al*, 2013). In addition, tobacco use in increasing in many developing countries, including sub-Saharan African countries. In Ethiopia, the tobacco company, National Tobacco Enterprise, recently increased their production of cigarettes from 4 billion to 6 billion annually, stating that the demand for cigarettes is increasing in the country (Sebsibe M, 2015). A recent study reported that the prevalence of cigarette smoking in Ethiopia is increasing among adolescents and that the proportion of female smokers is increasing; in this study, the prevalence of current smokers in adolescents was 17.2% and the prevalence of ever-smokers among adolescents was 28.6% in 2014 (Dereje N *et al.,* 2014).

According to the Ethiopian Demographic Health Survey (EDHS), few women (less than 1%) in Ethiopia smoke cigarettes or use tobacco of any kind, whereas seven percent of men age 15 to 49 use tobacco products of some kind; six percent say that they smoke cigarettes. The most

prevalent smokers in Ethiopia include men aged 40 to 49 in Harari (27% of them are smokers), in Somali (24 % smokers), in Dire Dawa (24% smokers) and in Afar (20% smokers) (Central Statistical Agency, 2011).

Tobacco use practices in Ethiopia appear to vary around the country, and since the data available on these is relatively minimal, published data on national tobacco use practices are necessarily only estimates. Indeed, though smoking prevalence in Ethiopia is said to be less than 10% in a recent study of 187 countries (Ng M *et al.,* 2014), another survey reported an adult smoking prevalence of 15.8% in the Gilgel Gibe Field Research Center (Alemseged F *et al.*, 2012). WHO estimates indicate that Ethiopia's national tobacco use prevalence is 7.6% in males and 0.9% in females (WHO, 2008). Most of these studies on smoking in Ethiopia have been conducted in urban populations or specific groups such as students (Misganaw A *et al.*, 2011; Central Statistical Agency, 2011) while rural areas and towns, where the majority (84.0%) of the population lives (Fisher S *et al,* 2008) are relatively neglected in tobacco research (Slama K, 2008), although one study, for example, in the Oromia region of Kersa rural town showed 28% of residents were current smokers. Of these, 68% of smokers expressed an interest to quit while 37% had tried to quit previously but without success. This study also showed a high exposure to second-hand smoke (52%) in homes and a third of this indoor smoking took place daily (Ayalu A *et al*, 2013).

To date, little is known about either the prevalence of smoking or passive smoking or their relationship with other substances of abuse such as khat and alcohol as risk factors for CVD in rural Ethiopia, although a study conducted in Addis Ababa showed daily smoking and regular khat chewing were significantly associated with each other (Tesfaye F *et al*, 2008).

Lung cancer is relatively uncommon in Ethiopia, but if the increase in smoking prevalence continues, it will likely to become a worsening health problem. It was estimated that there were almost 3000 lung cancer deaths per year in Ethiopia (Winkler V *et al*, 2011). There is a 20-year lag between smoking increases and corresponding increases in lung cancer prevalence, so an increased smoking prevalence in the next 5 years will reveal itself as a lung cancer epidemic in 20 years, time.

Therefore this study is designed to study smoking and passive smoking practices, as well as the prevalence of cardiovascular risk factors and metabolic syndrome among tobacco users, khat and alcohol users in a small town, Ogolcho, in Ethiopia.

1.3. Cardiovascular disease (CVD) and Smoking

Behavioral risk factors, that is, those that can be prevented or changed by a person‘s lifestyle, are responsible for 80% of all diagnosed CVD (WHO, 2011). Smoking is the leading behavioral risk factor of CVD. Tobacco use has been attributed to account for 14% of deaths from heart and circulatory disease (Health and Social Care Information Centre, 2012); with the risk being significantly lowered within two years of smoking cessation (Salonen JT, 1980). Compared with non-smokers, smokers have a 2 to 4 time‘s bigger risk of heart disease and of stroke (U.S. Department of Health and Human Services, 2004b).

In developed countries, over 30% of the population-attributable risk for myocardial infarction is directly attributable to smoking (Yusuf S, *et al.,* 2004).

Inhaling tobacco smoke causes numerous immediate responses within the heart and its blood vessels. Within one minute of starting to smoke, the heart rate begins to rise. This is mainly attributable to nicotine, the chief addictive substance in cigarettes. Nicotine stimulates the body

to produce adrenaline, making the heart beat faster. Nicotine also increases blood pressure, which is a measure of the tension created upon the walls of the arteries by the blood (Primatesta P, 2001). The increase in heart rate and blood pressure means that smokers‘ hearts often have to work harder than non-smoker‘s hearts, resulting in an increased risk of heart failure.

Smoking tobacco also results in increased exposure to carbon monoxide (CO), a colorless, odorless gas which is produced from the incomplete burning of combustible products, in this case tobacco. Carbon monoxide is the most abundant chemical of the 7,000 different constituents of tobacco smoke and can make up 3-5% of its volume (Hoffman D, 2001). When levels of carbon monoxide in the blood increase the ability of the body to carry oxygen is significantly decreased. This is because carbon monoxide attaches itself to hemoglobin (the oxygen-carrying pigment in red blood cells) much more tightly than oxygen does. This results in tissues being starved of oxygenated blood, which causes tissue damage. Smokers are also likely to experience shortness of breath and increased heart rate as a result of high carboxyhemoglobin levels.

One major contribution to the increased risk of CVD among smokers is tobacco‘s effect on increasing overall blood cholesterol levels.

This occurs as a result of the chemical acrolein, often used in pesticides, and present in tobacco smoke, which affects the way the body processes cholesterol, allowing greater amounts to remain in the blood system (Kato T, 2007). This compound, along with other less well defined tobacco chemicals, also decreases the ratio of high-density lipoprotein to low-density lipoprotein Gossett E, 2009; Campbell S *et al*, 2008). Low density lipoproteins (LDLs) and other fatty substances over time cause atherosclerotic changes, including foam cell and plaque formation and arterial narrowing. As the atherosclerosis progresses, blood flows less easily through rigid and narrowed arteries. If an unstable plaque ruptures it may lead to the formation of a thrombosis

(clot), with sudden blockage of an artery causing a heart attack, a stroke or acute gangrene of the legs. The speed of this process is increased further by many of the toxins in tobacco, which increase formation of reactive oxygen species (ROS), causing increased formation of pathogenic oxidized LDL, increasing foam cell formation, worsening plaque formation and causing damage to the blood vessel walls, allowing plaques to form at a faster rate than in a non-smoker (Mitchell B, 1999).

The risk of thrombosis is also raised due to tobacco‘s effect on fibrinogen levels and its effects on increased platelet aggregation which increases the risk of intravascular thrombus formation (Kannel WB *et al.*, 1987; Hunter E, 2001). Finally, it has been shown that smoking causes the body‘s blood vessels to constrict (vasoconstriction) by decreasing nitric oxide, and therefore by causing blood vessel constriction and increasing endothelin-1, which causes constriction of blood vessels (Kioski W *et al.*, 1994). The net result is raised blood pressure and a reduction in blood supply to tissues.

In line with active smoking, it is generally accepted that passive smoking, also referred to as secondhand smoke or environmental tobacco smoke (ETS), which is breathing in someone else‘s smoke, leads to increased prevalence of CVD as well as other tobacco-related diseases. Despite the well-known risks of smoke exposure and implementation of smoking bans in public places in many countries, millions of children worldwide are still exposed to environmental tobacco smoke in their homes (Metsios GS *et al.*, 2011). Such passive smoking has been implicated in deteriorating cardiovascular status in children in terms of lower high-density lipoprotein levels and deteriorating vascular function (Scientific Committee on Tobacco and Health, 2004). Different studies confirmed that exposure to second-hand smoke is a cause of CVD in non-

smokers (Board on Population Health and Public Health, 2010 ; Sims M *et al*., 2010; Raupach T, 2006).

1.4. Smoking and Cancer

There are more than 7000 substances in cigarette smoke; of these, 70 of them have been identified as carcinogens by the International Agency for Research in Cancer and for which there is –sufficient evidence for carcinogenicity" in either laboratory animals or humans. More than 20 are well known lung carcinogens (International Agency for Research on Cancer, 2010). The carcinogens in cigarette smoke, in particular polycyclic aromatic hydrocarbons, nitrosamines and heavy metals, among others, causes DNA damage, and repeated exposure to tobacco smoke overwhelms DNA repair mechanisms and results in genetic alterations that disrupt normal cellular growth and regulation, resulting in cancer.

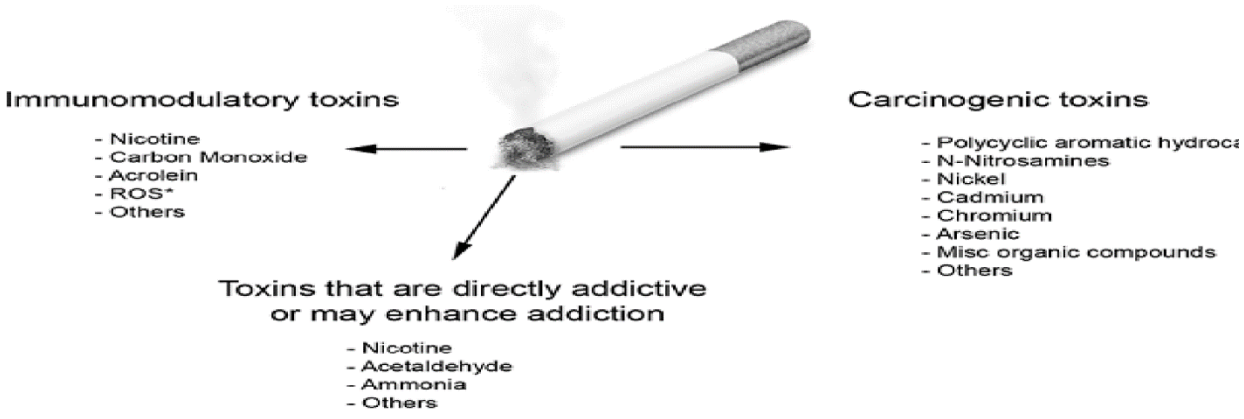

Figure 1. Major Substances and Carcinogens in Cigarette Smoke

1.4.1. Smoking and Lung Cancer

Smoking is the predominant etiologic risk factor for lung cancer (Alberg A *et al*., 2007). Approximately 90 % of lung cancers are caused by tobacco smoke inhalation (Alberg J and

Samet J, 2013).Incidence and mortality attributed to lung cancer has risen steadily since the 1930s, predominantly due to the popularity of cigarette smoking. In the past 100 years, lung cancer has therefore been transformed from a rare disease into a global problem (American Cancer Society, 2012). Lung cancer is the number one cancer killer in the world, causing 1.37 million deaths per year. There are six million new cases of lung cancer, or 12.7% of the world‘s total cancer incidence.

There are two broad histologic types of lung cancer depending on how the cells look under the microscope: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). The non-small cell carcinomas consist of three subtypes: squamous, adenocarcinoma and large cell carcinomas.

Non-small cell lung cancers account for 85 percent of all lung cancer cases (U.S. National Institutes of Health, 2006).They usually spread to different parts of the body more slowly than small cell lung cancer. Adenocarcinoma of lung comprises over 40% of all lung cancers, and is the most common subtype of lung cancer. Over 70 % of lung adenocarcinomas are due to cigarette smoking. Large cell carcinomas account for only 9% of lung cancers, but again are mainly (over 80% of them) due to cigarette smoking. Squamous carcinomas constitute 30% of all lung cancers, and over 90 % of squamous lung carcinomas are due to smoking.

Small cell lung carcinoma (SCLC) accounts for up to 20percent of all lung cancers (U.S. National Institutes of Health, 2006). This type of lung cancer grows more quickly and is more likely to spread to other organs in the body. It often starts in the bronchi and towards the center of the lungs. SCLC is mainly attributable to smoking:98% of SCLC arise in smokers. Only 6.2 percent of the people who develop small cell lung cancer survive for 5 years.

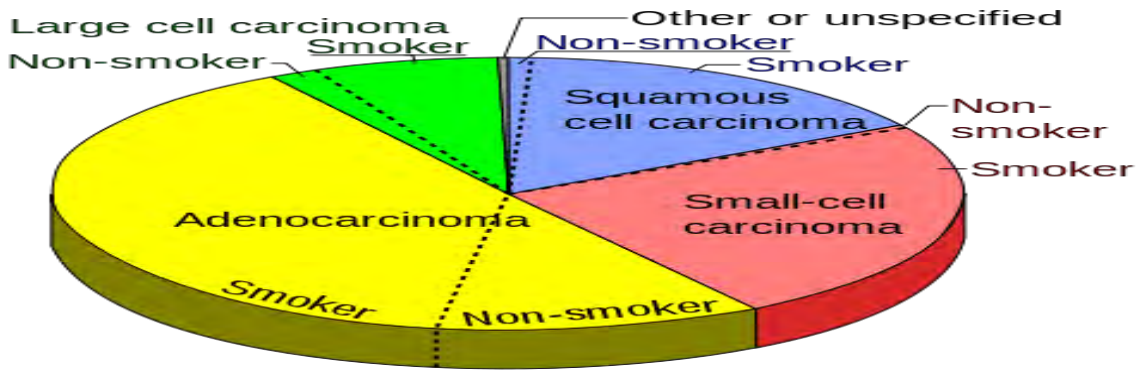

Figure 2. Proportions of different types of lung cancer caused by cigarette smoke

Thus, all of the four major types of lung cancer, squamous carcinoma, small cell carcinoma, large cell carcinoma and adenocarcinoma, are largely due to cigarette smoking.

A recent study demonstrated there is no difference in the risk of lung cancer between people who smoke medium tar filter, low tar filter, and very low tar filter cigarettes (Harris J *et al.*, 2004). The increased incidence and proportion of lung adenocarcinoma in recent decades may be associated with the appearance of cigarette filters in the 1950s. Cigarette filters decrease the levels of chemical constituents of cigarette smoke that are inhaled in a single puff. However, smokers compensate for this by taking deeper puffs, more puffs and by covering ventilation holes that are present in fliters, so maintaining their nicotine intake.This amounts to higher doses of carcinogens reaching deeper parts of the lungs (Herbst R et al., 2008; Thun M et al., 1997), with consequent increased risk of adenocarcinoma (Charames G and Bapat B, 2003). This demonstrates that cigarette filters do not make cigarettes safer: indeed they increase the chance that a smoker will develop adenocarcinoma.

The pooled evidence also indicates a 20 to 30 percent increase in the risk of lung cancer from secondhand smoke exposure associated with a non-smoker living with a smoker (US Department

of Health and Human Services, 2004a), because secondhand smoke contains all the same carcinogens that are present in the smoke inhaled by a smoker, but with less dose.

1.4.2. Other Tobacco Related Cancers

Cigarette smoke is the most common preventable cause of cancer and cancer mortality, responsible for approximately one third of all cancer deaths. Sufficient evidence has been accumulated to infer a causal relationship between tobacco use and cancers of the lung, larynx, oral cavity, pharynx, esophagus, pancreas, bladder, kidney, cervix, stomach, and acute, myeloid leukemia, with additional evidence suggesting a causal relationship for colorectal and liver cancer (US Department of Health and Human Services, 2004b). The risk of cancer further increases with the number of cigarettes smoked and the duration of smoking, as well as with exposure to second-hand smoke.

