Investigation of Urine Cotinine Levels as Indicators of Tobacco Use and Passive Smoking, with Parameters of Metabolic Syndrome, Among Adults in Chena Wacha

By:

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Submitted to: College of Health Science Department of Biochemistry

In requirement for Partial fulfillment of degree of Master's science in Medical Biochemistry

Addis Ababa University,

Addis Ababa, Ethiopia

March, 2015

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

This is to certify that thesis prepared by Anteneh Wondimu, entitled: Investigation of Urine Cotinine Levels as Indicators of Tobacco Use and Passive Smoking, with Parameters of Metabolic Syndrome, Among Adults in Chena Wacha, Submitted in requirements for partial fulfillment of degree of Master's science in Medical Biochemistry companies with regulation of university and meet acceptance standards with respect to originality and quality.

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_____

Chair of department/ graduates program coordinator

DECLARATION

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

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Advisors: Dr. Frank Ashall and Dr. Daniel Seifu

Abstract

Background: Active and passive cigarette smokers produce the nicotine metabolite, cotinine, which can be detected in the urine. Smoking increases the risk of metabolic syndrome, includes abnormalities in lipid profile, blood glucose, blood pressure, BMI and waist circumference.

Objective: The study, investigate urine cotinine levels as indicators of tobacco use and passive smoking, with parameters of metabolic syndrome among adult in Chenna wacha, SNNPR.

Method: A cross sectional study was done on a total of seventy smokers (46 active and 24 passive). Smoking history was obtained from the participants which was then used to calculate smoking consumption in pack-years. In this study, current active smokers were classified based on the number of pack-years as into mild to moderate (1 – 10 pack-years) and heavy (more than 10 pack-years. Parameters of metabolic syndrome were determined using standard methods, while urine cotinine was determined using COT® one step cotinine test device at a cut-off of 200 ng/mL. Data analyses were performed with SPSS16.0 system.

Result: The finding of the study indicates that 88.7 % of active smokers and 11.3 % of passive smokers were positive for urine cotinine, with substantial agreement between cotinine test results and self-reported cigarette use (kappa 0.63). Among five children taken from four families in which there was an active smoker in the home, two out of the five (40%) were positive for urine cotinine. The study also shows that the prevalence of metabolic syndrome, based on the occurrence of at least three components of metabolic syndrome, was 5/46 (10.8 %) in active smokers and 2/19 (10.5 %) among passive smokers. Taking mild to moderate smokers as reference, heavy smokers were relatively underweight with statistical significance (OR 7.90; p= 0.004). Adult passive smokers were not significantly underweight compared with mild-to-moderate smokers (OR 2.09; p= 0.234). As the number of pack-years of smoking increased among smokers, they had an increased number of parameters of metabolic syndrome.

Conclusion: This study shows that urine cotinine measurements are a practically convenient and valuable tool for objectively assessing smoking and passive smoking in Ethiopia, and detected passive smokers, including children as young as 6 years old. Some parameters of metabolic syndrome showed a significant association with the level of smoking.

Key words: *Cotinine, urine, metabolic syndrome*

Acknowledgements

First of all I thanks to my GOD helping me in every part of my life. I would like to express my deepest gratitude to my advisor Dr.Frank Ashall for his dedicated advice, closer help and warmest treatments beside the idea and suggestion he shared. His support, encouragement motivation and giving his time to go to research field and sharing his experience to the project from the beginning up to the completion of this study is kindly appreciated. I have a special respect and appreciation to him, for his help and advice. I thank him from the bottom of my heart for all his deeds that are difficult to express in words.

I would like to express my deepest gratitude to my advisor Dr. Daniel Seifu for his acceptance to work with me and for his valuable comments and dedicated advice from beginning up to the completion of this study.

I transfer my real appreciation and gratitude to Mis Juliet Ashall for her help in sending the kits from USA, and Ato Abirham Geremew who helped me in commenting of my paper. I am grateful to Ato Getahun Tsegaye for his cooperation in processing of sample. I also greatly acknowledge my family members Wondimu Tadesse, Manayesh Wondimu, Tadele Desalegn, Saba Wondimu and Jimmawork Abadiga, they were the back bone of my research, who transfer the objective and significance of my research to community.

My deepest heart-felt gratitude goes to my caring Helen Mergia, for her encouragement, and love. I am grateful to all of my friends, with whom I passed during my study, whose discussions and friendships were socially valuable in the study.

I am also thankful to Haramaya University for the sponsorship for M.Sc. study.

Lastly my appreciation goes to the participants of the study for their cooperation and sincere responses.

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List of Abbreviations

AEP: Acute Eosinophilic Pneumonia

ATP: Adult Treatment Panel

BAT: British American Tobacco

BMI: Body Mass Index

BP: Blood Pressure

CDC: Centers for Disease Control

CHD: Coronary Heart Disease

CI: Confidence Interval

COPD: Chronic Obstructive Pulmonary Disease

CSA: Central Statistical Agency

CVD: Cardiovascular Disease

DBP: Diastolic Blood Pressure

DDT: Dichlorodiphenyltrichloroethane

DNA: Deoxyribonucleic acid

EDHS: Ethiopian Demographic and Health Survey

ETS: Environmental Tobacco Smoke

FBG: Fasting Blood Glucose

GFR: Glomerular Filtration Rate

GTS: Green Tobacco Sickness

GTSS: Global Tobacco Surveillance System

HDL: High Density Lipoproteins

HIV: Human Immune Deficiency Virus

IDF: International Diabetes Federation

kg: kilogram

LC-MS: Liquid Chromatography Mass Spectrometry

LDL: Low Density Lipoproteins

MS: Metabolic Syndrome

NCEP: National Cholesterol Education Program

NRT: Nicotine Replacement Therapy

pH: Power of Hydrogen

SBP: Systolic Blood Pressure

SHS: Secondhand Smokers

TB: Tubercles Bacillus

TG: Triglycerides

USA: United States of America

VLDL: Very Low Density Lipoproteins

WHO: World Health Organization

1. INTRODUCTION

1.1 Background of the Study

Tobacco use is currently considered one of the greatest problems in public health worldwide and it is one of the most preventable causes of death (WHO, 2009). For example 13% of the tuberculosis (TB) cases in the world each year may be attributable to tobacco exposure. Recognizing this, the International Union against Tuberculosis and Lung Disease included tobacco cessation as integral part of all TB control programs, and it is supported by the Bloomberg Initiative to reduce tobacco use, and has put together several resources for tobacco cessation in TB patients (Slama *et al.*, 2007).

The World Health Organization (WHO) reported that currently tobacco use kills 6 million people a year-an average of one person every six seconds and accounts for one in 10 adult deaths worldwide (WHO, 2009) and this figure is expected to increase to 10 million deaths a year by 2020. According to WHO estimates, approximately 47% of men and 12% of women smoke cigarettes worldwide in 2010. Citing the death of 6 million individuals worldwide every year due to tobacco related diseases the WHO states that tobacco use should be considered a pandemic. If left unchecked, tobacco will kill one billion people in this century. The Bloomberg initiative to reduce tobacco use is focused on reducing the public health impact of tobacco use globally by implementing proven tobacco control policies in low and middle income countries where 80% of tobacco related deaths occur (WHO, 2013).

Since 1980, large reductions in the estimated prevalence of daily smoking were observed at the global level for both men and women, but because of population growth, the number of smokers increased significantly. As tobacco remains a threat to the health of the world's population, intensified efforts to control its use are needed. Despite the growing problem of global tobacco use, accurate information on the prevalence and patterns in the world's poorest nations remains sparse. For sub-Sahara Africa, in particular, a weak knowledge base limits the targeting of strategies to combat the potential growth of tobacco use and its harmful effect on future mortality.

Sub-Saharan Africa is in the early stages of the tobacco epidemic, characterized by low rates of smoking for women and men but increasing popularity of cigarettes among men (Mathers *et al.*, 2006). Tobacco use in Africa is on the increase as the tobacco industry shifts its marketing focus from the West to Africa and Asia. This is worrying as the fragile health systems in many African countries are unable to adequately cope with the double burden of infectious diseases and chronic diseases including those that are caused by tobacco use (Tumwine, 2011).

The WHO identified a package of cost effective tobacco control measures called for by the Framework Convention on Tobacco Control called the MPOWER package it includes: Monitor tobacco use and prevention policies, Protect people from tobacco smoke, Offer help to quit tobacco use, Warn about the dangers of tobacco, Enforce bans on tobacco advertising, promotion and sponsorship, Raise taxes on tobacco. These measures have been proven to reduce tobacco use and should be implemented in every nation. These solutions require nations to enact comprehensive smoke free laws, help tobacco users to quit, ban all tobacco advertising, promotion and sponsorships, implement graphic health warnings on tobacco products and raise the price of tobacco products by significantly increasing tobacco taxes (WHO, 2013). The new study compared what would happen from 2010 to 2030 if countries did nothing more to implement the MPOWER measures and if countries had fully implemented MPOWER as of 2010. If countries did nothing more, the global tobacco epidemic would continue to grow. The global number of smokers would increase by 10 percent; from around 794 million in 2010 to 872 million smokers worldwide by 2030.

Over the past five years MPOWER has been promoted in low and middle income countries, though many of the regulations recommended in the WHO Framework Convention on Tobacco treaty have not been properly instigated. This is critically important, as tobacco use has increased in many low and middle income countries even as it has stabilized or declined slightly in some high income countries. However, in high income countries rates of tobacco use, though declining, have leveled off and all these countries cannot afford to fall behind in protecting their people against the harms of tobacco use. The effects of these changes in consumption are already becoming apparent in terms of tobacco related deaths so governments must act now to reduce the

devastating global consequences of tobacco products on health, lives and economies (WHO, 2013).

Abuse of drugs is believed to be extremely rare in Ethiopia but use of locally growing psycho-stimulants such as tobacco, khat and cannabis is growing. The health hazards of these substances and prevalence of their use have not been well studied. According to 2005 report of Ethiopian Demographic and Health Survey (EDHS), among men with the age range of 15–49 years in Ethiopia, 9% of them use tobacco. Even though, there is no complete data on the prevalence of tobacco use among women EDHS reported that less than 2% of women in Ethiopia smoke cigarettes (CSA, 2006). But according latest report of WHO report, 6.3% Male and 0.5 % female were current adult tobacco user in Ethiopia and approximately 90% of individuals who become tobacco users initiate the behavior during adolescence (WHO, 2013).

Studies in Ethiopia have shown that the probability of dying from all causes was 2.3 times higher for current male tobacco user than males neither active nor passive smokers. The same study indicate that the risk of dying from all causes including lung cancer is about two times higher for current female smokers compared to those females who are neither active nor passive smokers (Kebede *et al.*, 2005). Factors that promote adolescent initiation are parental or older generation cigarette smoking, ethnic background, occupation and social milieu, family history, the availability of tobacco, and the social acceptability of tobacco use. The level of acceptance of tobacco use in the home, peer group, workplace and community norms influence tobacco use (Kebede *et al.*, 2005).

1.2 Literature Review

1.2.1 History of tobacco in Ethiopia

It was not possible to get literature that narrates exactly when tobacco was first used in Ethiopia. Evidence that indicates the presence of tobacco in Ethiopia going back many years is that there are several groups of people in different parts of the country that use native tobacco in different forms, such as snuffing, chewing and pipe smoking (Gaya). However, around the beginning of 20th century a tobacco factory emerged for the first time in Dire Dawa, later, in 1931 (1923 E.C.). Prior to 50 years ago, tobacco was grown for commercial purposes by state owned farms and by farmers around these farms but now the National Tobacco and Matches Corporation, which was renamed as National Tobacco Enterprise, has been given the mandate to organize tobacco production and processing in the country (Mulatu *et al.*, 2006).

1.2.2 Tobacco market and its economic impacts

National Tobacco Enterprise (NTE) manufactures five different brands of cigarettes with the leading product Nyala, which accounts for around 85 per cent of the total market, NTE also produces Elleni, Gisilla and Delight which are made of lower grade tobacco. It also imports Rothmans cigarettes from British American Tobacco (BAT) and Marlboro from Philip Morris International. The tobacco market in Ethiopia is set for significant growth over the coming years, due to rising incomes and a range of measures designed to reduce the volume of contraband cigarettes being consumed in the country. The number of cigarette sticks sold in 2013 is set to increase by 13.3 per cent to 4.5 billion sticks, and for sales to reach 5.9 billion sticks in 2016, which would represent a CAGR (Combined Annual Growth Rate) between 2012 and 2016 of 8.5 percent. Sheba, a Yemeni company, agreed in June 2014 with National Tobacco Enterprise to pay 1.25 billion birr to boost its share from 22 percent to 60 percent share, with the government holding the remainder. The NTE has been seeing growing profits over the past years, with 246 million birr in 2011/12, 280 million birr the following year and 319.5 million birr during the 2013/14 (NTE, 2013).

1.2.3 Tobacco products

***Cigarettes*:** A cigarette is a combination of cured and finely cut tobacco, reconstituted tobacco and other additives rolled or stuffed into a paper wrapped cylinder. Many cigarettes have a filter on one end. It has about one gram of tobacco and takes about 10 minutes to smoke. Although only one to two milligrams of nicotine are delivered to the body from smoking one cigarette, cigarettes are highly addictive. This is because cigarette smokers usually inhale smoke directly into their lungs, which allows for rapid absorption of nicotine into bloodstream (Kozlowski *et al.,* 1998; Connolly *et al.,* 2000).

Cigars: Most cigars are made up of a single type of air-cured or dried tobacco. Cigar tobacco leaves are first aged for about a year and then fermented in a multi-step process. Fermentation causes chemical and bacterial reactions that change the tobacco. This gives cigars a different taste and smell. Cigars contain higher level of nicotine than cigarettes. The smoke of cigars is more alkaline than cigarette smoke and dissolves more easily in saliva. Therefore the desired dose of nicotine is achieved without the need to inhale the smoke into the lungs. Cigars are capable of providing high levels of nicotine at a rate fast enough to produce clear dependence, even if the smoke is not inhaled. The only reason cigars are not particularly addictive for most users do not inhale (Baker *et al.,* 2000).

Pipe tobacco: Pipes are often reusable and consist of a chamber or bowl, stem and mouthpiece. Tobacco is placed into the bowl and lit. The smoke is then drawn through the stem and mouthpiece and inhaled. However, smokers of pipes usually smoke on an infrequent basis and usually do not inhale pipe smoke. These habits make pipe tobacco less addictive than cigarettes for most pipe users. A study found that pipe smokers were at greater risk of other tobacco related diseases. They had a thirty percent risk of heart disease and nearly three times the risk of COPD (Henley *et al.,* 2004).

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***Hookah**:* Hookah is also called narghile, argileh, shisha, hubble-bubble, and goza. It is a pipe used to smoke shisha, a combination of tobacco and fruit or vegetable that is heated and the smoke is filtrated through water. The user inhales the water filtered smoke through a tube and mouthpiece. The water lowers the temperature of the smoke. It is popular due to the common

misconception that the nicotine content in shisha is lower than that of cigarettes and that water used in this form of tobacco intake works as a filter, removing all the hazardous chemicals such as CO, nicotine and tar. These common misapprehensions lead public to believe that shisha smoking is not a hazard their health and others (Sameerur *et al.,* 2012). Water pipe smoking delivers nicotine, the same highly addictive drug found in other tobacco products. Because of the way a hookah is used, smokers may absorb more of the toxic substances also found in cigarette smoke than cigarette smokers do. Secondhand smoke from hookahs can be a health risk for nonsmokers (Simone *et al.,* 2008).

Smokeless tobacco: The tobacco industry's marketing of these novel smokeless tobacco products indicates they are also attempting to counteract tobacco control policies, especially laws prohibiting smoking in public places and workplaces (Parascandola *et al.,* 2009). The two main types of smokeless tobacco are chewing tobacco and snuff. Chewing tobacco comes in the form of loose leaf, plug, or twist. Snuff is finely ground tobacco that can be dry, moist, or in sachets (tea bag like pouches) and is inhaled through the nose. Snus is also a smokeless tobacco similar to snuff but is placed between the gums and jaw in the mouth and nicotine is absorbed through the oral cavity from it. Users then suck on the tobacco and spit out the tobacco juices, which is why smokeless tobacco is often referred to as spit or spitting tobacco. The nicotine in this tobacco is absorbed primarily through the skin in the mouth. The amount of nicotine absorbed from one use of chewing tobacco is usually two to three times greater than that from a single cigarette. Although absorption is usually slower, the nicotine from chewing tobacco stays in the body longer. This makes chewing tobacco very addictive (Richter *et al.,* 2008).

There is much to be learned about the use and health risks of novel smokeless tobacco products but study found that novel smokeless tobacco products are being promoted as safer alternatives to smoked tobacco products. Consequently, the perception among the general public is that smokeless tobacco products are less harmful than those that are smoked (Parascandola *et al.,* 2009).

Electronic cigarette or E- cigarette (nicotine delivery system): The e-cigarette is a battery powered device that contains a cartridge filled with nicotine, flavor and other chemicals. The e-cigarette is not a tobacco product but a nicotine delivery system. The e-cigarette turns the nicotine and other chemicals into a vapor that is then inhaled by the user. The user will puff on it, similar to a cigarette and receive a vaporized solution of propylene glycol/nicotine. There is no tobacco or burning of tobacco involved and the e-cigarette produces no smoke. It does produce a fine, heated mist. The e-cigarette often looks like a real cigarette and some have a glowing tip. Other models look similar to a ball point pen. World Health Organization claims there are no studies showing that the electronic cigarette is a safe and effective nicotine replacement therapy (Etter *et al.,* 2011). The most common effects of use include mouth and throat irritation and cough. Short-term negative impacts on lung functions have also been documented (Vardavas *et al.,* 2012). In addition, acute nicotine administration increases heart rate, blood pressure, and impairs circulation (Benowitz and Gourlay, 1997).

To date, there have been reports of many hazardous chemical compounds generated from e-cigarettes, particularly carbonyl compounds such as formaldehyde, acetaldehyde, acrolein, and glyoxal, which are often found in e-cigarette aerosols and adversely affect health. These carbonyl compounds are incidentally generated by the oxidation of e-liquid (liquid in e-cigarette; glycerol and glycols) when the liquid comes in contact with the heated nichrome wire (Bekki *et al.,* 2014).

1.2.4 Smoking

Tobacco smoke is an aerosol consisting of a particulate phase of liquid droplets dispersed in a gas/vapor phase. These either pass through the cigarette as mainstream smoke, or some being condensed a short distance behind the burning cone, or they are emitted into the air from the burning end as side-stream smoke. When it is inhaled, a cigarette burns at 700°C at the tip and around 60°C in the core. This heat breaks down the tobacco to produce various toxins compounds by pyrolysis of the tobacco (Stojanovic *et al.,* 2004). The main point of entry of cigarette smoke into the body is via the airways, but many constituents, particularly from pipe and cigar smoke, dissolve in saliva and are absorbed in the buccal cavity or are swallowed. Cigar and pipe smokers generally do not inhale the smoke and it remains in the oral cavity, is dissolved

in the saliva and absorbed through the mucous membranes or swallowed. Alcoholic drinks have a solvent effect for the smoke constituents facilitating their absorption (Smith *et al.,* 1999). Tobacco company use various additives to improve taste and decrease harshness mostly in the form of sugars, humectants, ammonia compounds, cocoa, and licorice which many believe added to entice younger people to smoke (Keithly *et al.,* 2005).

1.2.5 Nicotine

Nicotine is named after the tobacco plant *Nicotiana tabacum*, which in turn is named after Jean Nicot, a French ambassador, who sent tobacco and seeds from Portugal to Paris in 1550 and promoted their medicinal use (Akehurst, 1968). *Nicotiana tabacum*, the major tobacco plant species that is the major source of tobacco in cigarettes and other tobacco products, is a member of the nightshade family of plants. Tobacco produces by far the greatest quantity of nicotine of any plant source. The leaves of a typical tobacco plant may contain from 20,000 to 40,000 parts per million of nicotine. The nicotine content of tobacco constitutes about 0.3 percent to 0.5 percent of the plant when measured by dry weight. Close relatives of the common tobacco plant also contain nicotine in their leaves, including Aztec Tobacco (*Nicotiana rustica*) and tree tobacco (*Nicotiana glauca*) (Pego *et al.,* 1995).

The tobacco leaf is composed of 85-90 % water, mineral matter and organic compounds. The organic compounds may be considered as organic acids, carbohydrates and alkaloids (including nicotine). From which alkaloids make the tobacco to be used by human beings. Alkaloids are a group of nitrogen containing bases. Most of them are drugs. Only a few (like caffeine) are derived from purines or pyrimidines, while the large majorities are produced from amino acids (Geoffrey, 1981). Among the alkaloids, nicotine is recognized as an essential component in tobacco cultivation. It is found in the nightshade family of plants (*Solanaceae*), a study performed by the Centers for Disease Control and Prevention revealed that some foods that we consume on a regular basis contain nicotine. However it is found predominantly in tobacco but occurs in lower quantities in tomato, potato, eggplant (aubergine) and green pepper. It is also found in the leaves of the coca plant. When vegetables are thoroughly cooked, the nicotine will diffuse into the cooking water and less will be ingested. Also, most of the nicotine in vegetables, for example potatoes, is present in the skin and lost when they are peeled. Dietary intake of

nicotine in nonsmokers is therefore not of practical importance in the interpretation of the role of passive smoke inhalation when one is determining nicotine and cotinine levels in body fluids. It is the principal tobacco alkaloid comprising about 95% of the total alkaloid content (Hukkanen *et al.*, 2005).

Nicotine is synthesized from the polyamine putrescine, which is produced either directly from ornithine or indirectly from arginine. The first step in nicotine biosynthesis is the conversion of putrescine to *N*-methylputrescine, catalyzed by putrescine *N*-methyltransferase (Hashimoto and Yamada, 1994). The methyl group comes from methionine. Oxidative deamination of N-methylputrescine is catalysed by diamine oxidase, producing N-methyl-γ- aminobutanal. *N*-methylputrescine is then oxidized by *N*-methyl putrescine oxidase and cyclized to form a pyrrolidine ring, while quinolinic acid phosphoribosyltransferase serves in the pyridine ring synthesis that provides nicotinic acid (Hibi *et al.*, 1994; Shoji *et al.*, 2010). The pyridine ring of nicotinic acid is derived from 3-phosphoglyceraldehyde and aspartic acid (Samuelsson, 1999). Use of labeled nicotinic acid showed that the only hydrogen atom lost was that from carbon-6 and that the pyrrolidine ring system was attached to the carbon atom to which the carbocylic acid group was previously attached. Nicotine is formed by a condensation of *N*-methylpyrrolinium and nicotinic acid, and further metabolized to form other alkaloids, such as nornicotine, nicotyrine, and myosmine (Häkkinen *et al.*, 2007; Shoji *et al.*, 2010).