1.4.3 Other Tobacco Related Diseases

In addition to substantial cancer risks and cardiovascular disease, tobacco use also increases risk for other life-threatening chronic illnesses, including chronic obstructive pulmonary disease (bronchitis and emphysema) as well as asthma and has adverse health effects related to fertility, bone density, vision, and peptic ulcer disease (US Department of Health and Human Services, 2004b). Chronic obstructive pulmonary disease occurs among individuals with a long-term history of smoking and it affects 15 to 20% of smokers. Various combinations of these comorbidities are observed frequently in conjunction with smoking (Global Initiative for Chronic Obstructive Lung Disease, 2006).

The reason that people repeatedly expose themselves to the toxins and carcinogens in tobacco smoke is not because of free choice, but rather because of nicotine addiction. Those who experience the vast majority of illnesses resulting from tobacco use are those who are the most dependent on nicotine.

In line with active smoking, there is sufficient evidence confirmed by different studies in non-smokers to infer a causal relationship between second hand smoke exposure and cancer, cardiovascular disease, pulmonary diseases and other tobacco related disease among lifetime non-smokers that were previously attributed only to the long-term effects of active smoking (Glantz and Parmley, 2001; Board on Population Health and Public Health, 2010; Sims M *et a*l., 2010; Raupach T, 2006).

There has been increasing interest in the effects of passive smoking on the health of children (Brady *et al*., 2007; Al-Sayed EM and Ibrahim S, 2014). Children who spend much of their early life in home with their parents who smoke, therefore, are more likely to experience more intense and prolonged smoke exposure from parental smoking. Exposure to second-hand tobacco smoke puts children at risk of many tobacco related disease, such as lower respiratory infections (Baker et al., 2006), inflammatory bowel disease (Mahid *et al.,* 2007), sleep disturbances (Yolton *et al*., 2010), bronchitis, pneumonia), otitis media (Scientific Committee on Tobacco and Health, 2004), meningococcal meningitis (Murray RL *et al*, 2012b) and leukemia (Chang *et al.,* 2006). Exposure to second-hand tobacco smoke is also linked to a variety of behavioral issues and intellectual impairment in children (Yolton *et al*., 2005).

Exposure to second-hand tobacco smoke during pregnancy has a role in the induction of fetal growth retardation and low birth weight. Besides, exposure of a fetus to tobacco toxins from its

smoking mother causes subsequent disturbances in postnatal growth and development (Gomes PR and Seraphim PM, 2010).

Despite the well-known risks of second hand smoke exposure and active smoking, millions of individuals including children and pregnant women worldwide are still exposed to environmental tobacco smoke in their homes with cramped housing, overcrowding, poor ventilation in developing countries due to poor enforcement of smoke-free laws (Metsios GS *et al*, 2011). This non-voluntary health harm has spurred legislation in many states to protect non-smokers in workplaces, bars and restaurants. Currently, many countries, including Ethiopia, have laws prohibiting smoking in bars and restaurants. Thus, regulation of indoor smoking has become one of the pillars of tobacco control, along with aggressive counter-advertising and taxation, shown to be effective in decreasing smoking prevalence (Myers ML, 2013; Glantz S *et al*, 2012).

In addition to active and passive exposure to cigarette smoke, the tobacco industry causes a number of hazards to those who cultivate, harvest and process tobacco. Although some of these occupational related hazard like pesticide exposure and musculoskeletal trauma affect workers in other types of agricultural production, tobacco farming is associated with green tobacco sickness (GTS), which is acute nicotine poisoning. It occurs when workers, especially child labourers on tobacco farms, absorb nicotine through the skin as they come into contact while harvesting leaves of the mature tobacco plant. GTS is characterized largely by nausea, vomiting, headache, muscle weakness, and dizziness. (McKnight RH & Spiller HA, 2005).

1.5. Smoking, HIV and Tuberculosis as a Co-epidemic

Tuberculosis (TB), smoking and HIV are escalating epidemics in developing countries. The association between TB and HIV is well established (WHO, 2007). These associations are of

considerable relevance to public health and disease outcomes in individuals with TB. People who smoke are more prone to contracting TB, and patients with TB are more prone to developing symptoms of TB, less responsive to anti-TB medications, have worse side-effects of TB medications, and are more likely to die from tuberculosis. Moreover, tobacco smoking, a modifiable risk factor, is associated with poorer outcomes in HIV-associated opportunistic infections, of which TB is the commonest in developing countries. Thus, there is a deleterious and synergistic interaction between TB, HIV and tobacco smoking in a large proportion of the world's population, and many health experts believe that TB, HIV and tobacco smoking should be considered as a co-epidemic, and it recommended that all TB and HIV patients be screened for tobacco use, encouraged to quit if they do smoke, and discouraged from starting to smoke if they are non-smokers (Lin HH *et al*, 2007; Bates MN et al, 2007).

1.6. Non-Communicable Diseases (NCDs)

1.6.1 NCDs as a Global Health Problem

Non-communicable diseases (NCDs), including cardiovascular disease, diabetes and malignancies, can be prevented significantly by proper screening of populations for their risk factors, and by educating patients to implement healthy lifestyles, in particular by eating a healthy diet, increasing exercise and avoiding tobacco use (WHO, 2005; WHO, 2008). In addition to screening for tobacco, alcohol and other substance use, and smoking and dietary practices, other preventive medical tests, including blood pressure measurement, fasting blood glucose and fasting lipid profile, as well as measurement of waist circumference, body mass index (BMI), constitute basic parameters that can be useful for detecting early risk factors for cardiovascular disease and diabetes. Hypertension and diabetes can be treated relatively easily

and inexpensively in most countries, including Ethiopia, and screening patients for these preventive parameters is useful for both treating them and educating them on lifestyle such as healthy diets and exercise.

According to a WHO report onthe Africa region, NCDs cause 23% of all deaths and cardiovascular diseases accounted for the major proportion of deaths (42%). Other important causes of death, among NCDs, include malignant neoplasms (16%) and respiratory diseases (12%)(WHO, 2008)

Non-communicable diseases have been a difficult group to define. Even the term ―non-communicable diseases" is a misleading term, because it includes some diseases notably, cancers of the liver, stomach, and cervix that are at least partly caused by infectious organisms, and it usually excludes mental illnesses, despite their large contribution to long-term disability.

However, the four major behavioral risk factors for NCDs are: tobacco use, excessive alcohol consumption, poor diet, and lack of physical activity. Those risk factors are associated with four disease clusters grouped under NCDs, namely cardiovascular diseases, cancers, chronic pulmonary diseases, and diabetes that account for about 80% of deaths from NCD diseases (Lozano R *et al.*, 2012).

According to WHO estimates, NCDs contributed to 36 million deaths globally in 2008, and accounted for 63% of 57 million total deaths (WHO, 2011). About 80% of deaths related to NCDs occur in low- and middle-income countries, which also have a high proportion of deaths in middle age; such countries account for 90% of the 9 million NCD related deaths that occur before 60 years of age (WHO, 2013).

Cardiovascular diseases (CVD) account for the largest fraction of deaths related to NCDs, followed by cancer, chronic obstructive pulmonary disease (COPD), and diabetes (Murray CJ *et al.,* 2012a).

The economic consequences of NCDs are huge, because of the combined burden of health care costs and lost economic productivity due to illness and premature deaths. A study commissioned by the World Economic Forum concluded that the world will sustain a cumulative output loss of $47 trillion between 2011 and 2030 because of NCD and mental illness, about $30 trillion of which will be attributable to CVD, cancers, chronic pulmonary diseases, and diabetes (Bloom DE *et al.*, 2011). NCDs are also a major cause of catastrophic health expenditure among the uninsured (Heeley E *et al.*, 2009).

Measurable phenotypes such as high blood pressure, hypercholesterolemia, and obesity mediate much of the relationship between these risk factors and the incidence of NCDs. There are proven preventive strategies for NCDs. These measures would significantly prevent disease and delay complications that would otherwise demand costly and lifelong medical care. On the other hand, if the emergence and prevention of risk factors remain unintervened and the health services left undirected, the problem can increasingly cause more human suffering and escalate the cost of treatment.

To be comprehensive, a program for the prevention and control of NCDs must integrate policies designed to foster a societal environment in which people are encouraged to make and maintain healthy living choices, promote health literacy so that people can protect and improve their health, and provide health services focused on early detection and cost-effective management of NCDs.

Population-based interventions include policy measures such as increasing taxation of tobacco and alcohol, reducing salt and saturated fat and eliminating trans-fats in processed foods, and creating smoke-free and exercise-friendly public spaces, and improving diet practices such as encouraging a Mediterranean diet rich in fish, fiber, fruit, vegetable and legumes and low in red meat and baked product consumption. Among the risk factors identified as major causes of NCDs (Ezzati M *et al*., 2012), dietary risk factors and physical inactivity are partially determined by individual preferences but are substantially influenced by the manufacturing and marketing practices of the food industry and by the built and social environments that permit or impede physical activity.

Mass-media messaging as well as health promotion in specific settings (schools, workplaces, and community centers) may be used to provide health education. Preventive interventions include risk-factor assessment and treatment with behavioral interventions and medication for persons at high risk.

Tobacco is the second largest cause of deaths and disability worldwide, and tobacco control could prevent about a third of all deaths from cancer in the United States (Jemal A *et al.*, 2008) and could also rapidly reduce deaths from cardiovascular and chronic pulmonary diseases. The WHO Framework Convention on Tobacco Control provides proven tobacco-control strategies that need to be implemented within and between nations (WHO, 2003; Myers ML, 2013; Glantz S *et al*, 2012).

1.6.2. Non-communicable Diseases (NCDs) in Ethiopia

Prevalence of NCDs is increasing at a rate much higher than that of infectious diseases, maternal and prenatal diseases and nutritional deficiency diseases combined (Muluneh AT *et al.,* 2012*;* Nigatu *T,* 2012*;* Alemseged F *et al.*, 2012; Misganaw A *et al.*, 2014).

Despite escalating rates of different NCDs in Ethiopia, they are neglected and priority of health care focuses on communicable diseases which have been considered more urgently important than NCDs. Although NCDs are believed by many to be a problem of rich societies, in reality they represent an under reported and neglected burden on health in the developing countries and one that is ever increasing (WHO, 2009). For example one study in Ethiopia reported a prevalence of NCDs of 8.9%, with the specific observed prevalence of 0.5% for diabetes mellitus (DM), 2.6% for hypertension, and 3.0% for cardiovascular diseases (Muluneh AT *et al*., 2012). Another study reported 2.6% prevalence of DM and estimated increment of DM to 3.5% after two decades (Motala AA *et al.,* 2009).

The prevalence of hypertension was reported 10.1% in urban and 9.7% in rural areas of Sidamo zone (Giday A *et al.,* 2011). Status report on hypertension in Africa Consultative review for the 6th Session of the African Union Conference of Ministers of Health on NCDs reported a prevalence in Ethiopia of hypertension of 33% in males and 30% in females (Van de Vijver S *et al.,* 2013). Another study in Gondar, Northwest Ethiopia in 2012 reported 28.3% prevalence of hypertensive of whom more than a third (37.0%) did not know they had hypertension; which agrees with other studies showing the occurrence of a ―silent epidemic" of high blood pressure in developing countries, particularly in Ethiopia (Awoke A *et al.*, 2012).

A study in Ethiopia among adults in Addis Ababa also reported a prevalence of hypertension, an important CVD risk factor, of 31.5% among males and 28.9% among females (Tesfaye F *et al*., 2009).

1.7. Metabolic Syndrome, Insulin Resistance and Obesity

The term "Metabolic syndrome" is now used exclusively to define a constellation of abnormalities that are associated with increased risk for the development of NCDs, particularly CVD. The chief components of the metabolic syndrome are central adiposity, dyslipidemia (increase in plasma triglycerides (TG)) and LDL, decrease in high density lipoprotein cholesterol (HDL-C), hypertension and glucose intolerance.

Metabolic syndrome, a condition characterized by abdominal obesity, dyslipidemia (high serum triglyceride and low HDL levels), elevated blood pressure, and hyperglycemia and it‘s become one of the major public health problems worldwide (Alberti KG *et al.,* 2009). This syndrome increases the risk of developing type 2 diabetes and cardiovascular disease and is correlated with all-cause mortality (Wilson PW *et al.*, 2005;Lakka HM *et al.*, 2002).

The pathophysiology of Metabolic Syndrome is very complex and has only been partly clarified. Usually, it is observed in people who are obese, advanced in age, sedentary, and have a measure of insulin resistance. The most significant factors in order are: age, genetics, sedentary lifestyle (reduced physical activity and overindulgence of caloric intake), poor diet. Obesity is a risk factor for insulin resistance, which is associated with visceral obesity. Visceral fat differs from subcutaneous fat in that visceral fat produces large amount of adipokines, which are growth factors and cytokines, some of which affect the expression of genes involved in insulin

sensitivity of peripheral tissues, including adipose and skeletal muscle(Hallfrisch J, 1990; Reiser S *et al.*, 1989).

Common findings associated with the Metabolic Syndrome include: Fasting hyperglycemia, type 2 DM or impaired fasting glucose, impaired glucose tolerance, or insulin resistance, high blood pressure, central obesity (also known as visceral, male-pattern or apple-shaped adiposity), overweight with fat deposits mainly around the waist, decreased high density Lipoprotein cholesterol and increased Triglycerides.

There are many classification systems for the Metabolic Syndrome, which have been put forward by varying authorities including World Health Organization,National Cholesterol Education Program Adult Treatment Panel III (2001), NCEP III, the European Group for the Study of Insulin Resistance EGIR, (1999) and American Heart Association/Updated NCEP (Kuzuya T *et al.*, 2002; NCEP, 2001; Balkau B *et al.,* 1999; Grundy SM *et al*, 2004). Even though the classification criteria differ among different authorities in terms of reference ranges, nearly all of them include the same kind of combination of parameters including abdominal obesity, blood pressure and biochemical indicators, including lipid profiles (TG, HDL) and fasting blood glucose level. In this study, participants with three or more of the following five criteria will be considered to have the Metabolic Syndrome:

1. Abdominal obesity, determined by elevated waist circumference (≥ 90 cm in men and ≥80 cm in women),
2. Elevated serum triglycerides (≥150 mg/dL) or on drug treatment for hypertriglyceridemia,

3. Reduced HDL-C (<40 mg/dL in men and <50 mg/dL in women) or on drug treatment for reduced HDL-C,
4. Elevated blood pressure (systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg) or on antihypertensive drug treatment; and
5. Elevated fasting glucose (≥ 110 mg/dL) or on drug treatment for elevated glucose.