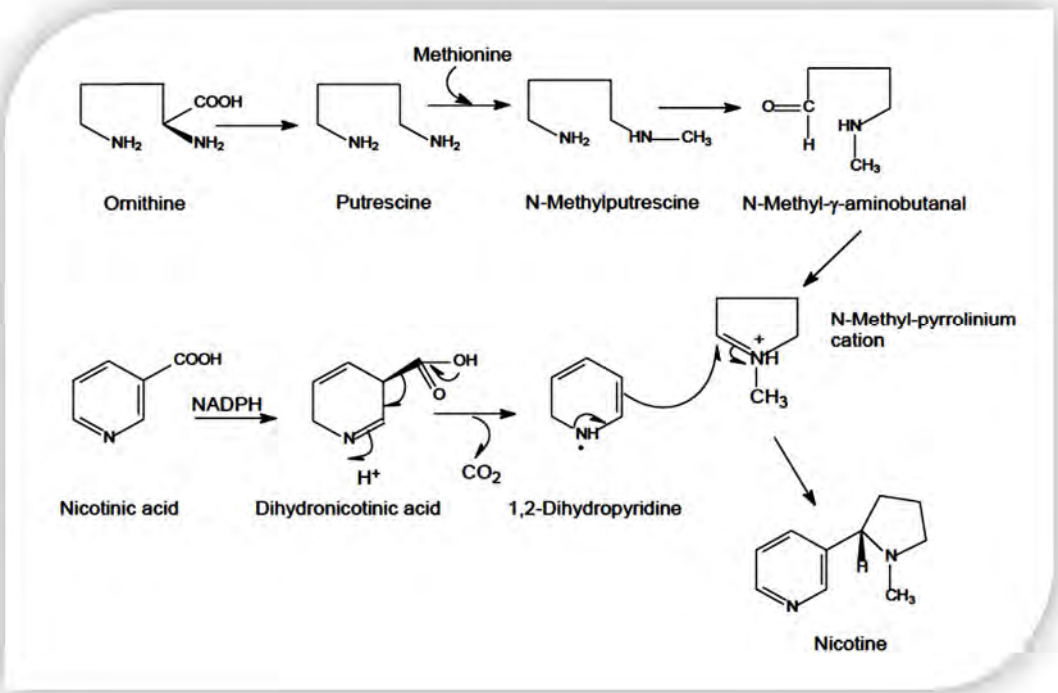

Figure 1.1 Biosynthesis of nicotine in *Nicotiana tabacum* (Samuelsson, 1999)

1.2.5.1 Nicotine pharmacokinetics

Nicotine is the major addictive substance in cigarette tobacco. Nicotine is given off by burning tobacco and carried into the respiratory tract on tar droplets and in the vapor phase. As a weak base, nicotine may exist in an ionized or a non-ionized form. The relative proportions of these two forms determined by the pH of the smoke, and affect the amount of "freebase" nicotine, which is most readily absorbed into the body. At the acidic pH of most cigarette smoke (which favors the ionized form of nicotine) absorption occurs predominantly in the lungs, but with the alkaline smoke produced by cigars and pipe tobacco, nicotine being predominantly non-ionized ("freebase" form) it is absorbed mainly in the mouth. Absorption into the bloodstream is rapid and concentrations of nicotine in the blood rise rapidly during smoking (Benowitz *et al.,* 2008).

Absorption of nicotine across biological membranes depends on pH. Nicotine is a weak base with a p*K*a of 8.0. In its ionized state, such as in acidic environments, nicotine does not rapidly cross membranes. The pH of smoke from flue-cured tobaccos, found in most cigarettes is acidic

(pH 5.5–6.0). At this pH nicotine is primarily ionized. As a consequence, there is little buccal absorption of nicotine from flue-cured tobacco smoke, even when it is held in the mouth. Smoke from air-cured tobaccos, the predominant tobacco used in pipes, cigars, and some European cigarettes are more alkaline (pH 6.5 or higher) and considerable nicotine is unionized. Smoke from these products is well absorbed through the mouth. It has recently been proposed that the pH of cigarette smoke particulate matter is higher than previously thought, and thus a larger portion of nicotine would be in the unionized form, facilitating rapid pulmonary absorption (Pankow, 2001).

The rapid absorption of nicotine from cigarette smoke through the lungs, presumably because of the huge surface area of the alveoli and small airways, and dissolution of nicotine in the fluid of pH 7.4 in the human lung facilitates transfer across membranes. After a puff, high levels of nicotine reach the brain in 10–20 second, faster than intravenous administration producing rapid behavioral reinforcement (Benowitz *et al.,* 2008). In addition, tobacco industry adds additives to tobacco, including ammonia, increase the bioavailability of nicotine by converting the ionized form to the more easily vaporized and absorbed neutral ("freebase") form (Rabinoff *et al.,* 2007).

Nicotine is metabolized extensively, primarily by the liver. In humans 40 to 60% of nicotine is eliminated as trans-3 hydroxycotinine, 22 to 35% as cotinine and cotinine glucuronide and 8 to 10% as nicotine (Raunio *et al.,* 2008). Nicotine, cotinine and 3'-hydroxycotinine are further metabolized by glucuronidation. Nicotine and cotinine undergo N-glucuronidation, while 3'-hydroxycotinine undergoes 0-glucuronidation. There is considerable individual variability in the metabolism of nicotine. Thus individuals with deficient C-oxidation have an unusually long half-life of nicotine because only 8% of nicotine converted to cotinine (Benowitz *et al.,* 2008). The metabolism of nicotine to cotinine is a two-step process via an intermediary metabolite, the nicotine iminium ion.

- The first step is metabolism by a cytochromes CYP450 enzyme, most likely CYP2A6.
- The second step is metabolism by cytoplasmic aldehyde oxidase.

Nicotine is hydroxylated at the 5' position, yielding an unstable intermediate 5'- hydroxyl nicotine in equilibrium with Delta1'(5') iminium ion. This first step is catalyzed by cytochrome P450 2A6, with cytochromes P450 2B6 and P450 2D6 also contributing to the hydroxylation. 2)

5'-Hydroxynicotine is then oxidized to cotinine, the major nicotine metabolite, by aldehyde oxidase. 3) Cotinine can then be converted either to cotinine-Gluc (*via* a detoxification mechanism) or trans-3'-hydroxycotinine. This compound can also be detoxified to trans-3'-hydroxycotinine-Gluc **(**Hecht *et al.*, 2000).

In humans, cotinine and its metabolites represent 70 to 80% of the metabolism products resulted from the nicotine absorbed by a smoker, which will be excreted mainly by the kidneys, but also by perspiration, maternal milk and oral fluids (Curvall *et al.*, 1990; Benowitz, 1996). Cotinine has become increasingly accepted as a short-term marker of nicotine exposure because of its relatively long half-life (approximately 20 hours, comparing with 2 hours for nicotine). It is also less susceptible to fluctuations during exposure to tobacco smoke and can be conveniently measured in blood, urine and saliva. Cotinine measurements from human fluids can provide an assessment of recent exposure to environmental tobacco smoke, but they do not indicate the duration of exposure nor do they indicate the intake of other components of tobacco smoke (Figueiredo *et al.*, 2007).

The enzyme that is deficient in the case of deficient nicotine oxidation has not yet been identified. Being a very strong toxic agent and addictive drug nicotine is the main component of cigarettes. The contributions to nicotine and cotinine renal clearances have been estimated in a twin study (Benowitz *et al.*, 2008). The study found a substantial contribution of genetic factors to the net secretory/re-absorptive clearances of nicotine and cotinine. It suggest either that the re-absorption of nicotine and cotinine are active processes and are influenced by the genetics of re-absorptive transporters, or that the active secretory component of renal clearance exerts a substantial effect on the clearance.

The blood level of cotinine is not a good marker of nicotine content of blood. In contrast urine excretion of cotinine is a good marker as it is less influenced by the flow of urine and pH and also it is non-invasive methods. The elimination half-life that is relevant to the accumulation of nicotine during the use of tobacco or nicotine products averages 2-3 hours. Thus nicotine levels accumulate over 6-8 hours during regular tobacco use or nicotine dosing. However, there is also a very long terminal half-life, 20 hours or more, presumably reflecting slow release of cotinine

from body tissues. It is stable in body fluids and has a long half-life (15-19 hours), low plasma protein binding (2.6%), and dose-independent disposition kinetics. These factors make cotinine a good marker for estimating both active and passive exposure to tobacco smoke (Curvall *et al.*, 1990).

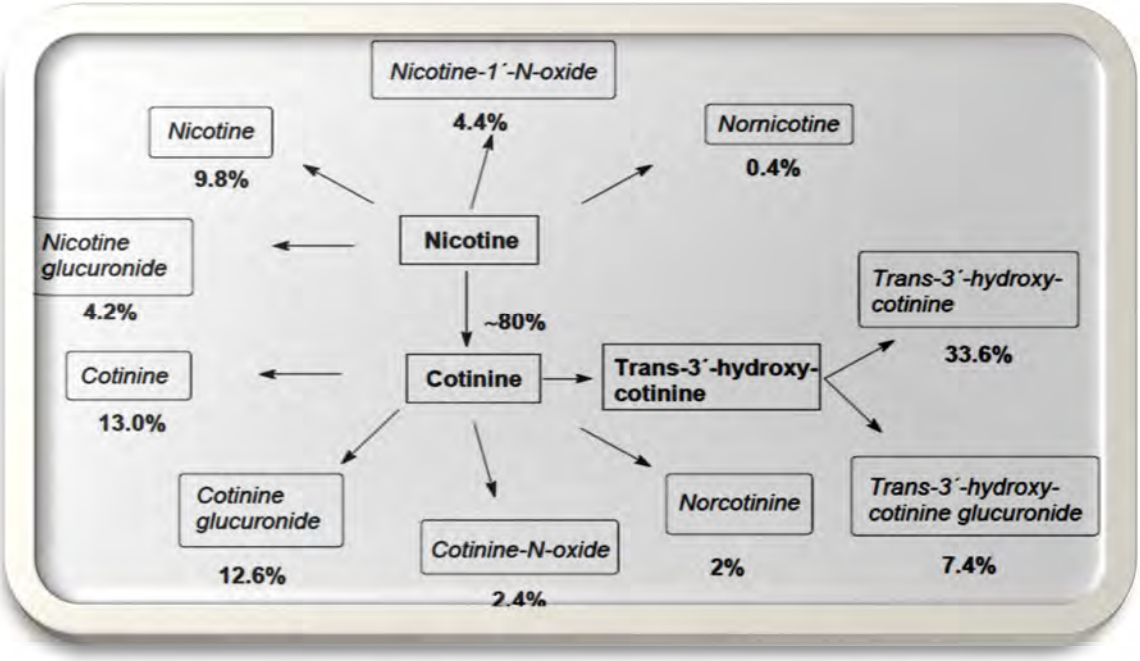

Figure 1.2 Quantitative scheme of nicotine metabolism. Compounds in italics have been detected excreted in urine (Benowitz *et al.*, 2008).

When nicotine is taken into the body through the lungs, it is absorbed via the lung alveoli. All, or nearly all nicotine, will be absorbed into the systemic circulation and reach body organs, including the brain, liver and kidneys. The liver converts nicotine to a large number of metabolites (Figure 1.2), the main one being produced by a hydroxylation attack leading to the opening of the pyrrolidine ring and reclosing of the ring to form cotinine (approximately 80% of the metabolites).

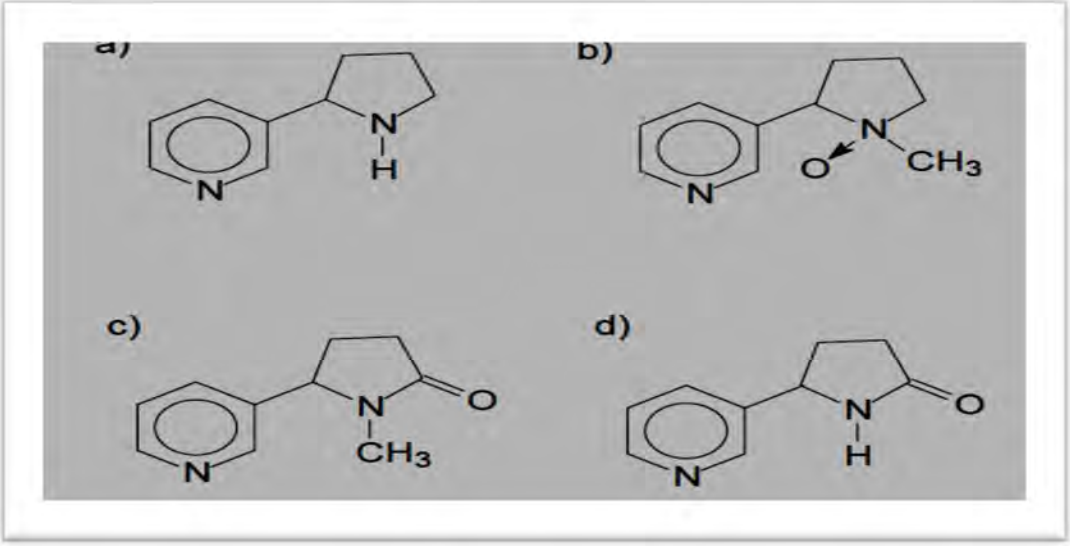

Figure 1.3 Chemical structures of the nicotine metabolites: a) nornicotine; b) nicotine-1-oxide; c) cotinine; and d) norcotinine (Benowitz, 1996).

1.2.6 Health effects of tobacco

A few risk factors account for a large proportion of chronic diseases (Capewell *et al.,* 2000). Smoking, high BP, and high cholesterol causes approximately 75% of heart attacks and strokes (Vartiainen *et al.,* 1995). Data on selected major risk factors are essential for planning primary prevention programs and for predicting the future burden of chronic diseases. The long lag time between exposure to risk factors and occurrence of chronic diseases suggests that risk factors should be primary targets for public health interventions (Bonita *et al.*, 2002).

Using healthy diet, regular physical activity and avoidance of tobacco use one can control non-communicable diseases including 80% of all CVD and type 2 diabetes and over 40% of cancer could be are becoming major problems of public health in most developing countries as a result of the effects of globalization and epidemiologic transition; however, there is little evidence regarding metabolic syndrome and other non-communicable diseases in these countries (Boutayeb A and Boutayeb S, 2005; Miranda *et al.*, 2008).

Consumption of tobacco is a risk factor for six of the world's eight leading causes of death (Lopez *et al.,* 2006). Cigarette smoke contains over 4000 compounds, including dozens about 70

carcinogens even more other poisons and over 300 polycyclic aromatic hydrocarbons. Among other chemicals, smoke contains cadmium, arsenic, butane, ammonia, lead, acetone, carbon monoxide, pesticide residues such as dichloro diphenyl-trichloroethane (DDT), polycyclic aromatic hydrocarbons (PAHs), formaldehyde, benzene, nickel, chromium, 2-naphthyl-amine, vinyl chloride, 4-aminobiphenyl and beryllium (Jaleel *et al.*, 2007).

Nicotine is generally accepted as the principal pharmacologically active component of tobacco smoke. Nicotine itself is odorless and colorless and it can stimulate the release of biogenic amines and result in vasoconstriction and decreased tissue perfusion when inhaled or injected (Alshaarawy *et al.*, 2013). Acute nicotine toxicity is widespread in tobacco farm workers who are exposed to nicotine, which is absorbed directly through the skin from handling tobacco leaves. This causes Green Tobacco Sickness, a common acute presentation of nicotine toxicity associated with severe distressing nausea, vomiting, abdominal pain, weakness, dizziness, headache, even psychosis. The millions of children who work on tobacco farms worldwide are particularly sensitive to this distressing illness (McKnight and Spiller, 2005).

Tobacco use leads most commonly to diseases affecting the heart vascular system and lungs, with smoking being a major risk factor for heart attacks, strokes, peripheral arterial disease, chronic obstructive pulmonary disease (COPD, including emphysema and chronic bronchitis), cancer (particularly lung cancer, cancers of the larynx and mouth, esophageal cancer and pancreatic cancer). The WHO states that much of the disease burden and premature mortality attributable to tobacco use disproportionately affect the poor, of the 1.22 billion smokers, 1 billion of them live in developing or transitional economies (WHO, 2008).

Chronic obstructive pulmonary disease (COPD): The global burden of disease and risk factors project confirmed that, in 2004, COPD was the fourth leading cause of death worldwide, accounting for 5.1% of total deaths. A further estimate is that, in high-income countries, 73% of COPD mortality is related to smoking, while in low to middle income countries only 40% is related to smoking (WHO, 2008). Chronic obstructive pulmonary disease (COPD) is the clinical condition arising from either chronic bronchitis or emphysema (associated with loss of lung tissue), or both. Smoking is the main cause of COPD; the risk of COPD is increased by a factor

of 10–14 in tobacco user compared to individuals who are neither active nor passive smokers (Doll *et al.*, 2004).

Recent predictions based upon current exposures to risk factors and disease trends suggest that COPD will become the third commonest cause of death globally by the year 2030, eclipsing deaths from human immune deficiency (HIV) and TB (WHO, 2008). This dramatic prediction is based on the fact that the epidemic will center on the large populations of developing countries in Africa, Asia, the Indian subcontinent and China, where there is not only an increasing uptake of tobacco smoking through the targeted marketing strategies of tobacco companies, but also in many countries a high and rising prevalence of cofactors such as air pollution and respiratory infections, important in the pathogenesis of COPD (Orozco *et al.*, 2006).

Smoking and cardiovascular disease*:* Exposure to nicotine also provokes some unwanted health consequences such as increased blood pressure and increased heart rate. Tobacco use is a major risk factor for coronary heart disease (CHD) with a dose response correlation between CHD morbidity and mortality and the number of cigarettes smoked (Kannel, 1981). Tobacco use induced increased insulin resistance may underlie the clustering of the metabolic and hemodynamic abnormalities that have potent atherosclerotic properties, designated metabolic syndrome. Thus, the metabolic syndrome may partly account for the observed link between tobacco use and atherosclerotic disorders (Dzien *et al.*, 2004) though components of tobacco may also directly contribute to diseased arterial endothelia. Exposure to tobacco smoke in childhood may play a role in the development of early atherosclerosis (Kallio, 2009). All the risk factors which include hypertension, diabetes mellitus, dyslipidemia, and smoking were associated with elevated risk of Cardiovascular Diseases CVD. Patients with alterations in the lipid profile had a higher risk of Venous Thromboembolism (VTE) whereas subjects who smoke presented twice the risk of venous thrombosis (Aránzazu *et al.*, 2013).

Nicotine as addictive drug: According to the American Heart Association nicotine provokes changes in the brain that makes tobacco users want it more and more. Nicotine is a powerful psychoactive agent that has a variety of central and peripheral nervous system effects as well as effects on the cardiovascular, endocrine, gastrointestinal, and skeletal motor systems. The action

of nicotine on the brain occurs rapidly after smoking and this is believed to provide optimal reinforcement for nicotine dependence. The role of nicotine is to maintain the addiction and other substances in tobacco smoke, particularly tar and some additives (such as ammonia and acetaldehyde) and some of the gaseous components such as carbon monoxide are the main direct causes of disease (Leone *et al.*, 2010). The addictive properties of tobacco smoke are attributed to nicotine, the principal tobacco alkaloid in smoke. Minor tobacco alkaloids include nornicotine, anatabine and anabasine (Hukkanen *et al.*, 2005).

***Smoking and renal disease**:* Data suggest that tobacco use increases the risk of chronic kidney disease, as manifested by proteinuria and/or lowered glomerular filtration rate (GFR), even in generally healthy adults. The mechanisms of smoking induced renal damage are not entirely clear; however, it is postulated that they involve acute haemodynamic changes, such as increased arterial blood pressure and intraglomerular pressure as well as chronic effects such as inflammation, endothelial dysfunction and arterial stiffness (Orth, 2004). Kidney failure by itself leads to an increased CVD risk, which is further aggravated by smoking (Efstratiadis *et al.*, 2008).

***Smoking and diabetes**:* Several hypotheses have been proposed to link tobacco use and incidence of diabetes. Smoking induces insulin resistance or hyperinsulinemia and leads to type 2 diabetes (Nakanishi *et al.*, 2000). Secondhand smoke exposure in childhood and adulthood are associated with a higher rate of type 2 diabetes (lajous *et al.*, 2013).

***Smoking and cancer**:* The tobacco alkaloids are not generally considered carcinogenic, but are accompanied by carcinogens in each puff of smoke. Study indicates nicotine has only limited capacity to initiate tumor formation; it can facilitate the progression and metastasis of tumors pre-initiated by tobacco carcinogens in immune competent mice (Davis *et al.*, 2009). Tobacco use has also been associated with an increased risk of chronic pancreatitis and pancreatic cancer, suggesting that tobacco smoke may be directly toxic to the pancreas (WHO, 2008). Smoking can cause cancer almost anywhere in the body, including the: mouth, nose, and throat, larynx, trachea, esophagus, lungs, stomach, pancreas, liver, kidneys and ureters, bladder, colon and rectum, cervix, bone marrow and blood (leukemia) (CDC, 2014).

1.2.7 Cotinine as a biomarker for intake of nicotine

Biomarkers refer to indicators of a particular disease state or a particular physiological state of an organism. In cigarette smoke, there are more than 4,000 chemicals, among which nicotine and its major metabolite cotinine to be biomarkers of tobacco smoking and environmental tobacco exposure (Jaleel *et al.*, 2007; Leone, 2005). The presence of cotinine in biological fluids indicates exposure to nicotine. Because of the long half-life of cotinine it has been used as a biomarker for daily intake, both in tobacco user and in those exposed to secondhand tobacco smoke (Benowitz, 1996). There is a high correlation among cotinine concentrations measured in plasma, saliva, and urine and measurements in any one of these fluids can be used as a marker of nicotine intake. Urine cotinine is widely used as a biomarker due to its higher concentration in the urine matrix as compared to other matrices, resulting in accurate detection in samples (Lafay *et al.*, 2010). There is, however, individual variability in the quantitative relationship between steady state cotinine levels and intake of nicotine. This is because different people convert different percentages of nicotine to cotinine (usual range 50–90%), and because different people metabolize cotinine differently at different rates (usual clearance range 20–75 mLmin^{-1}) (Benowitz, 1996). While cotinine functions fairly well as a marker of nicotine intake, it is not perfect due to individual variation in metabolism. Cotinine metabolism is affected by factors such as race, gender, age, genetic variation in the liver enzyme cytochrome (CYP2A6), and/or by the presence of pregnancy, liver or kidney disease (Benowitz *et al.*, 2008).

1.2.8 Metabolic syndrome

Although the clinical importance of metabolic syndrome is recognized today many uncertainties remain and a lively debate on its diagnostic criteria is ongoing. Metabolic syndrome was identified 40 years ago; the actual criteria that define this syndrome have been formulated very recently. Metabolic syndrome remains an evolving concept with different work groups presenting varied criteria for this condition. The state of controversy is reflected in the fact that the American Diabetes Association and the European Association for the Study of Diabetes made a joint statement in 2005 that no existing definition of this condition meets the criteria of a syndrome (Kahn *et al.*, 2005).

From a physic-pathological perspective, this syndrome is associated with multiple metabolic changes, physical findings and hemodynamic disorders, such as reduced glucose tolerance, type 2 diabetes, insulin resistance, arterial hypertension, fatty liver, altered lipid metabolism (hypertriglyceridemia, hypercholesterolemia, reduced high-density lipoprotein, and elevated low-density lipoprotein), obesity, waist circumference and abnormalities of the coagulation system. Table 1.1 presents the 2009 consensus criteria proposed by International Diabetes Federation Task Force on Epidemiology and Prevention, National Heart, Lung, and Blood Institute, American Heart Association, World Heart Federation International Atherosclerosis Society, and International Association for the Study of Obesity (Alberti *et al.,* 2009).

Table 1.1, 2009 Consensus criteria for metabolic syndrome and diagnosed if three or more of the following criteria are present.