1.8. The Framingham Cardiovascular Risk Score

The Framingham risk score uses the following parameters: sex (male or female), age, smoking status (yes or no), systolic blood pressure (in mm Hg), use of blood pressure medications (yes or no) were calculated for smokers only, serum triglyceride (in mg/dL) and serum total cholesterol (in mg/dL) to calculate the percentage risk of a person suffering from a myocardial infarction during the next ten years. It is based on a study of tens of thousands of individuals over many decades in the USA, but can be generally applied to other populations (Mahmood et al, 2014). The risk is based on a complex empirically derived equation, based on the results of the Framingham study, and can be determined by inserting the patients‘ parameters into an online site; the risk factor is then determined automatically (National Heart, Lung and Blood Institute, 2014). Performing Framingham scores with smokers and comparing them with the situation if they did not smoke allows evaluation of the contribution of smoking to the patients cardiovascular risk.

1.9. Nicotine

1.9.1. Nicotine and Cotinine Metabolism

Although most of the toxicity of smoking is related to other components of cigarette smoke, it is primarily the pharmacologic effects of nicotine that produce the addiction to tobacco. Sustained tobacco use leads to different metabolites that play important role for development of different tobacco related disease.

Nicotine is a base containing a pyridine and a pyrrolidine ring; each ring possesses a tertiary amine (Brewer B *et al.*; Yildiz D, 2004). It is absorbed through alveoli after inhaled from smoke into the circulation. During smoking, high levels of nicotine reach the brain in 10 to 20 seconds after a puff, faster than with intravenous administration (Dome P, 2010; Baker R *et al.*, 2004) because of the large alveolar surface and large blood perfusion of the pulmonary circulation.

After entering the circulation, nicotine is subjected to extensive metabolism by liver (Figure 3), primarily by the liver enzyme CYP2A6, which is responsible for the metabolism of about 90% of nicotine and to a lesser extent by CYP2B6 and CYP2E1 to different major and minor metabolites. Unmetabolized nicotine excretion via the urine only accounts for about 5% of total elimination. The rate of nicotine metabolism is influenced by many factors, such as age, gender, food consumption, race, hepatic or renal diseases, pregnancy, and tobacco ingredients (Balfour DJ, 2004; Garret BE *et al.*, 2001).

On average, 70 to 80% of the nicotine is metabolized to cotinine and to five other metabolites: nicotine-1-N-oxide (4%), nicotine glucuronide, nornicotine (0.4%), nicotine isomethonium ion, and 2-hydroxynicotine (Hukkanen J et al., 2005). Cotinine is further metabolized to cotinine-N-oxide and trans-3´-hydroxycotinine by CYP2A6, among others. Trans-3´-hydroxycotinine is the

most abundant metabolite in urine, accounting for on average 38% of the metabolites of cotinine (Dempsey D et al., 2004).

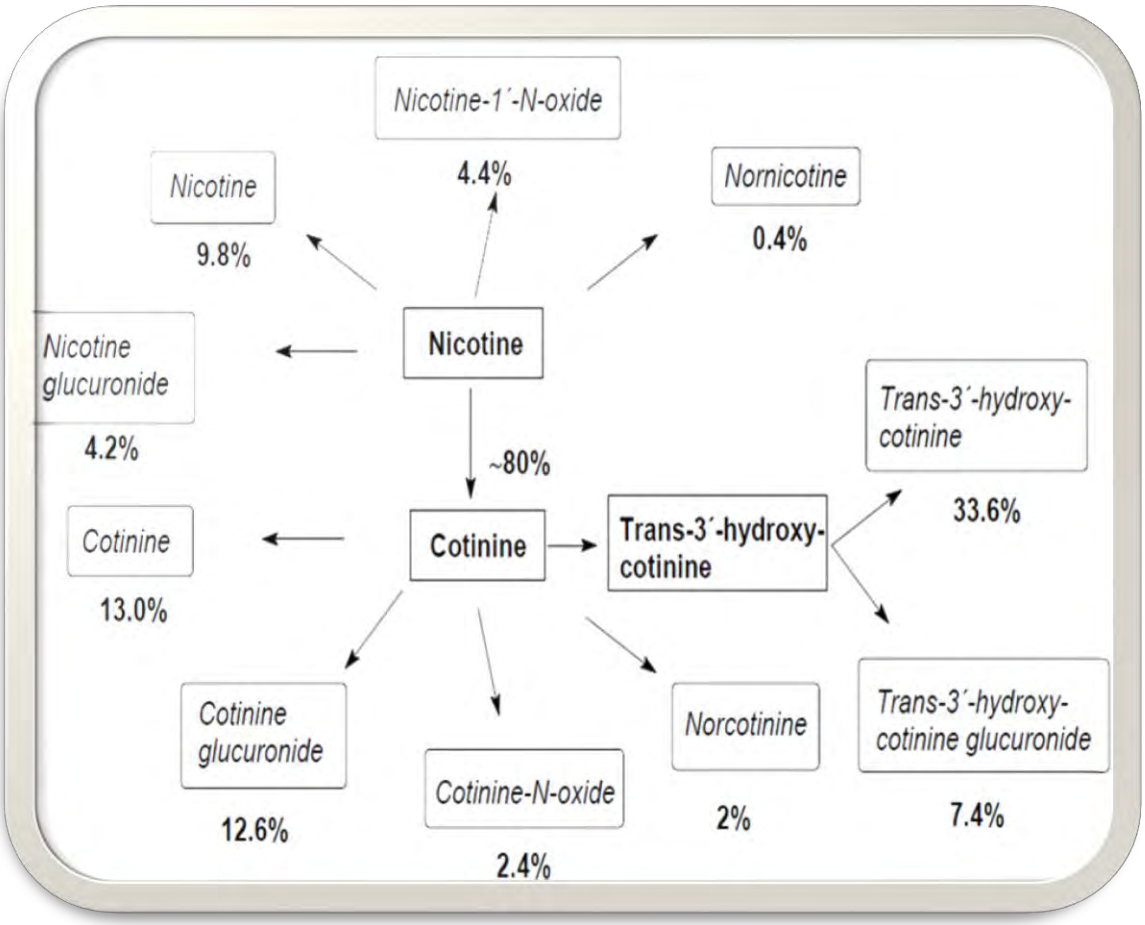

Figure 3. Metabolism of nicotine in the human liver.

The plasma half-life of nicotine is approximately 2 hours. In the brain, the distributional half-life of nicotine is 10 minutes. Distributional half-life describes the time that it takes a nicotine dose to fall 50% from its peak level in the brain as the nicotine is distributed to other body compartments with a high affinity for nicotine (for example, the liver, spleen, kidney, lung) The half-life of cotinine is much longer than plasma half-life of nicotine. On average it is ~16 h

(Dome P, 2010; Baker R *et al*., 2004), but it varies between indivuduals, and is generally considered to be detectable for up to 3 days after the last cigarette was smoked.

Cotinine is widely used as a quantitative marker for exposures to nicotine, and is useful as a diagnostic test for the use of tobacco and as a measure of compliance with treatments for smoking cessation. Cotinine levels are fairly stable throughout the day in smokers and its plasma half-life is longer unlike other metabolites' specifically nicotine, making cotinine a convenient and reliable marker for current smoking. Cotinine can be measured in the blood, saliva, or urine of people while they are using tobacco, based on their intake of nicotine from tobacco (Hukkanen J *et al*., 2005).

1.9.2. Nicotine Addiction

Nicotine is the most abundant component of all types of tobacco products and smoke. It is also the main addictive constituent of tobacco and is non-carcinogenic. It is a potent activator of brain's ~~"r~~eward system," eliciting cravings for continued tobacco consumption. Addiction is defined as a situation in which a drug unreasonably controls behavior (U.S. Department of Health and Human Services, 1988;Tobacco Advisory Group of the Royal College of Physicians, 2000), including tobacco-seeking behavior. Tobacco users often desperately seek tobacco or nicotine products when nicotine is not immediately available. Nicotine produces pleasurable and stimulating effects, relieves stress and anxiety, improves performance and offsets boredom.

Nicotine addiction is particularly related to the release of dopamine and other neurotransmitters in the brain, caused by the action of nicotine on nicotinic cholinergic receptors. Nicotine withdrawal symptoms, which develop early on after smoking is initiated, include irritability, restlessness, anxiety, problems of getting along with friends and family, difficulties

concentrating, increased hunger and eating, constipation, and craving for tobacco. These reinforce the smoker‘s need for nicotine. Within about 3 weeks after stopping smoking, withdrawal symptoms are powerful, but if a person can quit for longer than 3 weeks or so, many neurotransmitter changes due to nicotine return to normal in the brain. Despite this, most (over 80%) smokers cannot easily quit, in particular because even years after quitting smoking there are ―association" behaviours related to a person linking cigarette smoking with pleasant experiences, such as socializing with friends, drinking coffee, and relaxing, which increase the urge of an ex-smoker to resume smoking months or years after quitting. Approximately 80% of smokers who attempt to quit on their own relapse occurs within the first month of abstinence, and only approximately 3% remain abstinent at six months. This illustrates the powerful force of tobacco addiction and the chronic nature of the disorder. (Benowitz NL et al, 2008).

In addition to being addictive by itself, nicotine acts as a ―gateway drug" that enhances the addictiveness of other drugs, in particular cocaine. The idea that one drug can enhance the addictiveness of another drug is known as the Gateway Hypothesis, and evidence is accumulating to support the hypothesis. For example, 90% of cocaine users smoked cigarettes prior to becoming cocaine users. Also, use of other drugs of abuse commonly follows smoking addiction. Extensive studies in mice have shown that nicotine primes the cocaine response of the brain by increasing cocaine-induced transcription of the c-fos gene as a result of decreased histone deacetylation (Li H *et al*, 2014; Levine A *et al*, 2011).

1.10. Carbon Monoxide Exposure in Ethiopia

In addition to exposure to tobacco smoke, carbon monoxide from combustion of wood, charcoal and other biomass fuels is a potential threat to people who burn fires or stoves in or close to their

homes. In one study of ten homes in Addis Ababa, for example, WHO guidelines for maximal recommended carbon monoxide exposure were exceeded in most of the homes (Keil et al, 2010). Little information is, however, available on carbon monoxide exposure in Ethiopian homes, though in most rural parts of Ethiopia as much as 80% of cooking is done indoors using charcoal or wood (Keil et al, 2010). The incidence of carbon monoxide poisoning in Ethiopia was estimated to be almost 30,000 cases yearly in 2004 (Right Diagnosis from Healthgrades, 2015).

Carbon monoxide levels in the air between 1 and 70 parts per million (ppm) are usually asymptomatic, but as levels rise above this symptoms (headache, fatigue, nausea) and toxicity become increasingly serious. Nevertheless, there is evidence that a person‘s ability to perform tasks is reduced even at 15 to 20 ppm, and at 21 ppm and above, cardiorespiratory symptoms appear in some people. A level of 9 ppm over an 8-hour period in any one year is the maximum allowable level by the U.S. Environmental Protection Agency in the USA outdoor environment, but occupational health organizations allow up to a maximum of 50 ppm over an 8-hour work period (U.S. Centers for Disease Control, 1978;Iowa State University, 1997).

1.11. Objectives

1.11.1. General objectives

To assess active tobacco and passive smoking prevalence among family members of smokers, and to screen for several major cardiovascular risk factors.

1.11.2. Specific objectives

- To assess urine cotinine levels in active smokers
- To assess urine cotinine levels inpassive smokers
- To assess smoking habits, perceptions on tobacco, and other substance use in active smokers.
- To assess smoking habits, perceptions on tobacco, and other substance useinpassive smokers
- To determine major cardiovascular risk factors
- To determine lipid profile in active smokers.
- To determine lipid profile in passive smokers
- To evaluate Fasting blood glucose in smokers

Chapter **2**

Materials and Methods

2.1. Study design

A cross-sectional study was conducted among tobacco users, passive smokers and non-smokers in rural population of Ethiopia at Ogolcho town.

2.2. Study Area

This study was conducted in Ogolcho town which found in southeastern Ethiopia, located in the Arsi Zone of Oromia region. It has latitude and longitude of $8^0 9^0$N $38^0 49^0$E/8.050N 39.00E with elevation of 1636 meters and 158 km distance from Addis Ababa.

The total land area of Ogolcho town is 20 km^2 and the population of Ogolcho is about 13,000. Occupations of Ogolcho residents include mainly farmers, followed by merchants and civil servants. In the town there is one health center, two primary schools, one secondary school and one preparatory school. Ogolcho town was selected as study area for this project because the MSc student (Gobena Dedefo) is familiar with the area, having been brought up in a nearby village (Shenen).

The study was initially planned to involve visiting peoples‘ homes and drawing blood and performing measurements of blood pressure, height, weight, etc in their homes. However, sterility and accessibility and practical hindrances made this difficult, fortunately, a local health administrator kindly offered use of a room in a local Ogolcho clinic, where blood draws, interviews and other measurements were conveniently and safely done.

2.3 Study Period

This study was conducted from May 2014 to May 2015.

2.4. Population

2.4.1 Source population

The source population were all active and passive smokers; plus non-smokers live in Ogolcho town**.**

2.4.2 Study population

The study subjects were residents of Ogolcho town older than 18 years, who use tobacco and other substances, with their passive smokers‘ family and friends (including children) plus non-smoker subjects who were older than 30 years.

2.5 Sampling method

Individuals chosen for the study were recruited from streets and homes in the town of Ogolcho, Oromia. All smokers over 18 who volunteered were included in the study, as well as non-smokers over 30 years old. Passive smokers were generally of any age (including children) and were chosen because they were relatives, friends or coworkers of the chosen smokers and were exposed to their cigarette smoke.

2.6. Sample size determination

The sample size of smokers was calculated using single population proportion based on the following assumptions: WHO estimate indicates Ethiopia national tobacco use prevalence of 7% (WHO, 2008).

- ✓ Significance level calculated at 95%CI
- ✓ Margin of error tolerated is 5 %(0.05)

$$n = \frac{Z^2\ 1-\alpha/2\ P\ (1-P)}{d2}$$

Where:

- n is n minimum required sample
- Z is Z-score at 95% CI (1.96)
- P is population proportion (7%)
- d is margins of error (0.05)

$$n = \frac{(1.96)^2\ (0.07) \times 0.93}{(0.05)^2}$$

$$= \frac{(3.84)\ (0.651)}{0.0025} = \frac{0.249984}{0.0025}$$

$$n = \frac{99.99}{} \approx \underline{100}$$

To avoid non response rate 10% was added, so the total sample would be 10 +100 =110.

In practice, less than 110 smokers or other participants were recruited for the study, due to difficulties getting that number of people to volunteer for blood draws. This limits the statistical value of the study, but nevertheless enough participants were found to make the study worthwhile and produce some interesting and important results.

2.7. Inclusion/ Exclusion Criteria of Study Units

There were two arms of the study. The first arm involved a study of smokers only, together with passive smokers associated with them. For this part of the study, all smokers over 18 were included, and passive smokers of any age, including children, were included. The second arm of the study involved examining other cardiovascular risk factors (hypertension, diabetes and

prediabetes, dyslipidemia, high BMI and waist circumference) in Ogolcho, and for this part of the study, smokers aged 18 and over, as well as non-smokers aged 30 and over, were included.

2.8. Variables

2.8.1 Dependent

Active or passive exposure to tobacco and other substance (khat and excess alcohol consumption), TC, HDL-C, LDL-C, TG, fasting blood glucose, carbon monoxide levels in homes, urine cotinine test results, anthropometric parameter such as BP, WC, BMI.

2.8.2. Independent

The socio demographic factors: age, sex, educational background, and occupation.

2.9. Data collection, handling and laboratory methodology

Each subject was interviewed a questionnaire privately to obtain information about socio-demographic factors and lifestyle factors such as smoking, passive smoking and alcohol consumption, khat chewing and other substance use. Blood samples were also collected after an overnight fast (12 to 16 hours) from smokers and non-smokers older than 18 years and for fasting blood glucose and fasting lipid profile analysis.

2.10. One-step Cotinine test

One step cotinine test islateral flow, one-step immunoassay for the qualitative of detection of cotinine, the major metabolite of nicotine in human urine. It has a cut-off concentration of 200ng/mL cotinine, meaning that it is considered accurate for detecting most concentrations of cotinine above 200 ng/mL. This product is used to obtain visual, qualitative results.

2.10.1. Summary and explanation of the test

Tobacco smoking results in the absorption of nicotine through the lung and buccal/nasal epithelium, after which nicotine is metabolized into 20 metabolites that are excreted in urine. Cotinine a major nicotine metabolite, accumulate in the body with regular smoking. It is reported that cotinine is stable in body fluids, has a relative long half-life of approximately 17 hours, and can be detected in a smoker or passive smoker for up to three days after the last episode of inhalation of cigarette smoke. Therefore the detection of cotinine is less dependent on the time of sampling than that of nicotine and other metabolites, which have shorter half-lives. Cotinine has been widely used as a biomarker of recent tobacco exposure.

The one step cotinine test is based on an immunoassay that is used for qualitative detection of cotinine in human urine. It is based on the principle of highly specific immunochemical reaction of antigen (cotinine) with (anti-cotinine) antibodies. It is simple and convenient test for the rapid qualitative detection of cotinine in human urine above its 200ng/mL cut-off concentration. It does not readily detect cotinine concentrations below 200 mg/mL.

2.10.2. Principle of the One Step cotinine test

The One Step cotinine test applies the principle of a competitive immunoassay. The test device contain a membrane strip that is precoated with cotinine antigen at the test line region. The cotinine antibody- colloidal gold conjugate pad is present at the end of the membrane, where the urine sample is applied. In cotinine-free urine, or urine containing less than 200 ng/mL of cotinine, the purple colored antibody-colloidal gold conjugate and urine move chromatographically by capillary action across the membrane. This solution migrates to the test line containing cotinine antigen and forms a visible purple line as the antibody-gold complex

binds to the cotinine antigen at the test line. The formation of a visible purple line in the test zone indicates a negative result (urine cotinine less than 200 ng/mL). When cotinine is present in urine, it competes with cotinine in the test band region for limited antibody-gold colloid binding sites and prevents the anti-cotinine antibody-gold colloid conjugate from binding to the cotinine at the test line. When a sufficient concentration (above 200 ng/ mL) of cotinine is present in the urine, it will prevent attachment of the colored antibody-colloidal gold conjugate at the test line region. Therefore, the *absence* of a coloured (purple) band in the test region indicates a positive test result, that is, urine with a concentration above 200 ng/ mL.

A control line should always appear near the top of the test device, regardless of the cotinine status in the urine. This line occurs in all samples, whether positive or negative, and confirms that the system is working properly. It is based on a different (non-cotinine) antigen, not specified by the company, which is immobilized in the control zone and binds to antibody-colloidal gold conjugate that differs from the anti-cotinine antibody-gold conjugate.

After collection of urine specimen in a clean dry container, two drops of urine sample were dispensed into sample well. The result was read between 3 to 8 minutes after addition of samples (Figure 4).

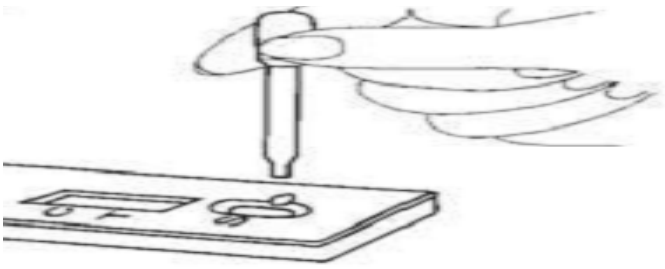

Figure 4. Dispensation of urine sample into one step cotinine test device

2.10.3. Interpretation of result

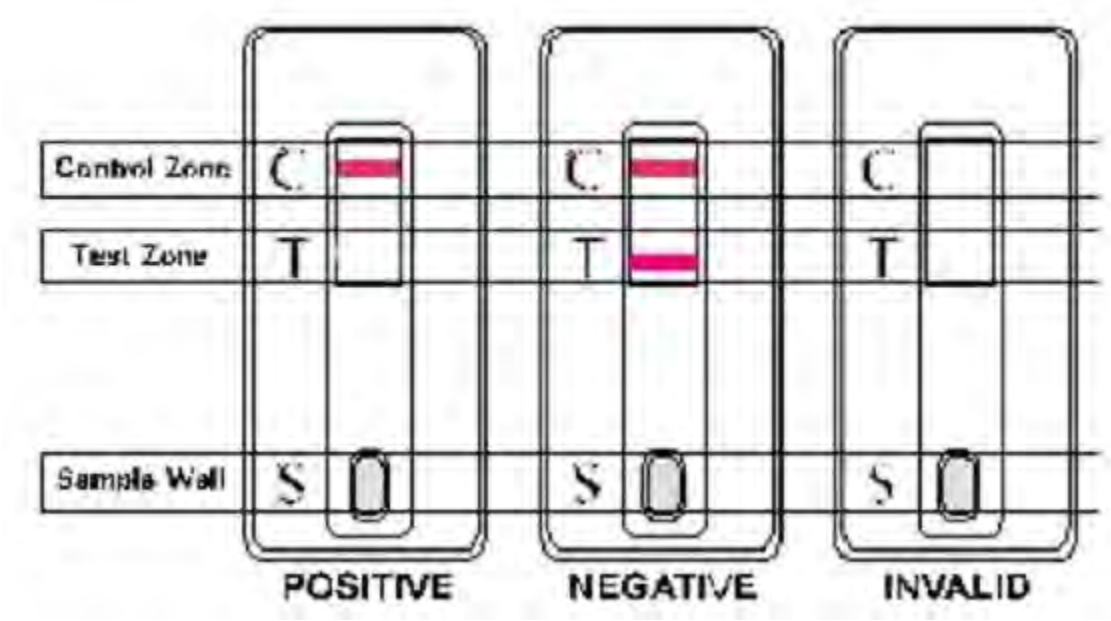

Figure 5: One step urine cotinine test result demonstration

Positive: Test is positive if colored line appears in the Control zone (C) only. No color in Test zone.

Negative: Both the test line (T) and the control line (C) should be colored if the test is negative.

Invalid: The test is invalid either control or test zone is not colored.

2.11. Serum Preparation and Biochemical Analysis

2.11.1 Serum Preparation

For serum preparation, about 10 mL of venous blood were collected from antecubital veins of patients after a fasting period of at least 4 hours. The blood was collected in sterile serum-separating tubes without anticoagulant and left to form a blood clot at room temperature for 90 minutes. It was found that the blood clot settled well into the bottom of the tubes and centrifugation was not needed in order to remove the clear serum (supernatant), which was

transferred to sterile tubes and stored at 4^{o}C for up to 4 days before being transferred to a freezer at -70^{o}C until biochemical tests were done.

Serum sample was analyzed with Roche/Hitachi 912 automated analyzer and the following parameters were measured:

- TG, TC, HDL-C , LDL-C

2.11.2. Fasting blood glucose (FBG) Determination Principle

A Sensocard glucometer, which measures blood glucose levels accurately up to 600 mg/mL and is based on the glucose oxidase method, was used. Glucose reacts with oxygen in the presence of the enzyme, glucose oxidase, which oxidizes glucose to gluconolactone and this enzyme is temporarily reduced by electrons transferred from glucose (2 electrons per glucose molecule). The reduced glucose oxidase enzyme next reacts with an oxidized mediator, transferring electrons to an electrical system that creates electric current. The created current is directly proportional to the concentration of glucose in the sample, which is displayed digitally on the glucometer.

2.11.3. Triglyceride (TG) Determination

The current method for triglyceride determination employs a modified Trinder (Trinder P, 1969) color reaction to yield a fast, linear, endpoint reaction. Triglycerides in the sample are hydrolyzed by lipase to glycerol and fatty acids. The glycerol is then phosphorylated by adenosine-5-triphosphate (ATP) to glycerol-3-phosphate (G-3-P) and adenosine-5-diphosphate (ADP) in a reaction catalyzed by glycerol kinase (GK). G-3-P is then converted to dihydroxyacetone phosphate (DAP) and hydrogen peroxide by glycerophosphate oxidase (GPO).

The hydrogen peroxide then reacts with 4-aminoantipyrine (4-AAP) and 3, 5-dichloro 2 hydroxybenzene (3,5DHBS) in a reaction catalyzed by peroxidase to yield a red colored quinoneimine dye. The intensity of the color produced is directly proportional to the concentration of triglycerides in the sample Siedel J *et al.*, 1993; Tiietz NW, 1995; Shepherd MDS *etal.*, 1990; Trinder P, 1969).

Triglycerides + H_2O → Glycerol + Fatty acids

Glycerol + ATP → Glycerol-3-phosphate + ADP

Glycerol-3-phosphate → DAP + H_2O_2

H_2O_2 + 4AAP + 3, 5 DHBS → Quinoneimine + $2H_2O$

Method: Buffer reagent: PIPES buffer (pH 7.5) (50mmol/1), 4-chlorophenol (5mmol/l), 4-aminophenazone (0.25mmol/l), Magnesium ions (4.5mmol/l), ATP (2mmol/l), Lipases (>=1300U/l), Peroxidase (>=500U/l), Glycerol kinase (>=400U/l), Glycerol-3-phosphate oxidase (>=1500U/L), Sodium azide (0.05%) and 3mL of standard.

Ten microliters of sample were pipetted into a cuvette, containing 1000 µL buffer reagent, mixed and incubated for 10 minutes at room temperature. The absorbance of the sample and standard against the reagent blank was measured at 500 nm within 60 minutes.

2.11.4. Total Cholesterol Determination Principle and Methodology

The method uses Tinder‘s (Trinder P, 1969) color system of peroxidase/ phenol/ 4-aminoantipyrine. The intensity of the red colour produced is directly proportional to the total cholesterol in the sample when read at 500 nm.

$$Cholesterol\ Esters \xrightarrow{Cesterase} Cholesterol + Fatty\ acids$$

$$Cholesterol + O_2 \xrightarrow{C.oxidase} Cholest-4-en-3-one + H_2O_2$$

$$2H_2O_2 + HBA + 4AAP \xrightarrow{peroxidase} Quinoneimine\ (red\ dye) + 4H_2O$$

Enzyme reagent: Phosphate buffer (pH 6.5) (30 mmol/l), 4-Aminophenazone (0.3 mmol/l), Phenol (5 mmol/l), peroxidase (>5KU/l), Cholestrolesterase (>150U/l),Cholesteroloxidase (>100U/l), Sodium azide (0.05%).

Procedure: In a cuvette, 10µL of sample, 1000 µL of buffer reagent were mixed and incubated for 10 minutes at room temperature for 5 minutes. Absorbance of the sample was measured at 500 nm against the reagent blank and standard solutions were used to calculate the concentrations of total cholesterol in the serum samples.

2.11.5. HDL-C Determination Principle and Methodology

The method employs an immunoinhibition reagent method which measures HDL directly. The method is in a two reagent format. The first reagent contains anti-human β-lipoprotein antibody which binds to lipoproteins (LDL, VLDL and chylomicrons) other than HDL. This antibody inhibits the ability of the LDL, VLDL and chylomicrons to react with the enzymes in the system. The second reagent contains enzymes (identical to those in the method for total cholesterol determination), which then selectively react with the cholesterol present in the HDL particles. Consequently, only HDL cholesterol is subject to cholesterol measurement (Tiietz NW, 1995, Linsel-Nitschke P *et al.*, 2005).

2.11.6. LDL-C Determination Principle and Methodology

In the presence Mg^{++}, a sugar compound markedly reduce the enzymatic reaction of cholesterol measurement in VLDL and chylomicron. The combination of sugar compound with detergent enable selective determination of LDL cholesterol in serum.

LDL- cholesterol esters → cholesterol + free fatty acid

Cholesterol esters are then broken down quantitatively into free cholesterol fatty acid by cholesterol esterase.

LDL- cholesterol +O_2→ cholestenone + H_2O_2

In the presence of oxygen cholesterol is oxidized to cholestenone + hydrogen peroxide, H_2O_2

$2H_2O_2$ + 4-Aminoantipyrine +HSDA → Purple blue pigment + $5H_2O_2$ (Abs max = 585nm)

In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-aminoantipyrine and HSDA (sodium N (2-hydroxy- 3 sulphopropyl) - 3, 5 dimethoxyaniline) to form purple blue dye which is detected spectrophotometrically at 500 nm.

2.12. Anthropometric indices

Anthropometric measurement is the science of measuring the human body parts for height, weight, and size of component parts. It is to study and compare the relative proportions under normal and abnormal conditions. Anthropometric measurements are among the most frequently applied methods for assessing metabolic syndromes. Anthropometric indices includes many parameters, but for this study Body Mass Index (BMI), waist circumference (WC) and BP were considered.

2.12.1. Body Mass Index (BMI)

BMI is a standardized estimate of an individual‘s relative body fat calculated from his or her height measured without shoes and weight measured in kg with light clothing.Subjects were weighed on analytical balance and their height measured with a wall-mounted ruler. BMI calculated as weight in kilograms divided by the square of height measurement in meters.On the basis of BMI, individuals were classified as Underweight = <18.5 kg/m2, Normal weight = 18.5-24.9 kg/m 2, Overweight = 25-29.9 kg/m 2 (Getahun D *et al.*, 2007).

2.12.2. Waist Circumference (WC)

WC is the best and simple anthropometric index of abdominal visceral adipose tissue, it is accepted that the measurements of abdominal adipose tissue correlate better with cardiovascular risk factors than BMI. Waist circumference was measured with digital machine midway between the inferior angle of the ribs and the anterior superior iliac crest, which usually corresponds with the level of the superior aspect of the umbilicus (Ashwell M *et al,* 2005).

2.13. Blood Pressure (BP)

BP was taken by using a non-mercury automatic sphygmomanometer (Heuer), calibrated according to WHO standards. Measurements were taken from the left upper arm after subjects would be sitting for >5 min in accordance with the recommendation of the American Heart Association (35). Dual measurements were taken with a 5 min rest interval between measurements and the mean value was recorded.