Parameter	**Threshold level**
Elevated waist circumference	**Men ≥ 94cm** **Women > 80cm**
Elevated triglycerides	**1.7mmol/L or ≥ 150mg/dL**
Reduced high density lipoprotein	**< 1mmol/L or < 40mg/dL for men** **< 1.29 mmol/L) or < 50 mg/dL in females**
Cholesterol	**>200 mg/dL**
Elevated blood pressure	**Systolic≥ 135mmHg** **Diastolic ≥ 85mmHg**
Elevated fasting blood sugar	**≥ 5.6 mmol/L≥ 100mg/dL**

Source: Alberti *et al.,* 2009

1.2.8.1 Smoking and metabolic syndrome

Metabolic syndrome includes the constellation of various metabolic abnormalities and confers an increased risk for diabetes and cardiovascular diseases. Studies exploring the prevalence of metabolic syndrome have come up with varied findings, primarily due to differences in cut off

points for various components of the syndrome. It is estimated that around 20–25% of the world's adult population has metabolic syndrome (IDF, 2006). Exposure to tobacco smoke in all forms has been associated with metabolic syndrome. Moreover, the number of cigarette smoking per day showed significant association with metabolic syndrome (Suriyaprom *et al.,* 2010; Berlin *et al.,* 2012). Although the evidence base is much wider and stronger for the smoking, even the smokeless form of tobacco has been associated with increased prevalence of metabolic syndrome. Additionally exposure to SHS (also called environmental tobacco smoke, ETS) has been found to contribute to increased prevalence of this syndrome (Xie *et al.,* 2010; Cena *et al.,* 2013; Shrivastava *et al.,* 2014; Gharipour *et al.,* 2008).

Other studies shows tobacco use acts at multiple levels in the etio-pathogenesis of metabolic syndrome. There is a positive dose response relationship between the daily number of cigarettes smoked and the risk of metabolic syndrome (Khalid *et al.,* 2014). Abnormalities in lipid profile and glucose tolerance are directly correlated with smoking pack per years (Parchwani *et al.,* 2013). Both former and current tobacco user have an increased incidence of metabolic syndrome, the risk persist up to 20 years after quitting tobacco use (Wada *et al.,* 2007). Exposure to tobacco smoke is associated with a 4-fold increased risk of development of metabolic syndrome among adolescents who are either overweight or at risk of being overweight (Weitzman *et al.,* 2005).

Smoking and Insulin Resistance: Increased insulin resistance might underlie the metabolic and hemodynamic abnormalities contributing to metabolic syndrome. Tobacco use, even short term tobacco use has been associated with reduced insulin sensitivity and development of insulin resistance (Filozof *et al.,* 2004). Tobacco use increases the circulating levels of hormones such as cortisol, catecholamines, and growth hormone which have insulin antagonistic actions. High circulating levels of free fatty acids also interfere with insulin mediated glucose uptake. Autonomic dysfunction as reflected by increased heart rate has also been associated with insulin resistance (Palatini *et al.,* 1997). It was evident from the studies of different scholar, in contrast to control group, smokers and gutka/smokeless tobacco consumers had significantly higher levels of total serum cholesterol and higher fasting blood glucose levels (Mukherjee and Chatterjee, 2013). Cigarette smoking is associated with insulin resistance, impaired fasting glucose and lower high density lipoproteins (HDL) (Yu kang *et al.,* 2009). Similarly the number

of pack-years was positively associated with the risk for impaired insulin secretion in a dose dependent manner (Morimoto *et al.,* 2012; Rafalson *et al.,* 2009).

The mechanism of insulin resistance induced by smokeless tobacco use might be different from that of smoking as the level of growth hormone (an insulin counter-regulatory hormone) has not been found to be raised among users of smokeless tobacco forms (Xie *et al.,* 2010). Secondhand smoke exposure in childhood and adulthood are associated with a higher rate of type 2 diabetes (lajous *et al.,* 2013).

Smoking and Dyslipidemia: Tobacco use leads to increase in triglyceride levels and reduction in HDL cholesterol by increasing sympathetic activity. Tobacco use causes higher fasting plasma cortisol concentrations, resulting in an increase in visceral adipose tissue (Chiolero *et al.,* 2008; Gharipour *et al.,* 2008). The link between cigarette smoking and dyslipidemia can be explained by the elevated plasma free fatty acids (FFAs) which is caused by decreased lipoprotein lipase activity (Mjos, 1988), increased 3-hydroxy-3-methylglutaryl-CoA reductase activity, and increased glucose-6-phosphatase dehydrogenase activity (Cena, 2011). These FFAs stimulate the hepatic synthesis and secretion of cholesterol, which increases the production of very low density lipoprotein (VLDL) and then increases serum triglyceride concentrations and decreases HDL concentrations (Audrain and Benowitz, 2011).

Smoking and Adiposity: The relation between smoking and obesity is incompletely understood. On the one hand, nicotine acutely increases energy expenditure (EE) and could reduce appetite, which likely explains why smokers tend to have lower body weight than do nonsmokers and why smoking cessation is frequently followed by weight gain (Ward *et al.,* 2001). On the other hand, studies indicate that heavy smokers (ie, those smoking a greater number of cigarettes/day) have greater body weight than do light smokers (Chiolero *et al.,* 2007; John *et al.,* 2005; Bamia *et al.,* 2004).

Tobacco user tends to have a higher waist and lower hip circumference compared to individuals who are neither active nor passive smokers (Panagiotakos *et al.,* 2004). Waist circumference is closely related to the visceral adipose tissue distribution. The visceral adipose tissue distribution, in turn, is influenced by the serum cortisol (Pasquali and Vicennati, 2000) and sex hormone

concentrations (low estrogen and testosterone) (Haarbo *et al.*, 1991). There is increasing evidence smoking affects body fat distribution and associated with central obesity and insulin resistance (Houston *et al.*, 2006).

Smoking and Hypertension: Tobacco use as a risk factor for hypertension is not well established. But different studies support tobacco use leads to an acute increase in blood pressure. Nicotine acts as an adrenergic agonist, mediating local and systemic catecholamine release. Additionally, it causes release of vasopressin. Tobacco use also causes an increase in heart rate which found to be an independent risk factor for hypertension (Xie *et al.*, 2010). Serum cotinine levels were found to be positively associated with pre hypertension in the age, sex-adjusted model in one study (Alshaarawy *et al.*, 2013). Cigarette smoking plays a strong role in the development and progression of cardiovascular damage, primarily atherosclerotic lesions (Leone *et al.*, 2010).

1.2.9 Smoking cessation

Monitoring tobacco exposure by the use of urine nicotine metabolite analysis has become an informative tool for evaluating the effectiveness of regulations intended to limit public exposure (Hecht *et al.*, 2010). Understanding the various metabolic processes involved in nicotine uptake and clearance may aid in optimizing and customizing cessation programs to improve their success rates (Lerman *et al.*, 2006, Ebbert *et al.*, 2007).

Effective management of tobacco use requires an individualized comprehensive intervention plan. All users should be advised to quit. Those who have not yet started should be advised not to initiate. Various pharmacological and non-pharmacological interventions are available for treatment of tobacco dependence. The medications approved for treatment of tobacco dependence include the Nicotine Replacement Therapy (NRT), bupropion-sustained release (bupropion-SR), varenicline, clonidine, and nortryptiline (Grief, 2011). Cytisine was found to be superior to nicotine-replacement therapy in helping smokers quit smoking, but it was associated with a higher frequency of self-reported adverse events (Walker *et al.*, 2014). NRT is one of the most commonly used first line therapy for tobacco dependence. It prevents the withdrawals associated with abstinence from tobacco products. It avoids the harmful consequence of the non-

nicotine ingredients of tobacco products. It is freely available as a gum and other delivery forms include patch, inhaler, lozenges, and nasal spray. The dose of nicotine is determined by the amount of tobacco product used by the individual (Miranda *et al.,* 2013). Nevertheless, sustained smoking cessation is difficult to achieve, and less than 10% of smokers who try to quit, even with smoking cessation medications, maintain abstinence: over 90% resume smoking within a year.

1.2.9.1 Smoking cessation and metabolic syndrome

Tobacco use cessation lowers the risk of developing metabolic syndrome. It has been associated with a reduction in the triglyceride level and improved HDL level (Rosmond and Bjorntorp 1999). It also improves insulin sensitivity as well (He Y *et al.,* 2009). However, increase in body weight subsequent to quitting tobacco use could contribute to the increase risk of metabolic syndrome. The mechanisms of weight gain after quitting tobacco use include increased calorie intake, decreased resting metabolic rate, decreased physical activity, and increased lipoprotein lipase activity (Hishida *et al.,* 2009).

Additionally, nicotine, through its anti-estrogenic effects, induces lipolysis by stimulating the sympathetic nervous system (Corella *et al.,* 2002). An imbalance between lipid intake and lipid oxidation consequent to smoking cessation can also contribute to increased body fat. However, the potential benefits of tobacco use cessation are multiple. Hence, it is important to advise all users to quit. An appropriate dietary and exercise schedule should be formulated for those interested in quitting tobacco use to address quitting associated weight gain.

1.3 Statement of Problem

Tobacco use is currently considered as one of the greatest problems in public health worldwide and it is one of the most preventable causes of death. Globally the use and sale of substances such as alcohol and tobacco are causing substantial levels of health problems. Citing the death of 6 million individuals worldwide every year due to tobacco related diseases the WHO states that tobacco use should be considered as a pandemic (WHO, 2009). Tobacco users are considered to have an acute increase in blood pressure than neither active nor passive smokers (Slagter *et al.,* 2013). Serum cotinine levels were found to be positively associated with prehypertension in the age, sex-adjusted model in one study (Alshaarawy *et al.,* 2013). There is a positive dose response relationship between the daily number of cigarettes smoked and the risk of metabolic syndrome (Khalid *et al.,* 2014), it leads to abnormalities in lipid profile and glucose tolerance which correlated with smoking pack per years (Parchwani *et al.,* 2013). Tobacco use is also impaired response to glucose tolerance tests and insulin resistance (Yu kang *et al.,* 2009).

A few studies have addressed the health effects of using substances such as cigarettes and khat in Ethiopia, focusing on psychiatric morbidity and psychosocial problems and their association with unsafe sexual behavior by (Kebede *et al.,* 2005). Cigarette smoking and khat chewing among men in Addis Ababa indicates associated with high blood pressure, an established risk factor for cardiovascular disease by (Tesfaye *et al.,* 2008).

According to International Diabetes Federation (IDF) and Adult Treatment Panel (ATP) III criteria, the overall prevalence of metabolic syndrome was 12.5% and 17.9%, respectively in Ethiopia (Tran *et al.,* 2011). Prevalence of ever tobacco user was 12.2% among school adolescents in eastern Ethiopia (Reda *et al.,* 2012). 11.8% and 0.2% male and female respectively, were current tobacco user in Butajira town (Schoenmaker *et al.,* 2005). Studies have tended to focus on large cities and focused on the prevalence (Kebede, 2002; Seyoum *et al.,* 1999; Betre *et al.,* 1997) and the scale of tobacco consumption in small towns or rural areas, where more than 80% of the population lives is not well studied.

Moreover, assessments of tobacco users and passive smokers based on the levels of cotinine as biomarker of cigarettes smoking in conjunction with parameters metabolic syndrome were never

been done to the best of my knowledge. The increase in the number of tobacco users requires effort to know cotinine levels for tobacco users and passive smokers and its possible effect on metabolic syndrome. Therefore the research focused on determination of urine cotinine levels for adult tobacco users and passive smokers in conjunction with parameters of metabolic syndrome in Chena wacha.

1.4 Significance of the Study

Little information is available on the prevalence of tobacco use, passive smoking and metabolic syndrome in Ethiopian communities. The purpose of this research is to increase the knowledge about the relationship of urine cotinine levels and tobacco use and passive smoking as well as to investigate parameters of metabolic syndrome and its possible relationship with tobacco use in order to improve future smoking cessation and prevention interventions in Chena wacha. In view of the increase in the number of tobacco users there is a need to investigate urine cotinine levels of tobacco users and passive smokers and the possible influence of tobacco use and passive smoking on parameters of metabolic syndrome.