2.14. Determination of room air carbon monoxide levels

A Smart Sensor AR8700A carbon monoxide meter (Frank Electronics Co Ltd. of Shenzhen City, China) was used to determine carbon monoxide levels in the atmosphere of homes. It detects carbon monoxide levels from 1 to 1000 ppm.

2.15. Calculation of Framingham Risk Scores

Framingham risk scores were calculated using the NIH online site, in which the six parameters of an individual are used to calculate the score: age, sex, HDL cholesterol, total cholesterol, blood pressure and smoking habits. The actual equation differs slightly between women and men. For men, the Framingham risk score is $1 - 0.88936^{(\sum ßX - 26.1931)}$ where ß is the regression coefficient and X is the level for each risk factor. For women the score is $1 - 0.95012^{(\sum ßX - 26.1931)}$

2.16. Data processing and analysis

Collected quantitative data was coded, entered to computer, processed, edited, and analyzed using Microsoft excel and exported to SPSS version 20 statistical software for analysis. During analysis frequencies ofthe different variables were determined, cross- tabulations, odd ratio and chi-square test were used tocompare frequencies.

2.17. Ethical Considerations

Permission to conduct this study was obtained from AAU, ethical Committee of department of Biochemistry. Then a letter of support to local administrator of Ogolcho town about the study were written by department of Biochemistry and permission was obtained from local administration office to access data from study population. All eligible subjects were informed as their participation were voluntary and as the aim of this study was only to collect necessary

information which is helpful to assess prevalence of carbon monoxide exposure, tobacco and other substance use and change of lipid profiles and fasting blood glucose level as consequence of exposure and advance in age. Based on laboratory results and other investigations patients were advised to quit smoking, practice healthy life style in terms of food and exercise. Any patients with hypertension, dyslipidemia or other health problems were referred to a local health centre for further diagnosis and treatment follow-up. All patient information was kept confidential.

Chapter 3

Results

3.1. Socio-demographic Characteristics

As indicated in Table 1, the majority of current study participants were male 75% (70 out of 93), and 56 % of participants were aged 19 to 48 years. Mean age was 40.2 years (SD, 16.4) with 4 minimum ages and 75 and maximum ages. Most of the individuals studied were married (81%) and the remaining were single (17) and divorced (2%). Approximately 48% of participants had primary school education only, 29% had no formal education; the remainder had secondary (14%) and above secondary (9%) schooling. Most participants were farmers (46%) followed by students (25%), housewives (12%), merchants (11%) and government employees (6%).

Table 1: Socio-demographic characteristics of study participants in Ogolcho town, Ethiopia, 2015

Socio-demographic characteristics		number	Percent (%)
Sex	Male	70	75
	Female	23	25
Marital status	Single	16	17
	Married	75	81
	Divorced	2	2
Occupation	Farmer	43	46.2
	Government employee	6	6.5
	Merchant	10	10.8
	Housewife	11	11.8
	Student	23	24.7
Educational status	Illiterate	27	29
	Primary school level	45	48.4
	secondary school level	13	14
	Above secondary school	8	8.6
Age (years)	4-18	9	9.7
	19-33	24	25.8
	34-48	28	30.1
	>49	32	34.4

3.2. Cigarette Smoking Behaviors and Related History of Participants

According to Table 2, in the entire sample of 95 participants, 33% (31) were active smokers, 56.3% (54) passive smokers, 8.6% (8) non- smokers and 2% (2) purely shisha smoker. Among

active smokers 93.5 (29) were male and 6.5% (2) female. Of passive smokers 68.5 % (37) were male and 31.5% (17) female. Of non-smokers 50%, two were male and two were female. Two purely shisha smokers were males aged 22 and 23 years. (Table 2).

Most active 87% (27) and passive smokers 70% (38) used multiple substances, especially alcohol and/or khat. More than 80% of passive and active smokers use khat regulary. In addition to this, use of both khat and alcohol together were common in active smokers (40%) and passive smokers (32%).

Table 2. Cigarette Smoking Behaviors and Related History of Participants in Ogolcho Town, Ethiopia, 2015

Behavioral characteristics and Related History of Participants			**Number**	**Percent (%)**
Smoking status	Active smokers	Male	29	93.5
		Female	2	6.5
		Total	31	100
	Passive smokers	Male	37	68.5
		Female	17	31
		Total	54	100
	Shisha smokers	Male	2	2.1
	Non- smokers	Male	4	4.3
		Female	4	4.3
		Total	8	100
Use of other substances	Active smokers	Yes	27	87.
		No	4	12.9
		Total	31	100
	Passive smokers	Yes	38	70.4
		No	16	29.6
		Total	54	100
Specific substances used by smokers	by Active smokers	Alcohol	13	48
		Khat	25	92
		Alcohol and khat	11	40.7
		Total	27	100
	by passive smokers	Alcohol	18	47
		Khat	33	87
		Alcohol and khat	13	32.4
		Total	38	100

About 90 % active smokers smoked cigarettes, and only 10% of subjects also chewed tobacco leaves. The main reason given for smoking was for relaxation or entertainment (48.4 % of smokers), followed by peer pressure (35.5%), and 16 % of smokers reported that they smoked to relieve stress.

Active smokers responded that they smoked either daily (87%) or weekly (13%) despite their knowledge that cigarette smoking is harmful to their health and the health of others 91%. In this study most subjects had tried to quit smoking (58%). Their most important reasons to try and quit smoking were to be more healthy (50%), to reduce addiction/ other reasons (27.7%) or to spent money more reasonably (22%) (Table 3).

Table 3. Behavioral Characteristics of Active Smokers in Ogolcho Town, Ethiopia, 2015.

Behavioral characteristics of active		**Number**	**Percent (%)**
Types of tobacco used	Cigarette smoking	28	90.3
	smoking and Tobacco chewing	3	9.7
Main reason for using tobacco	Peer pressure	11	35.5
	Relieve stress	5	16.1
	Entertainments	15	48.4
Awareness of harmful effect of smoking	Yes	28	90.7
	No	3	9.3
Frequency of cigarette smoking	Daily	27	87.1
	Weekly	4	12.9
Tried to stop tobacco use	Yes	18	58
	No	13	42
Reasons for quitting tobacco use	Spend money reasonably	4	22.2
	to be healthy	9	50
	To reduce addiction/ others	5	27.7

The earliest age of cigarette smoking initiation (Table 4) was 15 years old, with a mean age for starting smoking of 27.9 years (SD = 11.3) and an age range of initiation 15 to 57. On average, each smoker smokes 10.6 cigarettes per day (SD = 7.3). A minimum one cigarette and a maximum thirty of 30 cigarettes were smoked per day. The mean duration of smoking was 19.8 years (SD=14.7) with a range of 1 to 50 years. On average the number of pack-years (number of packs of 20 cigarettes per day multiplied by the number of years smoked) for subjects in Ogolcho town were 12.7 (SD=13.9) with a range of 0.15 to 52.

Table 4. Tobacco use by active smoker in Ogolcho town, Ethiopia, 2015.

	Mean ±SD	**Median (Range)**
Cigarette smoked per day	10.6 ± 7.3	10 (1 to 30)
Total years tobacco used	19.8±14.7	20 (1 to 50)
number of pack years	12.7±13.9	10 (0.15 to 52)
age of starting smoking	27.9±11.3	24 (15 to 57)

Table 5. Behavioral characteristics of passive smokers in Ogolcho town, Ethiopia, 2015.

Behavioral characteristics		**Number**	**Percent (%)**
Places of cigarettes smoke exposure	At home only	22	40.7
	At work only	10	18.5
	Recreation centers	6	11.1
	Both home and at work	16	29.6
Duration of exposure	Less than one hour	5	9.3
	One to five hours	34	63.0
	>5 hours	15	27.8
Awareness of harmful effect of passive smoking	Yes	49	90.7
	No	5	9.3

Most passive smokers were exposed to cigarette smoke from their family, friends or relatives at home only (n=22) at work only (n=10), at both work and home (n=16), or in recreational places (n=6) such as a local drinking house, khat chewing house, or trading centers.

3.3. Urine Cotinine Test Results

The One Step urine cotinine test shows no false positive results in tests done by the supply company on hundreds of individuals who were not active or significant passive smokers; largely this is due to the assay being an immunospecific assay. However, false negative results (known smokers or passive smokers who show a negative cotinine test result) are frequent, because the test is not sensitive for urine cotinine levels below 200 ng/mL, and some smokers and passive smokers, especially those with relatively low exposure, have levels of cotinine below 200 ng/mL. Out of total 85 passive and active smokers (Table 6), 67 % were positive for urine cotinine using the One Step cotinine test. Almost all active smokers (90%) had a positive urine cotinine test using this kit, but for passive smoker around 54% individuals resulted in a positive urine cotinine test. Active smokers‘ urine cotinine tests were 1.67 times more likely to give a positive urine cotinine result as compared with passive smokers. Two purely shisha smoker were tested for urine cotinine test and one of them gave a positive result. This shisha smoker with a positive urine cotinine test used shisha 3-4 times a week, but the other shisha smoker with a negative test result used shisha only once per week. Both shisha smokers used alcohol and khat.

Table 6. One step urine cotinine test results in active, passive and shisha smokers in Ogolcho town, Ethiopia, 2015.

Smoking status	**Result**	**Number**	**Percent**
Active smokers	Positive	28	90.3
	Negative	3	9.7
	Total	31	100
Passive smokers	Positives	29	53.7
	Negative	25	46.3
	Total	54	100
Shisha[1] smoker only (not exposure cigarettes smokes	Positive	1	50%
	Negative	1	50%
	Total	2	100

[1]Shisha is tobacco that is smoked, of ten mixed with herbs and spices, using a waterpipe, in which the smoke is percolated through water before being inhaled, and so contains nicotine.

Table 7. Urine Cotinine Test Results with Respect to Dose of Cigarettes, Duration of Exposure, Age and Sex of Participants, in Ogolcho Town, Ethiopia, 2015.

Dosage of cigarette smoked and urine cotinine test results		Number (%)	Cotinine test results Frequency (%)		
			Positive	Negative	p -value
	Light	6 (20 %)	3 (10 %)	3 (10 %)	P< 0.05
	Moderate	14 (48.4 %)	14 (48.3 %)	0 (0 %)	
	Heavy	9 (31 %)	9 (31 %)	00 (0 %)	
	Total	29 (100 %)	26 (90 %)	3 (10 %)	
Duration of passive smoker exposed to passive smoke	Short[1]	6 (11.2 %)	5 (9.3 %)	1(1.9%)	p>0.05
	Medium[2]	36 (66.6 %)	18 (33.3 %)	18(33.3%)	
	Long[3]	12 (22.2 %)	6 (11.1 %)	6(11.1%)	
	Total	54 (100 %)	29 (53.7 %)	25 (46.3%)	
Cotinine result of passive smokers by age	Children	9 (16.7 %)	2 (22.2 %)	7 (77.8 %)	P<.005
	Adults	45 (83 %)	27 (60%)	18 (40%)	
Total		54 (100 %)	29 (53.7 %)	25 (46.3 %)	
Passive smoker cotinine results by sex	Male	37 (68.5 %)	25 (67.6 %)	12 (32.4 %)	P<.005
	Female	17 (31.55 %)	4 (23.5 %)	13 (76.5%)	
Total		54 (100 %)	29 (53.7 %)	25 (46.3 %)	

[1,2,3] Light cigarette exposure was considered, based on the American Medical Association,to be less than 5 cigarettes per day, moderate is 5-15 cigarettes per day and high exposure is more than 15 cigarettes per day. For passive smokers, exposure for less than one hour per day was considered as short duration exposure, 1 to 5 hours a day was medium exposure, and more than 5 hours exposure per day was long exposure.

From Table 7, overall 20 % of light, 48.4% of moderate and 31 % of heavy smokers were tested for one step urine cotinine. All (100%) heavy and moderate active smokers were positive for urine cotinine, whereas only 50% of light smokers gave positive urine cotinine test results.

Passive smoker participants were classified based on their daily duration of exposure to cigarette smoke. The result showed that 63% of passive smokers were exposed to cigarette smoke for one to five hours daily, 28% for more than five hours daily and 9% for less than one hour per day. 60% of passive smokers who were exposed to cigarette smoke for more than five hours daily were positive for urine cotinine, 56% of passive smokers exposed for between one and five hours daily had positive urine cotinine results, but only 20% of passive smokers exposed to cigarette smoke for less than one hour daily were positive for urine cotinine.

Among 54 passive smokers, most of them were adults (83.3%). About 22% (2 out of 9) of passive smoking children had a positive urine cotinine results but about 60% of adult passive smokers were positive for urine cotinine.

Most of urine cotinine test result for passive male smokers were positive (25 out of 37) but only small number of urine cotinine test result for passive female smokers were positive (4 out of 13). This may be due to small number of female participants studied, or as a result of female exposure to passive smoking being mostly at home from their husband or family, unlike male passive smokers, who are exposed to ETS at home, work and recreational place. This would increase the chance of a positive urine cotinine test result.

3.4. Framingham risk scores and effect of smoking

Framingham risk score is measurement of patient‘s risk of cardiovascular disease (CVD) or risk of having heart attack disease within ten years. Among the risks of developing CVD, cigarette

smoking is the most prominent factor, with age, LDL, diabetes, TG, sex, lack of physical exercise, poor diet, family history of heart attack, total cholesterol, HDL and systolic blood pressure being other risk factors. Framingham risk scores are calculated using six risk factors (HDL, total cholesterol, age, sex, systolic blood pressure and smoking status).

From Table 8: it is clear that smoker 11A and 29T had 21% and 20% Framingham risk score as smokers, but if they quit smoking their risk of developing CVD would reduce to 15% and 12%, respectively or from high risk of developing CVD to moderate risk. Furthermore patient 3G and 66T had 12 % and 16% Framingham risk score as smoker which put them at moderate risk of developing CVD, but if they quit smoking their risk of developing CVD would reduce to 5% and 7%, respectively which change the risk from moderate to low risk of developing CVD.

Although LDL (the bad cholesterol) is not included in Framingham risk score calculation, increased LDL is big risk factor for development of CVD: but in this study there was no difference between smokers and non-smoker mean LDL levels.

Table 8. Framingham cardiovascular risk factors, Framingham risk scores, effect of a smoking status on Framingham score, and serum LDL levels in some study subjects.

Codes refer to participant's identity, designed on a confidentiality coding system.

Code	**Age**	**Sex**	**SPB**	**TC**	**HDL**	**On BP pills**	**LDL**	**Framingham risk score as smoker**	**Framingham risk score if quit smoking**
3G	50	M	148	155	33	No	96	12	5
4K	34	M	119	155	46	No	60	1	<1
6I	30	m	122	172	44	NO	120	1	<1
7B	45	M	116	183	49	NO	111	6	2
8M	30	M	134	167	35	NO	89	1	<1
11A	61	M	178	209	50	YES	126	21	15
12J	42	M	140	160	31	NO	104	7	2
15J	67	M	126	131	54	NO	72	9	4
26M	30	M	128	139	43	N0	77	1	<1
29T	58	M	190	170	42	YES	99	20	12
31A	42	M	138	168	59	NO	90	4	1
32J	60	M	154	202	54	N0	114	16	7
50K	60	F	123	139	21	NO	85	6	3
60C	50	F	123	133	32	NO	75	2	1
61K	55	M	135	144	32	NO	77	12	6
66T	57	M	128	204	53	NO	124	16	7

Patients with less than 10% Framingham risk score are considered as at low risk to develop CVD within ten years, but patients with 10%-20% Framingham risk score are at intermediate and more than 20% at high risk of developing CVD within ten years.