1.5 Hypothesis

People who are active tobacco users and passive smokers release nicotine metabolite (cotinine) which can be detected using urine.

Smoking has been linked to metabolic syndrome; which typically it includes abnormal body mass index, hyperglycaemia, elevated blood pressure and abnormality in lipid profile.

2. OBJECTIVES

2.1 General Objective

The general objective of this study is to determine urine cotinine levels for adult tobacco users and passive smokers and to investigate parameters of metabolic syndrome in these individuals in Chena wacha.

2.2 Specific Objectives

- To determine urine cotinine levels of active and passive smokers in Chena wacha.
- To determine parameters of metabolic syndrome in active and passive smokers in Chena wacha
- To assess any possible relation between tobacco use and metabolic syndrome.
- To assess the agreement between self-reported smoking and urine cotinine levels.

3. METHODOLOGY

3.1 Study Area

The study conducted at Chena woreda, kaffa zone, SNNPR, South west Ethiopia 510 km far from the capital, Addis Ababa, which is found in kaffa Zone (Southern Nations and Nationalities).The residents are speaking Kaffi nono which belongs to omotic language. The topography is characterized by slopping and rugged areas with very little plain land. The altitude of the zone varies within the range of 700-3400 meters above sea level. It has a total area of 9,616,399.5m^2. It is enclosed between 8^{o}33" 00' -8^{o}36" 00' north latitude and 39^{o}17" 57' - 39^{o}21" 15' east longitude. Based on the 2007 census conducted by the CSA, Chena had a total population of 158,449, of whom 78,150 are men and 80,299 women; 11,629 or 7.34% of its population are urban dwellers (CSA, 2011).

3.2 Source Population

The source population for the study was all the active and passive smokers living in the Chenna wacha.

3.3 Study Population

The study participants were all active and passive smokers included in the study. A total number of seventy (70) individuals were included from the target population.

Inclusion criteria: The study subjects included were tobacco users and second hand smokers (passive smokers) living in the particular study area, invited to participate in the research.

Exclusion criteria: The exclusion criteria are significant clinically overt disease, and treatment that might disturb the measurements performed in the study, or unwillingness to participate and other tobacco user than cigarette.

3.4 Study Design and Period

A cross-sectional study was conducted among active and passive tobacco smoking adults living in Chenna wacha. The study was conducted between the months of October, 2014 and December, 2014.

3.5 Sample Size Determination and Sampling Technique

The sample size was estimated based on single population proportion formula developed by (Cochran, 1963)

$$n = \frac{\left(Z_{\frac{\alpha}{2}}\right)^2 P\ (1-P)}{d^2}$$

Where n_0 - is the sample size,

Z^2- is the abscissa of the normal curve that cuts off an area α at the tails (1 – α equals the desired confidence level, 95%),

d -is the desired level of precision,

p- is the estimated proportion of an attribute that is present in the population.

The sampled individuals were selected using a purposive non probability sampling method. A total of 70 individuals including 65 adult and 5 children, tobacco users and passive smokers, were selected from population living in the Chena wacha. The required sample size, 59, calculated for 95% C.I (confidence interval), 5% a sampling error and 4% estimated level of tobacco users in the targeted population and adding 11% non-response rate (70).

3.6 Method of Data Collection

Except for five children, taken from four families to assess passive smoking level in children using urine cotinine levels, all tobacco users and passive smokers who volunteered to participate in the research were also assessed for fasting blood glucose, blood pressure, BMI and waist circumference, but only 18 active cigarette smokers came back for blood sampling for the additional purpose of determining their lipid profile test that is used for assessment of parameters of metabolic syndrome. Urine from all participants was taken and evaluated for cotinine levels. These tests were done in conjunction with experienced health care professionals.

Besides this, evaluation of the self-reported smoking status and other lifestyle factors, including other substance use like alcohol and khat was done on a questionnaire basis. Smoking history was obtained from the participants which was then used to calculate smoking pack- years by

using formulae: Pack-years = {(Number of cigarettes smoked per day × Number of years smoked)/20}; where one pack of cigarettes is equivalent to 20 cigarettes. In this study, active smokers were classified into mild to moderate (Group I) and heavy (Group II) based on the number of pack-years as 1 – 10 and more than 10 respectively (Parchwani *et al.*, 2013).

Questionnaires

After informed consent was obtained, all individuals (active and passive smokers) completed the questionnaires. The questionnaire covers a range of socio-demographic factors and tobacco use and smoking habits and exposure. The questions on tobacco use included: Do you use any form of tobacco, and if so what types, how much, how frequently and for how long? Participants were asked the age of onset of tobacco use, whether they are exposed to passive smoking, and details of passive smoking such as frequency, extent and specific environments in which they passively smoke. To assess socio-demographic status, questionnaire on age, education, and occupation were asked. Measurements of blood pressure, BMI (weight, height), cotinine levels and fasting blood sugar were registered in the questionnaire. Questionnaires were adopted from Global Tobacco Surveillance System (GTSS, 2011) and translated to local languages (Annex 1).

Operational definition

Tobacco smoking: Is the practice of burning tobacco and inhaling the smoke. A more broad definition may include simply taking tobacco smoke into the mouth and then releasing it, as is done by someone with tobacco pipes and cigars.

Passive smoking (secondhand smoking): Is the involuntary consumption of smoked tobacco also refers (Environmental Tobacco Smoke). It usually refers to non-smoking individuals who breathe in the smoke of individuals who smoke.

3.6.1 Ethical considerations

Ethical clearance was obtained from Addis Ababa University College of Health Sciences Department of Biochemistry, protocol number M.Sc. Thesis 02/14. A letter of cooperation was written from department authorities. During distribution of questionnaires, subjects were informed that the information collected would kept anonymous to protect individual privacy and

the objective of the study was explained to the study participants prior to obtaining their informed consent. The subjects were briefed about the confidentiality of their response and the importance of providing correct information, and made fully aware participation help them get necessary advice regarding active and passive tobacco smoking and its health effects that possibly help them to quit smoking and to protect others from passive exposure. The selection of subjects and the collection of specimens from the subjects were done after prior notice and approval for all protocols involved, and made clear that the participants under study have the right to refuse participation or quit participation at any point of the interview, data-collecting, clinical and laboratory test if they so wish.

3.6.2 Variables

3.6.2.1 Dependent variable

- Cotinine levels
- Fasting blood sugar (FBS)
- Waist circumference (WC)
- Body mass index (BMI)
- Diastolic blood pressure (DBP)
- Systolic blood pressure (SBP)
- Total Cholesterol level (TC)
- Triglyceride (TG)
- High density lipoproteins (HDL)

3.6.2.2 Independent variables

- Age
- Smoking type
- Sex
- Number of cigarettes smoked per day or pack-years
- Educational status
- Occupation
- Years of smoke
- Frequency of exposure to smoking environment

3.6.3 Anthropometric analysis

There are several diagnostic criteria for metabolic syndrome but in this particular study I used the 2009 consensus criteria proposed by International Diabetes Federation Task Force on Epidemiology and Prevention, National Heart, Lung, and Blood Institute, American Heart Association, World Heart Federation International Atherosclerosis Society, and International Association for the Study of Obesity it includes, waist circumference, total cholesterol, triglyceride, HDL, fasting blood sugar and blood pressure but I also used (BMI) body mass index in addition the above parameters to measure metabolic syndrome. Anthropometric data (weight, height and waist circumference) were collected according to WHO steps manual (WHO, 2010).

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 scale and their height measured with soft measuring tape. The body mass index is then calculated as the individual's weight in kilograms divided by the square of the height in meters ($BMI = (weight\ in\ kg) / (height\ in\ metres)^2$). Subjects are considered underweight if their BMI is <18.5, normal if their BMI is 18.5-24.9, overweight if their BMI is 25-29.9, obese if their BMI is 30-39.9 and morbidly obese if their BMI is > 40.

Waist circumference is the best 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 a soft measuring tape on standing subjects at a point midway between the inferior angle of the ribs and the anterior superior iliac crest, at the superior aspect of the umbilicus.

3.6.4 Biochemical analysis

Cotinine Determination: Cotinine has a much longer half-life (20 hours) than nicotine (2 hours) and thus is considered a more useful marker in assessing tobacco use (Gilbert, 1993). Investigation done between different levels of smoking group based on questionnaire data and their urine cotinine levels, using COT® one step cotinine test device. DN: 1150311801 (distributed by Innovacon, Inc., San Diego, CA, USA) cotinine kits were used for urine cotinine

assessment. It is a lateral flow chromatographic immunoassay for the detection of cotinine in human urine at a cut-off 200 ng/mL.

The COT One Step Cotinine Test Device (Urine) is an immunoassay based on the principle of competitive binding. The test utilizes a monoclonal antibody to selectively detect elevated levels of cotinine in urine. Cotinine in the urine sample competes with the cotinine conjugate in the test kit for binding sites on the antibody. During testing, a urine specimen migrates upward by capillary action. Cotinine, if present in the urine specimen below 200 ng/mL, will not saturate the binding sites of antibody coated particles in the test device. The antibody coated particles will then be captured by immobilized cotinine conjugate and a visible colored line will show up in the test line region.

The colored line will not form in the test line region if the cotinine level in the urine sample exceeds 200 ng/mL because the urine cotinine will saturate all the binding sites of anti-cotinine antibodies. A cotinine positive urine specimen will not generate a colored line in the test line region because of cotinine competition, while a cotinine negative urine specimen or a specimen containing a cotinine concentration less than the cut-off will generate a line in the test line region. To serve as a procedural control, a colored line will always appear at the control line region indicating that that proper volume of specimen has been added and membrane wicking has occurred.

Procedure: The cotinine test device was placed on a clean and level surface. The dropper was held vertically and 3 full drops of urine (approx. 100 μL) were added to the specimen well of the test device. Air bubbles in the specimen well were removed. The result should be read within 5 minutes.

Figure 3.1 Typical COT test kit result: left, one band was positive, indicating more than 200 ng/mL cotinine in the urine sample, while right two bands present, indicating a negative uirine cotinine test result (Chenna wacha, November 19-27, 2014).

Blood pressure: Blood pressure was measured using a digital self-inflating sphygmomanometer (Heuer model, based on WHO recommendations) in the sitting position after at least a 5-minute rest. To improve the reliability of measurement two readings were taken with interval of minutes and the average was used (raised blood pressure: SBP $\geq$ 130 mm Hg or DBP $\geq$ 85 mm Hg) (Lemogoum *et al.,* 2003).

Blood sugar: Fasting blood was measured using glucose meter (Sensor card blood glucose monitoring system). The sensor card is designed to provide easy and accurate method for determination of capillary whole blood glucose content. This analysis applies enzyme glucose oxidase and based on advanced electromechanical technology that is specific for β-D-glucose

measurement. Test strips designed in such a way that blood sample absorbed in the reaction zone after blood has been applied to the test strip. In the reagent zone glucose oxidase triggers the oxidation of glucose in blood. Intensity of formed electron is measured by meter and correlates well with the concentration of glucose in the blood sample.

Procedure: The tip of the subject's middle finger was pricked with a sterile micro let lancet to get a drop of blood. After putting a glucose test strip into the blood glucose meter, the subject's blood was transferred to the test strip (sensor card) by touching the incipient blood droplet on the finger with the tip if the test strip designed to receive blood. The blood glucose meter gives the test results within a minute or less (raised fasting blood glucose (FBG) ≥ 100 mg/dL 5.6 mmol/L).