3.5. Prevalence of Other Cardiovascular Risk Factors

Table 9: Comparison of measured parameters of non- smokers with active and passive smokers in Ogolcho town, Ethiopia, 2015.

Measured parameters	Active smoker	Non smokers	Passive smokers
	Mean± SD with range	Mean± SD with range	Mean± SD with range
BMI (kg/m2)	19.9 ± 3.4 (14.7 to 28)	23 ±3.9 (18 to 28.2)	21.9 ± 3.4 (16.5 to 28.8)
SBP (mmHg)	133.5 ± 20 (96t o190)	118 ±12 (98 to 133	133.8 ± 25.2 (96 to 205)
DBP	81.6 ± 14 (32 to 110)	77.6 ± 8.5 (66 to 90)	79 ± 11 (60 to 107)
Waist circumstance (cm)	81 ± 10 (64 to 13)	90 ± 10.5 (72 to 106)	82.9 ±9.8 (66 to 102)
blood sugar (mg/dl	102.8 ± 16 (73 to 149)	96 ± 9.4 (84 to 108)	98.1± 14.9 (72 to 136)
TC(mg/dl)	157 ± 29 (105 to 209)	160 ± 32.7 (119 to 214)	154.7± 28 (112 to 215)
TG(mg/dl	195 ± 102 (55 to 377)	177 ± 72 (63 to 260)	139.5 ± 30 (88 to 286)
LDL(mg/dl)	87.5 ± 23 (45 to 126)	91.5 ± 21.3 (68 to 135)	88.2 ± 25 (54 to 150)
HDL(mg/dl)	34.8 ± 14.4 (7 to 59)	44 ± 18 (29 to 85)	42.2± 15 (18 to 81)

Insufficient number of subjects within each group such as active smokers, passive smokers and non-smokers were studied for comparison between the groups to be made statically. Therefore p-value were not determined.

Table 9 shows various cardiovascular risk factors other than tobacco use in the population studied. The results revealed that active smokers had TG levels with a mean of 195 ± 102 and a range of 55 mg/dl to 377 mg/dl. TC in active smokers had a mean of 157.2 ± 29, range 105 to 209; LDL cholesterol with a mean of 87.5 ± 23.05 and ranging from 45 mg/dl to 126 mg/dl; HDL Cholesterol with mean of 34.83 ± 14.4.

Non-smokers had a mean TG of 177 ± 72 (range 63 to 260 mg/dL); TC mean of 160 ± 32.7 (range 119 to 214 mg/dL); mean LDL of 91.5 ± 21.3 (range 68 to 135); a mean HDL of 44 ± 18 (range 29 to 85 mg/dL).

Passive smokers had a mean TG of 154.7 ± 28 (range 112 to 215); mean TC 154.7 ± 28 (range 112 to 215); mean LDL of 88.2 ± 25 mg/dL (range 54 to 150); mean HDL of 42.2 ± 15 mg/dL (range 18 to 81).

Mean of waist circumstance of both active and passive smoker were decreased by 27.7 as compared to non-smoker and tobacco use or exposure were negatively associated with abdominal obesity.

There was no significance difference in serum glucose level of active, passive and non-smokers.Approximately 69% of passive smokers had normal body weight, 17 % overweight and 14%. Mean BMI of passive smoker was 21. 9, but mean of BMI for non-smoker was 22.9 with 50% normal body weight, 12.5 underweight and the remaining 37.5% were overweight.

Table 10. Prevalence of Measured Preventive Parameters among participants in Ogolcho town; 2015, Ethiopia.

Parameters		Results	Active smoker	Non smokers	Passive smokers
			Number (%)	Number (%)	Number (%)
BMI (kg/m2)		Underweight	15 (48.4%)	1 (12.5%)	5 (17.2%)
		Overweigh	1 (3.2%)	3 (37.5%)	7 (24%)
BP		Normal	18 (58.1%)	7 (87.5%)	13 (44.8%)
		Hypertensive	13 (41.9%)	1 (12.5%)	16 (55.2%)
	Male	Normal	21(67.7)	2 (25%)	18 (62%)
		Elevated	8 (25.8%)	2 (25%)	3 (10.3)
WC	Female	Normal	2 (6.5%)	2 (25%)	2 (6%)
		Elevated	0 (0%)	2 (25%)	6 (20%)
Blood Sugar		Normal	30 (96.8%)	8 (100%)	29 (100%)
		High	1(3.3%)	0 (0%)	0 (0%)
TG		Normal	14 (45.2%)	3 (37.5%)	18 (62.1%)
		High	17 (54.8%)	5 (62.5%)	11 (37.9%)
TC		Normal	31 (100%	5 (62.5%)	29 (100%)
		High	0 (0%)	3 (37.5%)	0 (0%)
L D L		Normal	31 (100%)	8 (100%)	29 (100%)
		High	0 (0%)	0 (0%)	0 (0%)
HDL		Normal	16 (51.6%)	6 (75%)	22 (75.9%)
		Decreased	15 (48.4%)	2 (25%)	7 (24.1%)

Subjects with BMI of less than 18.5 kg/m2 were considered underweight, 18.5 to 24.9, normal weight, overweight 25 to 29.9 kg/m2, obese >30 kg/m2. Blood pressure less than 140/85 mmHg is normal, greater than 140/85 mmHg elevated. Blood sugar greater than 110 mg/dL was considered elevated, over 126 mg/dL diabetes. Waist circumference greater than 90cm is high for men and greater than 80 cm for women. TC greater than 200 mg/dL elevated, less than 200 mg/dL normal. TG greater than 150mg/dL high, less than 150 mg/dL normal; LDL greater than

130 mg/dL elevated. HDL lower than 40 mg/dL is abnormal for a man and less than 50 mg/dL abnormal for a woman.

As shown in Table 10: active smokers had a 25.8% prevalence of abdominal obesity, 48.4% underweight and 3.2% overweight. 54.8% of active smokers had elevated triglycerides, 42% were hypertensive, 48.4% decreased HDL and 3.2% increased fasting glucose. Only 10.3% of passive smokers had abdominal obesity, 17% underweight, and 24% overweight. Among passive smokers 55% were hypertensive, 38 % had elevated triglyceride and 24% low HDL. Prevalence of cardiovascular risk factors among non-smokers were: 12.5% elevated blood pressure, 50% abdominal obesity, 12.5% overweight and 37.5% overweight. Non-smoker dyslipidemia prevalence was: elevated total cholesterol in 37.5%, decreased HDL in 25% of non-smokers.

3.6. Carbon Monoxide Studies in Homes

Carbon monoxide levels were studied initially in several homes and were found to be less than 1 ppm in the general atomosphere even following a coffee ceremony, where the coffee was prepared on a charcoal stove inside the room. Because most homes, despite having small rooms, were well ventilated and stoves were either outside or close to an open door, and because it was difficult within the time constraints to visit multiple homes (study individuals were examined in a room provided by a clinic in Ogolcho), further homes were not tested.

However, measurement of carbon monoxide levels closer to the charcoal stove, while it was lit, showed that these were higher than levels in the rest of the room. Measurements taken at a distance of more than to 0.5 meters (50 cm) away from the stove were safe (less than 2 ppm carbon monoxide), whereas as the distance came closer to the glowing charcoal, the carbon monoxide level rose rapidly, as in Table 11.

Table 11. Indoor carbon monoxide concentrations (ppm) in the atomosphere at varying distances from a burning charcoal stove.

Distance from burning charcoal	Carbon monoxide concentration (ppm)
5 cm	>1000
10 cm	201
20 cm	57
30 cm	23
50 cm	2
100 cm	0
200 cm	0

Chapter 4.

Discussion

4.1. Passive and Active Cigarette Smoking Practices and Urine Cotinine Test

Among various biochemical markers used to detect tobacco metabolites in both active and passive smokers urine cotinine is considered as a good marker. This because it is stable in body fluids, with relative long half-life of approximately 17 hours.Cotinine can be detected in a smoker or passive smoker for up to three days after the last episode of inhalation of cigarette smoke. In the present study an attempt was made to screen urinary cotinine in active and passive smokers by using the One Step cotinine test kit and to determine the prevalence of other cardiovascular risk factors.

In this study the majority of active smokers studied were male (93.5%) and most (68.5 %) passive smokers were male. Few female subjects were active smokers; this may be due to socio-cultural and religious influence, which restrict female involvement in such activities. Among passive smokers, children were victims; two children aged 4 and 5 years old were positive for one step urine cotinine test.This finding is consistent with 2008 WHO report which indicated a low number of female active smokers (0.9%) in Ethiopia (WHO, 2008) but a higher number of female passive smokers (31.5%). Thus, the relatively high prevalence of female passive smokers could be explained by their frequent exposure to second hand smoke of their husbands, sons or other family members since all smokers usually smoke in their home and in other public places daily.

In the current study, the minimum age of smoking initiation was 15 years old and the mean age of smoking initiation was 27.9.The most common reason for smoking initiation was to

experiment with smoking (–~~tr~~y it"). This finding differs somewhat from the study by Derje*et al*., (2014),which reported an age interval of 14 to 15 years age at which majority of current active adult smokers started cigarette smoking.

More than half of current study subjects (58%) tried to stop tobacco use, mainly due to concerns about ill health effects of tobacco use, economic challenge and rid of bad smell and addiction. Thisfindingisinagreement with a study by Girma *et al*., (2010) which reported 57% of smokers in the study area (Dire Dawa) had the intention to quit cigarette smoking but the current study finding is low compared to a study byAyalu A *et al*, (2013) in Kersa, Oromia regional state, which showed 68% of the smokers expressed an interest to quit smoking.

In this study, a majority of respondents (90%) had good knowledge about adverse tobacco health effects. This finding is comparable with previous study conducted in Jimma among school adolescents, whichshowed almost all of the surveyed adolescents(94.6%) were aware that cigarette smoking is harmful to health Dereje *et al*(2014). Despite their knowledge, most patients reported that they were challenged to quit smoking. Many smokers participating in this study were seeking means to quit smoking, including requesting any medications that could help them to quit smoking. Some smokers even dipped their cigarettes in petrol (gasoline) to discourage them from smoking: this made them feel sick and nauseous. However, after a few days they reverted to smoking. This demonstrates how addictive smoking is to these individuals and how desperate they are to quit smoking. Nicotine addiction leads to the unfortunate situation where an otherwise rational, motivated, knowledgeable person who understands the risks of tobacco, continues to use it, and Ethiopians are no exception to this rule (Hymowitz N et al, 1997). Unfortunately, smoking cessation medications, including nicotine replacement medications, are not readily available in Ethiopia. We provided intensive health education

regarding ill health consequence of cigarettes smoking and most of the smokers were showed willingness to quit smoking.

A total of 85 urine samples were analyzed for cotinine: 54 from passive smokers and 31 from active smokers. Nearly 90% (28 out 31) of active smoker were positive for urine cotinine; all active smokers with a positive urine cotinine test were moderate or heavy smokers, whereas the only three active smokers with negative cotinine test results were light smoker. This finding showed cotinine urine positive test results were significantly associated with the number of cigarettes individuals smoked (OR = 3.38; P = 0.01).

In the group of passive smokers, nine were children and two of the children were positive for the urine cotinine test; but the majority of adult passive smokers were positive. The possible justification for increased positive urine cotinine test results in adult passive smokers, compared with that of children, could be due to adults‘ exposure to cigarette smoke at more than one different place, such as recreation or work places in addition to home which increase the dose of tobacco smoke exposure. Another possibility is that children and adults metabolize nicotine at different rates, resulting in differences in their urine cotinine levels. The results of a cross-sectional study in passive smokers among school children of Serbian population were consistent with current study finding. This study showed 20% urine cotinine positive results in children, compared with 22% in the study of this thesis. It is also established that cotinine-positive results are often higher in persons with stronger exposure to tobacco smoke (Stošić L *et al*, 2006).

In this study most passive smokers (41%) exposed to environmental tobacco smoke at home, where children and females spend the majority of their time, especially children, are susceptible to different tobacco induced diseases due to their relatively early development (lungs, for

example, are not fully developed until a child reaches 8 or 9 years old) or immature immune system. Workplace took the second rank (18%) and 11% account for different recreational centers like local arake and tella sale home khat chewing and shisha house. But according to a study conducted in Israel, in contrast to the current study finding, ranked workplace the main place of environmental tobacco smoke exposure (33.6%) followed by home (26%) (Goldsmith S *et al*, 2013). This difference in place of exposure to environmental tobacco smoke exposure could be due to sociocultural difference between two study populations.

Three out of six light smokers were positive for cotinine; but all others (moderate and heavy smokers) were positive for urine cotinine. Though a small number of patients were covered here, it does suggest that light smokers may be less prone to having a positive urine cotinine result using the One Cot test kit, which has a relatively low cut-off concentration (< 200 ng/ mL) (Zevin S *et al,* 1997).

In this study less than one fourth (23 %) of female passive smokers were positive for urine cotinine test, but more than half of male passive smokers (67.6 %) were positive for the test. This difference could be justified in terms of place of exposure difference between the two sexes in study area (Ogolcho town), or poor statistical power of the study. As already mentioned, children and women are exposed to cigarette smoke at home since they spent most of their time at home. Male passive smokers, however, are exposed to environmental cigarette smoke at different places due to their daily activities (at work place or local recreational centers) in Ogolcho. This would increase dose of smoke exposure and increase urine cotinine levels in these males.

Optimal urine cotinine levels that discriminate between smokers and non-passive non-smokers are less than 200 ng/mL, which was the level detectable with the One Step cotinine kit used in this study. This means that both active and passive smokers with urine cotinine concentrations

less than 200 ng/mL were missed by this study. Therefore, passive smokers with significant levels of cotinine above those seen in non-smokers who are not exposed to passive smoking are likely to be even more common than was found in this study. Kim S and Jung A., in a study of South Korean smokers and non-smokers, found that a urine cotinine concentration of 164 ng/mL was a valid cutoff concentration that discriminated between smokers and non-smokers (Kim S and Jung A, 2013).

A study conducted by Benowitz NL *et al*, (2009) in San Francisco, California studied cotinine levels in passive and active smokers.Of people who denied smoking, 32% were found to have had significant exposure to the smoke exhaled by smokers. The authors concluded that cotinine levels provide objective evidence of tobacco smoke exposure, resulting in more intensive intervention to encourage subjects to stop smoking and avoid Second hand exposure. However in present study in Ogolcho, the cutoff cotinine concentrations value used were high (<200ng/ml) as compared with Benowitz NL *et al,* (2009) (cotinine >14 ng/mL). Nevertheless cotinine is the best metabolite to asses cigarette smoke exposure and to encourage possible interventions.

Another study conducted in Israeli in 2013 indicated widespread second hand tobacco exposure in the nonsmoking Israeli adult population, especially among males, and younger and less educated participants, by using urine cotinine testing. The current study is in agreement with this finding which revealed high prevalence of smoking at home and public places which expose non-smokers, especially children and females, to second hand smokes.

Behera D *et al* (2003) reported high nicotine and cotinine excretion in urine in smokers as compared to non-smokers and they concluded that active smokers have high levels of cotinine in their urine. The current study in Ogolcho is also in agreement with this report, with more than 90% of positive urine cotinine test in active smokers.

Another study conducted by Thompson SG *et al.*, (2014) in India reported a linear relation between urinary cotinine concentrations and habitual consumption of cigarettes. In this Ogolcho study, the number of cigarettes smoked per day for active smokers, and duration of exposure to second hand smoke for passive smokers, also revealed positive association between urine cotinine positive test result and degree of passive or active smoking.

4.2. Cardiovascular Risk Factors Among Study Participants

In this study, cardiovascular risk factors in addition to smoking were fairly common, indicating that there are undiagnosed patients at risk for uncontrolled diabetes, obesity and hypertension, though comparisons between smokers, nonsmokers and passive smokers are not particularly powerful statistically due to the relatively small numbers of subjects in each group. Nevertheless, screening of individuals for cardiovascular risk factors would likely be beneficial in Ethiopia to reduce the burden of NCDs.

Study conducted by Elhashimi *et al.*(2013) showed elevated total cholesterol, triglyceride and low density lipoprotein decreased serum high density lipoprotein level in smokers as compared to non-smokers. Similar results were found in smokers group who smoked > 15 cigarettes per day compared with those who smoked less. With increased duration time of smoking the TC and LDL-C were increased, TG showed no change, while the HDL-C was decreased, showing greater risk of these persons to atherosclerosis and coronary heart disease. This study finding is similar with current study of this project.

Another study in Ramadi Municipality revealed that total cholesterol(TC), low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) were significantly higher in smokers as compared with non-smokers, while low-density lipoprotein cholesterol in smoking group

was significantly lower than in non-smoker group (p=0.04). Total cholesterol, low-density lipoprotein cholesterol were statistically affected by number of cigarettes smoked per day. Although a comparison between smokers and non- smokers were limited due to small sample size in this current study, the overall finding is similar with Dullah Kh *et al.,* (2009).

Furthermore a study conducted by Yohannes at the Gondar College of Medical Sciences showed a 10.4% prevalence of total cholesterol, 14% high blood pressure, 1.6% overweight (Yohannes G, 1998) in subjects tested, including both smokers and non-smokers. As compared to Yohannes results, the result of current study show a higher prevalence of total cholesterol (37.5%), high blood pressure (12.5%) and overweight (3.2%). The difference between the two studies may be due to lifestyle difference among the study subjects.

Moreover, a study conducted by Alemseged *et al.* (2012) revealed a 0.5% prevalence for diabetes mellitus (DM) and 9.3 %% for hypertension. Our findings indicated higher prevalence of elevated blood glucose levels (3.1%) and high blood pressure (12.5%). However, large scale population-based studies are needed to document the current magnitude of diabetes in Ethiopia.

Another study in Gondar, Northwest Ethiopia in 2012 reported a 28.3% prevalence of hypertension of whom more than a third (37.0%) did not know they had hypertension; which agrees with other studies showing the occurrence of a ―silent epidemic" of high blood pressure in developing countries, including in Ethiopia. In the current study of this MSc project almost all subjects did not know they had hypertensionand lacked knowledge about the disease and so did not seek treatment at the local health care facilities. In addition to this, the Status Report on Hypertension in Africa Consultative Review for the 6th Session of the African Union Conference of Ministers of Health on NCDs reported comparable results on prevalence of hypertension in Ethiopia.

A study by Lemba *et al*., (2012) in Addis Ababa city reported a 22% prevalence of hypertension; 15% of hypertensive participants never had had their blood pressure checked prior to the study. Contrary to this, in the current study in Ogolcho, more than 90% of participants, including individuals diagnosed with elevated blood pressure in this study, never had their blood pressure checked previously. A study conducted by Bonsa F *et al*, (2014) in Bedele town showed poor follow-up blood pressure measurements among diagnosed hypertensive subjects. These results indicate that there is an urgent need for screening programmes for hypertension as well as better treatment and follow-up of known hypertensive patients.

A study conducted by Tesfaye *et al*., (2009) in Addis Ababa city also reported a high prevalence of hypertension (31.5%) and 38% overweight. This agrees with the prevalence of overweight among adults in Ogolcho that was seen in this study (24%). Urbanization influences are apparent in the city, with an increasing use of motorized transport and sedentary types of occupation such as trade and office work. This is accompanied by shifting dietary and lifestyle behaviours, which contribute to weight gain, diabetes and hypertension and associated increased cardiovascular risks.

Studies of khat chewers among Ethiopian diaspora in the UK showed that khat can also act as a gateway drug for tobacco use, which is very concerning considering the increase in khat users in Ethiopia (Kassim S et al, 2014), and the high prevalence of khat users (over 80% of adult active and passive smokers) in individuals tested in this study.

4.3. Indoor Carbon Monoxide Levels

Carbon monoxide levels in several homes tested initially safe (less than 2 ppm), and because of time constraints, further homes were not tested. The test individuals were seen in room kindly

provided by a local clinic in Ogolcho, so blood draws and measurements were not done in people‘s homes but in this clinic room. In any case, homes were generally well ventilated insofar as charcoal stoves were either outside the home or close to an open door. However, measurement of carbon monoxide levels in the air showed a very high concentration (over 50 ppm) at a distance close to the burning charcoal stoves, but at a distance beyond 50 cm, levels were safe (less than 2 ppm). Therefore, those at significant risk for carbon monoxide poisoning would mainly be the people (for example, maids and other women in the households) who are sitting close to the stoves and even then they would have to breathe in air at a distance of less than 50 cm from the burning charcoal.However in one study of ten homes in Addis Ababa during coffee ceremony carbon monoxide exposure were exceeded WHO guidelines for maximal recommended in most of the homes (Keil et al, 2010).

5. Limitations

The present study has numerous limitations that should be considered or addressed, including:

1) The study was cross-sectional and therefore shows no definitive cause-and-effect relationships between parameters. Also the size of the population of individuals studied was small.

2) The study was based on self-reported responses of a questionnaire, and thus may be subject to recall bias and underreporting of tobacco use due to social desirability bias.

3) The cotinine test kit used had a cut-off concentration of 200 mg/mL in urine and this will fail to detect some smokers and passive smokers who have nevertheless elevated cotinine in their urine, because 164 mg/mL is considered by some researchers to be the proper cut-off limit between active/ passive smokers and non-smokers.

4) Comparisons of cardiovascular risk factor statitistics between smokers and non-smokers is limited because of the small sample sizes.

5) The study was carried out in a poor rural town where smoking and khat use are particularly common and may not reflect the situation in other towns or at the national level.

6. Conclusions

In conclusion, this cross-sectional analysis indicates that active as well as passive cigarette smoking, alcohol and khat consumption are highly prevalent in the rural town of Ogolcho. Passive smoking is common in homes and children are exposed to the cigarette smoke of their

fathers in particular, with levels of urine cotinine over 200 ng/mL, which is similar to levels found in smoking adults. Cardiovascular risk factors, including high blood pressure, dyslipidemia (especially low HDL), elevated blood glucose, elevated BMI and waist circumference are not uncommon in this population.

7. Recommendations

Smokers should be counseled that there is a health risk at any level of SHS exposure, both to themselves and to those around them, including their children, and public education programmes should be instigated to educate non-smokers and smokers about the health risks of tobacco use and tobacco smoke exposure. Moreover, adolescents in particular, should be enriched with the knowledge on the dangers of tobacco use.

The Ethiopian government ratified the WHO Framework Convention on Tobacco Control in 2014, and this involves enforcing certain tobacco laws banning tobacco advertising, sales of single cigarettes, sales to minors, smoking in public places, increased cigarette taxes, and enforcing tobacco companies to place health warning signs on cigarette packs. Therefore responsible bodies should enforced these laws to protect large population from second hand exposure and decrease the prevalence of active smoking prevalence with other related disease.

Further studies of active and passive smoking using a larger number of subjects is needed to delineate further the prevalence of cigarette smoking and other substance use in Ethiopia and the co-use of multiple substances such as tobacco, khat and alcohol.

Equipment is available to measure urine cotinine levels more precisely at any level, even less than 200 mg/dL (the cut-off value for the test kit used here). Therefore by using more sensitive

equipment to measure urine cotinine levels quantitatively is recommended to provide more accurate information about passive smoking.

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Annexes

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTEMENT OF BIOCHEMISTRY

Annex-I:

Consent form

Purpose

Chronic non communicable disease (CNCD) become is the main problem of developing countries in the world, like Ethiopia despite lack of sufficient information. The most important cause of (CNCD) in Ethiopia are life style, different habits like tobacco use, alcohol consumption and other substance use. The objective of this study was to assess, as a pilot study, prevalence ofcarbon monoxide exposure, tobacco use and passive smoking, other substance use, fasting

blood glucose, lipid profiles, blood pressure and other parameters of metabolic syndrome of a community in Ogolcho town Ethiopia.

Participation

Without your participation and voluntarism the feasibility of this research are under question, so we asking you and all other to voluntary participant in this study. What we expect from you is your willingness to give blood to be for examined metabolic syndromes status. The examination involves laboratory procedure with collection of 40µl blood from fingertip. Sample will be collected using sterile and disposable needles and test tubes.

Risks

Taking 4 ml of blood does not have any harm to your health but minor needle pain may last for seconds. If there comes any discomfort, we shall offer you necessary medical treatment freely.

Benefits

If we find any negative result possible medical care and treatments will be provided for participant by referring to local health service. Health education will be provided regarding the harmful effect of tobacco both on active and passive smokers, healthy diet and benefit of physical exercise to control chronic-non communicable disease.

Confidentiality

Any information collected from you will be kept confidential. Your identity will not be disclosed in any situation and study results will be present by using different code number instead of your name.

Sharing the Result

After analysis of the data, we will present the result of the study to the responsible bodies. The report will not bear any information about you; because we use code to disseminate the results to concerned bodies and for the purpose of publication.

Right to Refuse

Since your participation in this study is entirely depend your voluntarism you have right to refuse to accept this request.

Contact address

If you have any question or concern, you can contact Gobena Dedefo at any time using the following address:

Gobena Dedefo Addis Ababa University, Faculty of Medicine, Department of Medical Biochemistry:

Tel: +125913983634

Email: gobddefo@yahoo.com

Addis Ababa

Annex II

Consent Form

I, the under signed, confirm that, as I give consent to participate in the study, it is with a clear understanding of the objectives and conditions of the study and with recognition of my right to withdraw from the study if I change my mind.

I……. do hereby give consent to Dr. /Mr. /Mrs. /Miss……..to include me in the proposed research. I have been given the necessary information about the research. I have also been assured that I can withdraw my consent at any time without penalty or loss of benefits. The proposal is explained to me in the appropriate language I understand.

Unique number of the Participant ……..

Signature of the Participant ……

Name of the Investigator……

Investigators Signature…….

Date: ……..

Annex-III

Assurance of the Principal Investigator

I, who undersigned, agree to accept responsibility for the scientific ethical and technical conduct of the research project and for the provision of required progress reports as per terms and conditions of the research publications office in effect at the time of grant is forwarded as the result of this publication

Name of the student: _____

Date …… Signature…….

Assurance of Advisors

Name of the Advisors

1. Frank Ashall, (B.A. (Oxon), M.D., D.Phil).

Date ……Signature……

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTEMENT OF BIOCHEMISTR

Annex-IV:

Questionnaire:

Questionnaire **CODE: _____**

1. Socio-demographic factors

1. Name: _____ Age_____ Sex_____ Ethnicity_____ Marital status_____

2. Please fill the table below.

Occupation	Private_____	Government_____ ___	Unemployed__ _____	Other_____ __
Education	None_____ _	Primary ☐	Secondary ☐	Above ☐ secondary
Income	Per day_____	Per month_____		

\

Smoking/ tobacco use history

To be answered only by tobacco users

1. Do you use any form of tobacco? _____ if so what types? _____

2. On average, how much tobacco do you smoke per day and for how long how you used tobacco?

_____

_____

Cigarettes per day_____ for _____years. Number of pack-years_____

3. How old were you when you started to use tobacco? _____

4. Do you smoke inside your house, near/or in front of your families or friends **a.** Yes **b**. No

5. If yes how often do you smoke in your house, near/or in front of your families or friends?

a. Daily **b**. Weekly **c**. Monthly **d**. not known

6. What are the important reasons why you smoke or use tobacco?

a. It is enjoyable b. It is a sociable thing to do **c.** Smoking helps me feel good and relax

d. Makes me feel more like an adult **e.** helps me forget my worries f. Other

7. During the past 12 months, have you tried to stop tobacco use for a day or longer, because you wanted to quit smoking or tobacco use? **a.** Yes **b**. No, go to question no 10

8. Which were your most important reasons for quitting smoking/tobacco use?

a. Did not want to smell like a smoker **b**. Wanted to get rid of addiction **c.** Spend money more reasonably **d.** Wanted to be healthier. **e.** Wanted to be a better role model for children **f.** Other_____

9. Do you think cigarette smoking is harmful to your health? **a**. Yes **b**. No

10. Did you ever see or hear any anti-smoking advice anywhere? **A. Yesb**. No

11. If yes, where did you see or hear the information? **a**. Television **b**. Radio **c.** Doctor or nurse.

d. Newspaper/ magazine **e.** Health warning on cigarette packs **f.** Other

12. Were you ever advised by a doctor or nurse to quit tobacco use? **a**. No **b**. Yes

To be answered only by passive smokers

13. Do your family, relatives or friends smoke tobacco? **a**. Yes **b**. No

14. If so, where are you exposed to other peoples‘ tobacco smoking?

A. At home **b**. At work **c.** Public recreation areas **d**. Other_____

15. For how many hours, on average each day, are you closely subjected to other people's tobacco smoke? **a.** Less than one hour each day **b**.1 to 5 hours a day **c.** More than 5 hours a day

16. Do you think the smoke from other people‘s cigarettes is harmful to you, or that your own smoking is harmful to others around you? **a**. Yes **b**. No

To be answered by both active and passive smokers

17. Do you participate in any physical activity? If so which one a. walking **b.** doing any sport regularly **c**. doing hard work which requires high energy d. other_____

18. Which type of diet you eat? _____

19. Do you use other substances? If so which one **a**. alcohol **b**. khat **c**. other_____

20. Do you have any health problems? _____ if so which health problems do you have?

_____

_____

21. Do you take any medications? a. Yes b. No If so, what are they?

_____

_____

Measured Parameters

Cigarette pack-years_____

Height_____ m Weight_____ kg BMI_____ kg/m^2

Blood pressure (right arm): SBP_____ mmHg DBP _____mmHg

Blood pressure (left arm): SBP_____ mmHg DBP _____mmHg

Waist circumference: _____ cm

Blood sugar_____ _mg/dL

Lipid profile: TC_____ HDL_____TG_____LDL (mg/dL)