Lipid profile: For fasting lipid profile test 5 mL blood were drawn from antecubital vein of the arm of participant into anti-coagulant free blood collection vacutainer tubes. The whole blood was left to stand vertically for 45 minutes in cold place and the serum from the sample was separated using sterile pipette immediately. The extracted serum was stored at 2 to 8°c up to one week until processed for high density lipoprotein-cholesterol (HDL-c), triglyceride and total cholesterol. The values were compared with standards. Raised serum triglycerides (TG) level: ≥ 150 mg/dL (1.7 mmol/L): reduced HDL cholesterol: < 40 mg/dL (1.03 mmol/L) in males and < 50 mg/dL (1.29 mmol/L) in females and raised total cholesterol > 200 mg/dL

Triglyceride (TG) determination: *Method and principle*: The current method for triglyceride determination employs by Trinder (Barham & Trinder, 1972; Trinder, 1969). The triglycerides are determined after enzymatic hydrolysis with lipases. Indicator is quinoneimine formed from hydrogen peroxide, 4-amino-antipyrine and 4-cholorophenol under the catalytic influence of peroxidase.

Contents: 15mL; 100mL or 250mL Monoreagent**,** 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.

Triglycerides $\xrightarrow{\text{lipases}}$ glycerol + fatty acids

Glycerol +ATP $\xrightarrow{\text{GK}}$ glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O_2 $\xrightarrow{\text{GPO}}$ dihydroxyacetone phosphate + H_2O_2

H_2O_2 + 4-aminoantipyrine $\xrightarrow{\text{POD}}$ quinoneimine + HCL +H_2O + 4-chlorophenol

Procedure: Ten microliters of sample were pipetted into a cuvette, containing 1000 µL buffer reagent, mixed and incubated for 10minutes at 20-25^{0}C or for 5 minutes at 37^{0}C. Measure absorbance of the sample and standard against the reagent blank (ΔA) within 60 minutes.

$$C = 200 \times \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \text{ [mg/dL]}$$

Or

$$C = 2.28 \times \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \text{ [mmol/l]}$$

Total cholesterol determination: *Principle and methodology:* The method uses Trinder (1969) color system. The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidase. The intensity of the red color produced proportional to the total cholesterol in the sample when read at 500 nm.

Cholesterol ester + H_2O $\xrightarrow{\text{CHE}}$ Cholestrol + fatty acid

Cholesterol + O_2 $\xrightarrow{\text{CHO}}$ cholestene-3-one + H_2O_2

$2H_2O_2$ + 4-aminophenazone + phenol $\xrightarrow{\text{POD}}$ quinoneimine + $4H_2O$

Contents: 4x 30mL, 3x250mL or 4x100mL enzyme reagent, Phosphate buffer (pH 6.5) (30 mmol/l), 4-Aminophenazone (0.3 mmol/l), Phenol (5 mmol/l), peroxidase (>5KU/l), Cholestrol esterase (>150U/l), Cholesterol oxidase (>100U/l), Sodium azide (0.05%) and 3mL of standard

Procedure: In a cuvette, 10µL of sample, 1000 µL of buffer reagent were mixed and incubated for 10 minutes at 20-25^{0}C or for 5 minutes at 37^{0}C. Absorbance of the sample was measured at 500 nm against the reagent blank (ΔA) within 60 minutes and standard solutions were used to calculate the concentrations of total cholesterol in the serum samples.

$$C = 200x \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \text{ [mg/dL]}$$

$$\text{Or } C = 5.17 \times \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \text{ [mmol/l]}$$

HDL-C determination: *Principle and methodology*: The assay combines two specific steps: in the 1st step chylomicrons, VLDL and LDL cholesterol are specifically eliminated and destroyed by enzymatic reactions. In the 2nd step remaining cholesterol from the HDL fraction is determined by well-established specific enzymatic reactions in the presence of specific surfactants for HDL (Gordon *et al.*, 1977).

1st step:

LDL, VLDL, and Chylomicrons $\xrightarrow[\text{Specific conditions}]{\text{CHE + CHO}}$ Cholestenone $+H_2O_2$

$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$

2nd step:

HDL $\xrightarrow[\text{Specific surfactants}]{\text{CHE + CHO}}$ cholestenone + H_2O_2

H_2O_2 + chromogen $\xrightarrow{\text{peroxidase}}$ quinone pigment

Enzyme reagent (white cap): Good's buffer, pH6.6 (25^{0}C): (100 mmol/l), Sodium chloride (170mmol/l), Cholesterol esterase (1400U/l), Cholestrol oxidase (800 U/l), Catalase (600KU/l), Ascorbate oxidase (3000U/l), N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HDAOS): (0.56mmol/l), Preservative (0.1%w/v).

Substrate (green cap): Peroxidase (3500U/l), 4-Aminophenazone (4 mmol/l), Good's buffer, pH7.0 (25 degree centigrade): (100mmol/l), Preservative (0.1% w/v), Detergents (1.4% w/v), Sodium azide (0.05% w/v).

1x4 mL Calibrator: HDL-Cholesterol (concentration see vial label)

Procedure: Reagent and cuvettes were pre-warmed at 37^0C, and the following were mixed in a cuvette at 37^oC:10µL sample (or reagent blank, or standard), 750 µL of enzyme reagent. Then 250 µL of substrate to sample was added and the mixture incubates at 37^0C for 5 minutes. Absorbance at 500 nm ΔA of samples against reagent blank after 5min. Δ sample and calibrator =Δ sample or calibrator – A reagent blank

Calculation:

$$\text{C sample} = \text{C calibrator} \times \frac{\Delta \text{ A sample}}{\Delta \text{ A calibrator}} \text{ (mg/dL)}$$

Conversion factor: C (mg/dL) x 0.02586 = C (mmol/l)

LDL-C determination: LDL was calculated according to Friedwald's formula (Friedwald *et al.,* 1972)

$$LDL = TC - (HDL - C + VLDL)$$

VLDL-C determination: VLDL-C was calculated according to the formula proposed by Wilson (Delong *et al.,* 1986).

VLDL = TG/5 or 0.2 X TG

3.7 Statistical Tool and Analysis Employed

Data on active, passive smoking and parameters of metabolic syndrome was entered into excel and analyzed using (SPSS16.0). Descriptive statistics were used to describe the socio-demographic, clinical and laboratory characteristics. p = value and chi square test (x^2) were used to represent the values of the raw data. Continuous variables were expressed as mean, standard deviation, median and interquartile range (IQR**)** while categorical variables were expressed as number (percentage). Binary and multinomial logistic regression was used to examine the associations between smoking and components of metabolic syndrome. Logistic regression also was used to examine the effect of smoking and daily tobacco use on the risk of having abnormal body mass index. This approach generated odds ratios that predicted the odds of having abnormal body mass index for the different smoking statuses.

Agreement between self-reported smoking status and the cotinine results was assessed using the Kappa statistic. Based on the criteria suggested by Cohen's Kappa values were evaluated and determined a value of less than 0 as a poor, 0.00-0.20 as slight, 0.21-0.40 as fair, 0.41-0.60 as moderate, 0.61-0.80 as substantial, and 0.81-1.00 as an almost perfect agreement (Landis and Koch, 1977). A p-value of 0.05 or less is used as cut-off level for statistical significance.

4. RESULTS

4.1 Smoking Type Based on Socio Demographic Characteristics

A total of 70 participants which constituted 46 active and 24 passive tobacco smokers were included in the research. The socio-demographic characteristics of 65 adult participants were described in (Table 4.1). The 5 children studied had only their urine cotinine tested: metabolic syndrome parameters were not measured in these children.

The mean ages of participants were 39.5 years. Difference between the type of smoking occurred between different age groups, and the majority of active smokers were in the third and second group of age classification while the majority passive smokers in the second group. Sex was also other demographic variable assessed during the study and it shows statistically significant different (p value 0.001) indicating all active smokers were male while none of the female were active smokers. Regarding assessment of other substance use in addition to smoking cigarette, active smokers tends to use alcohol and khat with the statistically significant value of (p value= 0.001) than passive smokers. Assessing the percentage of other substance use, the result indicates that 44/46 (95.7%) active smokers and 9/19 (47.4%) of passive smokers use alcohol, while 11/46 (23.9 %) of active smokers and 4/19 (21.05 %) of passive smokers were khat users. Regarding other variables including, ethnicity, marital status, educational levels, occupation, health problem and income don't show significant different between active and passive smokers groups.

Table 4.1 Smoking type based on some socio-demographic characteristics of the study participants, Chenna wacha, November 19-27, 2014

Demographics Variables		**Type of smoking**		
		Active (46) No (%)	Passive (19) No (%)	Pearson chi^2 and p- value
Sex	Male	46 (85.19)	8 (14.81)	32.05 &0.001
	Female	0 (0.00)	11(100)	
Age	18-39	9 (81.12)	2 (18.18)	6.5 & 0.039
	40-49	18 (56.25)	14 (43.75)	
	≥50	19 (86.36)	3 (13.64)	
Education	Illiterate	19 (61.29)	12 (38.71)	2.63 & 0.269
	Primary	22 (78.6)	6 (21.43)	
	Secondary and above	5 (83.33)	1 (16.67)	
Occupation	Farmer	44 (72.13)	17 (27.87)	2.50 & 0.29
	Government	1 (100)	0 (0.00)	
	No	1 (33.33)	2 (66.67)	
Marital status	Married	35 (70.00)	15 (30.00)	0.062 & 0.8
	Not married	11 (73.33)	4 (26.67)	
Other substance use	Alcohol	33(82.50)	7 (17.50)	21.12&0.001
	Khat	0 (0.00)	2 (100)	
	Alcohol and khat	11 (84.62)	2 (15.38)	
	No	2 (20.00)	8 (80.00)	
Health problem	Yes	8 (88.89)	1 (11.11)	1.66 & 0.198
	No	38 (67.86)	18 (32.14)	

Variables statistically significant at p- value < 0.05

4.2 Smoking Status of Active Smokers Based on Questionnaire Data

Assessing smoking status of active smokers, the median number of cigarette smoked and median number of years smoked was 6 and 16.5 respectively. Among the national tobacco product Nyala brand was the most frequently smoked cigarette by active tobacco users in study participants. Regarding the level of smoking, the majority of study participants were mild to moderate smokers 31/46 (67.4 %) and the remaining 15/46 (32.6%) were heavy smokers (Table 4.2).

Table 4.2 Median values and interquartile range (IQR**)** of active smokers by self-reported age onset of smoking, frequency and amount of cigarettes smoking Chenna wacha, November 19-27, 2014

Variables			**No (%)**	**Median (IQR)**
Number of cigarette smoked per day		< 10	25 (54.35)	6 (10, 4)
		10- 20	13 (28.26)	
		>20	8 (17.39)	
Level of smoking by (pack -years)	Mild to moderate	1-10	31 (67.39)	6 (12, 2.4)
	Heavy smoker	≥10	15 (32.60)	
Age onset of smoking		<18	14 (30.43)	22 (28, 16)
		18- 24	15 (32.60)	
		≥25	17 (36.95)	
Years of smoking		1-20	33 (71.74)	16.5 (25, 8)
		21-30	7 (15.21)	
		>30	6 (13.04)	

4.3 Behavioral Characteristics of Active and Passive Smokers

Assessing the behavioral characteristics, most active cigarette smokers initiating smoking by peer pressure 34/46 (73.9 %) followed by enjoyment of smoking 7/46 (15.2%) and other reasons which account for 5/46 (10.9%) and includes the sense that smoking helps them to escape from snacking on food, especially when they go to hot areas for farming, and it also protects them from the cold environment when they wake up in early morning for farming. Among smokers, 33/46 (71.7%) of them tried to stop tobacco use for a day or longer mostly because of worried about health and use money reasonably. Even though most smokers' 42/46 (91.3%) recognize smoking is harmful for health majority of them 43/46 (93.5%) smokes inside house exposing family and friends for passive smoking.

Assessing the status of getting anti-cigarette information 40/46 (86.96%) of participants respond yes while 6/46 (13.04%) said no, when asked if they had encountered anti-smoking information.