Carbon monoxide level in home _____ppm Time of day_____

I. Odeeffannoo kandhimma Hawwaasummaa fi uummataa ilaalu

1. Koodii: _____ Umurii _____ Saala _____

Saba_____

2. Gabatee armaan gadii guuta

Hojii	dhuunfaa ☐	Hojjattuu guyyaa ☐	Kanbiroo	
Sadarkaan baruumsaa deebii kennituu	homaa kan hinbaratin ☐	sadarkaa tokkoffaa ☐	sadarkaa lemeffaa ☐	Sadarkaa lemeffaa ☐ dhaa ol
Galii	Galii guyyaa_____	Galii ji‘aa____		

2. **Gare tamboo xuxxan**

Debii warroota fayadamoo tamboo ta’anin debi’u

1. Tamboo gosa adda addaa ni fayyadamta? Gosa akami fayyadamta? _____

2. Guyyaatti hagam fayyadamta? Hamam waliraa fageesitee fayyadamta?_____

3. Waga meqatii tamboo xuxxuu jalqabde?_____

4. Toraban kessatti guyyaa meeqa xuxxaa?_____

5. Guyyota lamaan darban kessatti tamboo xuxxee?

a) eeyyen

b) laki

6. Ji‘ootaan kudhaa lamaan darbaan keessatti itti fayyadama Tamboo xuuxuu dhiisuu guyyaa tokkoof yaaltee beekta‘a ?

a) Eeyeen b) hin beekuu Gara gaafii saddeetaffaa deemii

7. Sababni Tamboo Xuuxuu dhiisuu yaalteef sababa maaliitin?

a) Akka nama arsuu hin urgoofneef

b) Araada irraa bilisa ta‘uuf

c) Itti Fayyadama Maalaqa koo sababeefachuuf

d) Da‘aiimaniif fakkeenyaa gaarii ta‘uu dhaf

e) Fayyaa ofitiif yada‘udhaaf

f) Kan bira

8. Itti fayyadama Tamboo xuuxuuyalu keetiif rakkoo (miidhaa)Fayyaa kee irratti qaqabuu yaadudhan ogessaa Fayyaa mariachiftanii beektu?

Namoota Tamboo hin xuuxne garuu karaa Nanna’atiin miidhaa tamboo xuuxuutin kan saaxilamaniin qofa guutamu.

9. Maatii kee hiriyyoota kee keessaa namnii itti fayyadama Tamboo xuuxuu ta‘e jira?yoo jiraate

10. Namoota itti fayyadama tamboo ta‘an waliin kan itti wal qunnamtu Nannoo kamiti?

a) Mana keessaatti

b) Iddoo Hojjiti

c) Iddoo Bashanannatti

d) Kan bira

11. Tilmaaman guyyaa keessaatti yeroo meeqaaf Namoota Tamboo xuuxaan wajjiin yeroo kee dabarfattaa?

Namoota Tamboo hin xuuxaniifi Namoota Tamboo itti hin fayyadamne garuu karaa naana’atiin miidha Tambootiif kan saaxilamaniin guutamuu

12. Tamboo alaa wantoota araada nama qabsisaan fayyadamtaa?yoo fayyadamtee?

a) Alkoolii

b) Jimaa

c) Kan biroo

13. Dhibeen Fayyaa isin qunamee baakaa?yoo beeknaa ta‘e

a) Dhibee Asmii
b) Dhibee Haffuraa baffachuu.
c) Dhibee Guraa
d) Kan birroo

Ani Gobena Dedefo Uninversiitti Finffinee Barataa Faakaliitti Fayyaa kan barachaa jiruu yoon ta‘u Gosa barnoota baayyoo keemisteriittin Digrii waggaa Lamaaffa guutuu dhaaf qoranoo ebaaf kan ta‘uu bareeffamaa.

Namoota tamboo fayyadamaanti hin fayyadamiinee garuu karaa birraattin namoota fayyida kanaattif saxilamaaniif tanboo xuxu dhan dhibee dhuufu danda‘uufi hin dandeenyee akkasumaas dhibee salphaatti ittisuu danda‘uumata duree jedhuu irraatti qoranoo gageessuu.

1. **Kaayyoo qoranoo barreeffamichaa**

Waalumaa galaatti kanoon qoranoo barreeffamaa kana dhudhaalee namoota tamboo keemikaala fayyadamaniifi tamboo kan hin fayyadamiine garuu karaa birootiin itti fayyadamaa tambootif namoota saxilamaan irraa jirachuufi jirachuu dhabu fi sababa tanboo xuxaniifi dhibee dhufuu danda‘uu haala salphaa taleen dandeentti ogummaa fayyaa fayyadamun addan basuu.

2. **Waa’ee hirmaatoota**

2.1 tratiiba/dura-dubaan

Yoo barreeffamaa qoranoo kana irraatti hirmaachuudhaaf heyamamaa taatanii jechaa daqiiqaa 10 Fudhatuu waaliitti deddeebisuu gochuun. Itti aansuudhaan iddattoo dhigaa keenudhan nafaa (qamaa) keessatti yokan hansaa duudhalee tanboon keemiikalaa jirachuufi jirachuu dhabuu ni mirkaneessina. Akkasumaas iddattoo fincaanii kenuudhaan tanboo fayyadamuudhan dhibee dhuufu dandamuu ni beekinaa.

2.2 **Ulaagaa Namoota Barreefama Qo'anna kana irraatti hirmaachuu ykn hirmaachu dhabu danda'aan addaan baasuuf.**

Barreeffamaa Qorannoo kun namoota itti fayyadamtoota fi namoota hin fayyadamnee tamboo akkasumas saxilamtoota itti fayyadamtoota tambootiin namoota midhamaa kan hammatuu yoo ta'an namoota qorannoo kana keessatti hin haammatamne ammo warraa midhama Fayyaa ol'anaa qaban ykn galma barreefama qo'annoo kana akka galma hin geenyee yaada faalleesuu (burjaajjeesuu) dhiiyeesuu kan danda'aan namooti hordoofii yaalii fayyaa qaban yoo ta'aan fi barreeffama Qo'annoo kana irratti hirmaachuuf namoota fedha hin qabnee dha.

3. **Rakkoo Qoranoon geesisuu danda'u**

Yoo qorannoo kana geggeesinuu iddattoo dhiigaa muurasaa fudhanee yoo qoranuu hanga tokko dhukibiin kan itti dhagamuu maalee dhibee cimaa irraan kani ga'u miti. Yaa ta'utti gaaruu yoo iddattoo dhiigaa fudhanee qorannuu dhibbee fiduu ykn mulachuu kan danda'u dha.

4. **Faaydaa**

Barreeffama qo'annaa kana keessatti hirmaachun faaydaalee asiin gaditti argaman kenna.

- Tamboo kallattiidhaan ykn al-kallattiidhaan fayyadamuudhaan hanqina fayyaadhaa kan isinirra gahe mirkaneessuu dandeessuu? Isinirra gahee yoo jiraate rakkichi babal'achuudhaan duratti gara ogeessa fayyaa deemuuf murteessudhaaf isin gargaara.
- Akkasumas kallattidhaaniis ta'e karaa naanna'aan ittifayyadama tamboo ta'u keessaniin midhaawwan isiin irraa gahuu danda'u ilaalchiisee gorsa gahaa ni argatuu.
- Kanamalees raga isiin keeniitan kun akka aanaatii gaggeesiitoota sagantaallee fayyaa (qopheesiitoonii)itti fayyadamuummaa Tamboo gadii hiriisuuf ykn dursanii ittisuuf hojiiwwan hojjechuuf ragaallee barbaachiisoo argachuu ni danda'u.

5. **Iccitummaa**

- Ragaallee nuuf keenniitan dhimma armaan olittif Eerame qofaaf kan oluu fi Iccitummaan isaan kan eegameedha.barreefama Qo‘annoo kannatti hirmaachuuf yoo hin barbaanee mirga itti hirmaachuu dhisuu kan kabajamee dha.
- Hirmaachuudhaafis hayyamamaa taatanii garuu jidduutii (yeroo barbaadaniitii)dhimma kan isiin hin toolee yoo argitan dhisanii keessaa bahuu mirga qabduu.

6. **Mirga raga Argachuu**

Hayyama barreeffama Qo‘annoo kana gaggeessuuf kan keennamuu Univarstii Finfineeti Mummee(Biochemistry)irraayi.Xallayaa deeggarsaas kan keennuu I/G Mummee kanaa yoo ta‘u kaayyoo Barreeffamaa Qo‘annoo kanaa keessattii namooti hirmaachuu qaban Adeemsa barreefama Qo‘annoo keessatti rakkollee kamyuu dhalachuu hundaa irraa Eeguufii .

Ahaadii Waliigaltee

Annii Barataa Gobena Dedefo Barreeffama Qo‘annoo Unniivarstii Finfinnee gaggeessuu irratti hirmaachuudhaaf hayyamammaa dha.

Annii Barreefama Qo‘annoo kan qopheessee namootaa kallatti Tamboo itti fayyadaman fi karaa naana‘aan itti fayyadaman irratti haftee Tamboon keessatti keemikaala jiraachuu isaa mirkanneesuufi namoonii Tamboo itti fayyadamuu isaanitin kan ka‘ee midhaawwan fayyaa irratti qaqabisiisu danda‘aan addaan baasuuf kan qophaa‘ee ta‘u isaa sirriitti hubadheen jira.

- Qorannoo kana keessatti hirmaachuuf kan danda‘aan namoota 113 keessaa tokkoo anii ta‘u kootii.
- Dhimma hirmaadheef fedhii fi Faayyidaa (kanfaltii)akka hin barbaanee ni beekaa.barreeffama Qo‘annaa irratti hirmaachuufis ta‘e hirmaachuu dhabuudhaaf Eerga murteesee booda waan natty hin toolee yoo jiraate rakkoo tokkoo malee addaan kutuuf kan danda‘u ta‘u kootiif hubadhee jira.

- Barreefama Qo‘annoo kana nama Qopheessuu raga kan narraa argatuu akkaata kamiyyuu maqaa koo itti fayyadamudhan faayyidaa irratti olchuu hin danda‘u.Kanaafuu barreefama Qo‘annoo kana irratti namoota hirmaatan Iccittii isaanii seerraan ni Eegamaa,Ittifayyadamummaa Ragaa seera ittifayyadamummaa raga kan hordoofuu ta‘e Iccitti nama dhunfaafis ta‘e jaarmiyaallee kan Eeggamuu dha.
- Ahaadii waliigaltee kanaa garagalchaan kan naaf keennamee yoo ta‘u,barreeffamaan dubbiisee argadhee fi ibsa naaf keennamee irraa hubadheen yaadoota gaafii natty ummanif deebii gahaa waan argadheef barreefama Qo‘annaa kana irratti hirmaachuudhaaf walii galeen jira.

Mallattoo Hirmaataa

Mallattoo

Qopheessaa Barreefamnn Qoa‘naa _____

Annex: V: Advice or health education to Oolcho town residents on harmful effect of tobacco use and its products.

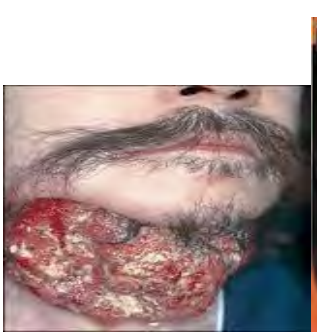
1) Smoking kills millions of people every year. In fact, one in every ten deaths in the world is caused by smoking. A half of all smokers will die from smoking-related diseases, and many more will suffer for years from illness.

2) Smokers often die from lung cancer, heart attacks and strokes. Smoking can also cause mouth cancer, throat cancer, and cancer of the pancreas, loss of lung tissue with severe difficulty in breathing, loss of legs, weak bones, premature aging, and many other health problems.

3) People who do not smoke themselves, but breathe in the smoke from others who do smoke, can suffer, and even die, from smoking-related diseases. This includes children, who have an increased risk of dying, getting pneumonia and ear infections from breathing in adult smokers‘ cigarette smoke.

4) Ethiopia has one of the lowest smoking rates in the world, but the number of smokers is going up. Stay healthy and happy for yourself and your children, and do not smoke! Keep your children healthy by advising them about the health dangers of smoking! Help your neighbours, friends, family and youth not to smoke, and make Ethiopia proud to have one of the world‘s lowest smoking rates!

5) Tobacco is addictive and dangerous, so any type of tobacco use, including cigarettes, cigars, pipes, shisha, chewing tobacco, is dangerous to your health and to your family‘s health.



This smoker died from throat cancer lung cancer kills many smokers smoking kills!

Namichu Kun kanserri kokketin du‘e namooni tambo xuuxan heddduun isaani kanseri sombaatin du‘u Tamboo xuxun nama ajjesa

Gorsa hirmattota qoranno kanatiif waa’ee miidhaa tamboo xuxxuu ilaachisee kennameef.

1) Tamboo xuxuun namoota miliyoona heddu waggatti ajjesa. Addunyarratt namoota 10 keessa tokko sababa tambootiin du‘a. 50% namoota tamboo xuxani dhibe tamboo xuxuun wal qabateen du‘u. Kan hafan immoo dhibee addd addatiin wagga dheeraf dararmu.
2) Namoonni tamboo xuuxan yeroo bay‘ee kan isaan ajjesu kanserii sombaa,dhibee onnee fi dhiigni gara sammu deemu qabu yoo dhaabbatuudha. Kana malees tamboo xuxuun kansarii afaani, kokke fi hadhooftu fida. Dabalatanis tishuu sombaa dadhabsiisa, Lafees human dhoorka, dafnee akka dullomnus nu taasisa.
3) Namoonni ofii hin xuunne, garuu namni bira yoggu aarsu yoo bira taa‘an dhibee tamboo fiduu danda‘u hundaanu ni qabamu, du‘aafis ni saaxilamu. Daa‘imman gam kanaan baay;ee hubamu. Dhibee gurraa,daranyoo samboo fa‘aa hubamu. Carraan du‘uus baay‘ee bal‘aadha
4) Etoophiyaan baay‘inna namoota tamboo xuxani xiqqa qabdi. Garuu yeroo yerootti dabalaa jira. Fayyaa keessan eegadha jiraadha,ijoolle keessaniifi ofiifis. gammachuun jiraadha. Tamboo xuxxurra ofii fi ijoolle keessanille tiksaa Miidha tamboo xuxuun fidu ijoolle keessan barsiisuudhan fayya isaan eega. Olla, hiriyyaa fi mmatii keessan akka tamboo xuunne taasisa. Namootni tamboo xuxaan Etoophiya keessa muraasa ta‘uu isaatti boona

5) Tamboon arada cimaa kan nama qabsiisu yoo ta‘u; dabaltaanis dhibeewwan fayyaa nama dararan heddu nama qabsiisa. Kanafuu ofii kessanii fi maatille tamboo gosa kamittu fayyamuu irraa qusadha.