Regarding the source of the information, radio accounts for 22/40 (55%) while warnings on cigarette packs cover only 9/40 (22.5%). The results of the study indicate that 18/19 (94.7%) of passive smokers responded that passive smoking is harmful but they were involuntarily exposed to smoking environment mainly due to husband smoking inside house. The frequency by which passive smokers were exposed to a smoking environment was 16/19 (84.2%) for constant exposure and the home was the most frequent area of exposure for passive smokers 12/19 (63.2%).

Table 4.3 Baseline data according to behavioral characteristics of active and passive smokers Chenna wacha, November 19-27, 2014

Behavioral characteristic of active smokers		**No (%)**
Smoking at home	Yes	43 (93.48)
	No	3 (6.52)
Reason of starting smoking	For relaxing	7 (15.22)
	Peer pressure	34 (73.91)
	Other	5 (10.87)
Want to quit smoking	Yes	33 (71.74)
	No	13 (28.26)
Reason to quit	Not smell like smoker	2 (6.06)
	Use money reasonably	13 (39.39)
	Better role model	1 (3.03)
	Worried about health	14 (42.42)
	Other	3 (9.09)
Is smoking harmful	Yes	42 (91.30)
	No	4 (8.70)
Have you encounter any anti-cigarette information	Yes	40 (86.96)
	No	6 (13.04)
From where did you get anti-cigarette information	Television	1(2.5)
	Radio	22 (55)
	Cigarette packs	9 (22.5)
	Other	8 (20)
Behavioral characteristic of passive smokers		
Site of tobacco exposure	Home	12 (63.16)
	Work place	7 (36.84)
Duration of exposure	Sometimes	3 (15.79)
	Always	16 (84.21)
Is passive smoking harmful	Yes	18 (94.74)
	No	1 (5.26)

4.4 Agreement between Self-Reported Smoking Status and Urine Cotinine Result

The study used urine cotinine as marker to investigate self-reported active smoking and passive exposure to tobacco smoke. Interpretation of biochemical validation test for urine cotinine level was done at 200ng/mL cutoff point and result shows high proportion of active smokers were positive for the urine cotinine test which is 88.6 % while 11.3 % of passive smokers were positive for cotinine test as described in Table 4.4

Table 4.4 Agreement between self-reported smoking status and urine cotinine result Chenna wacha, November 19-27, 2014

Self-reported Smoking status	**No**	**Cotinine result**	
		No (%)	**No (%)**
		Positive	Negative
Mild - moderate smokers	31	24 (54.55)	7 (26.92)
Heavy smokers	15	15 (34.09)	0 (0.00)
Passive smokers	24	5 (11.36)	19 (73.08)
Total	70	44 (100)	26(100)

Note: Pearson chi2=29.83, p-value=0.001
Kappa (0.63): substantial test agreement between questionnaires response and cotinine test result.

In the present study five children's taken from four volunteer families who had exposure to smoking environment in the house because of frequent smoking habits of fathers in the house and result indicates 2/5 (40%) children were positive for urine cotinine.

4.5 Characteristics of Participants According to Metabolic Syndrome (MS)

As defined by 2009 consensus criteria for metabolic syndrome, overall prevalence of metabolic syndrome was 7/65 (10.8%) with at least three components of metabolic syndrome were, 5/46 (10.8 %) and 2/19 (10.5 %) among active and passive smokers respectively. The result of present study shows only one passive smoker had high body mass index this was omitted for analysis purpose and the result show there is a decrease in BMI (more underweight) proportionally with

increase in the number of cigarettes smoked per day (cigarette pack-years), taking mild to moderate smokers as reference heavy smokers becomes underweight with statistical significance (OR: 7.90, p =0.004) and passive smokers but not significant (OR: 2.09, p= 0.234) while for all BMI classes and smoking statuses, the percentage of subjects with elevated blood pressure, elevated blood glucose and elevated waist circumference don't show significant different (p> 0.05) between mild to moderate, heavy and passive smoking. The result indicate that longer frequency of passive smokers exposure to smoking environment is statistically significant with increased diastolic blood pressure (p = 0.027) but don't show statistically significant for systolic blood pressure, fasting blood sugar, waist circumference and body mass index (p> 0.05).

However there were no consistent effects of the amount of tobacco smoked on blood pressure and blood glucose. Analyzing the prevalence of each component of 2009 consensus criteria for metabolic syndrome the study found that abnormal body mass index value 27/65 (41.5%) followed by elevated fasting blood sugar 26/65 (40 %), elevated DBP 24/65 (36.9 %), low HDL cholesterol 6/18 (33.33%), elevated TG 4/18 (22.22%), elevated SBP 10/65 (15.38%) and high waist circumference 3/65 (4.62 %), both in active and passive smokers.

Table 4.5 Characteristics of the participants according to the metabolic syndrome (MS) and smoking status Chenna wacha, November 19-27, 2014

Parameters	**Active smokers (%)**		**Mean(SD)**	**Passive smokers (%)**	**Mean(SD)**	**P (value)**
	Mild to moderate	Heavy smoker				
BMI			19.03 ±1.84		19.1 ± 2.6	0.017
Underweight	8 (25.8)	11 (73.3)		7 (36.84)		
Normal	23 (74.2)	4 (26.7)		11 (57.89)		
Overweight	0 (0.00)	0 (0.0)		1 (5.26)		
Waist circumference			77.6 ± 6.59		77.8 ± 7.3	0.28
Normal	30 (96.77)	15(100)		17 (89.5)		
High	1 (3.23)	0 (0.0)		2 (10.5)		
Fasting blood sugar			95.8 ± 10.6		99.9 ± 10.3	0.08
Normal	23 (74.2)	7 (46.7)		9 (47.4)		
High	8 (25.8)	8 (53.3)		10 (52.6)		
DBP			81.80 ± 8.7		82.4 ± 10.3	0.65
Normal	20 (64.5)	11 (73.3)		11(57.9)		
High	11 (35.5)	5 (26.7)		8 (42.1)		
SBP			127.3 ± 14.6		123.9 ± 14.5	0.7
Normal	25 (80.65)	13 (86.7)		17 (89.5)		
High	6 (19.35)	2 (13.3)		2 (10.5)		
TC			115.23 ± 30.5			
Normal	18(100)					
High	0 (00.)					
TG			118.3 ± 52.78			
Normal	14 (77.78)					
high	4 (22.22)					
HDL			41.5*(48.4, 38.5)			
Normal	12 (66.67)					
Low	6 (33.33)					

Variables statistically significant at p- value < 0.05

*Data are in median (inter-quartile range) since distribution was skewed

4.6 Prevalence of Metabolic Syndrome Components Based on Smoking Years

Among smokers grouped under different years of smoking 2/7(28.6%) of 21-30 years smoker and 2/6 (33.4%) of >30 years smoker show metabolic syndrome (three parameter), while 2/7 (28.6%) of 21-30 years and 3/6 (50%) of >30 years of smokers shows two parameters of metabolic syndrome. But when consider smoking years in the range of 1-20, 18/33 (54.5%) of them with shows one parameter of metabolic syndrome and 8/33 (24.2%) were normal.

The present study shows the number of each components of metabolic syndrome increase with increased cigarette smoking years indicating the more years smoked there is more chance to develop metabolic syndromes (figure 4.1).

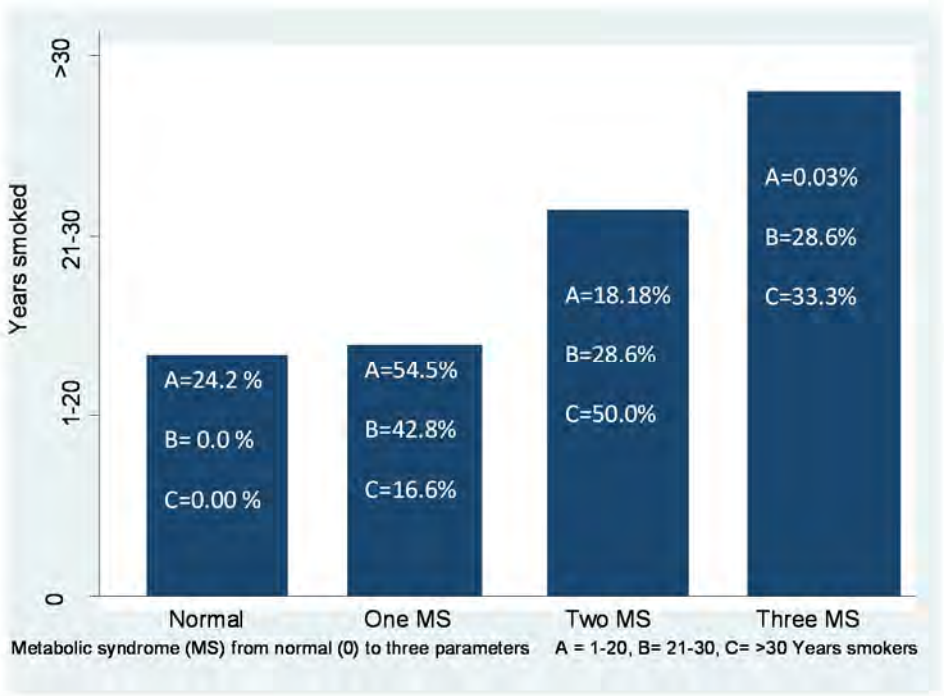

Figure 4.1 Years of smoking and components of metabolic syndrome November 19-27, 2014

5. DISCUSSION

Self-reported smoking is often underestimated by the reporting of a reduced amount of smoking or by the denial of smoking itself, often because smoking is consider as bad habit in society and so smokers may be embarrassed to admit the extent of their habit. Therefore, biochemical evaluations are generally performed to verify the validity of self-reported smoking. The present study investigated urine cotinine as a biomarker of tobacco smoking and passive exposure of nonsmokers to smoking environment using 200ng/mL cutoff point cotinine test strips in conjunction with parameters of metabolic syndrome. The present study is in line with findings of different studies. Urine cotinine is widely used as a biomarker of smoking or passive smoking due to its higher concentration in urine as compared to other body fluids, resulting in accurate detection in samples (Lafay *et al.,* 2010; Danch *et al.,* 2008). A study by Jung *et al.,* 2012 using data sets from Korea National Health and Nutrition Examination Survey (KNHANES) 2007-2010 supported the validity of using urine cotinine levels for assessment of nicotine dependence in active smokers and environmental tobacco exposure in passive smokers, when used in addition to self-administered smoking questionnaire.

Although the incidence of misclassification of smoking status decreased with an increase in the number of measurements, a single measurement of the urine cotinine level may accurately represent long-term exposure to tobacco smoke (Lee *et al.,* 2011). In several other studies smokers, non-smokers and ex-smokers were found to be different in their urine nicotine and urine cotinine levels, and the levels generally correlated well with degree of tobacco use or exposure (Man *et al.,* 2009; Babhadiashar *et al.,* 2014; Matsumoto *et al.,* 2013). A study by Gill *et al.,* 2014, in which forty asthmatic children were screened by urine cotinine testing reported 70% subjects had urine cotinine levels ≥1ng/mL though only one parent reported smoking in the home suggesting ETS exposure. Another study in Japanese subjects showed the occurrence of 20% of homozygotes or heterozygotes of the CYP2A6*4 allele, which has impaired nicotine metabolism and subsequently may underestimate the actual exposure to secondhand smoke. This report supported the idea that combination of urine cotinine measurement and self-report of parents' smoking seems to be accurate to assess the exposure to second hand smoking in mass screening (Ino *et al.,* 2011).

The present study used a cutoff value 200ng/mL of urine cotinine, for validating of active and passive tobacco smoking, the result indicates that 88.6 % of active smokers and 11.3 % of passive smokers were positive for urine cotinine using this test, with a test agreement of kappa 0.63 between questionnaire response and urine cotinine result. The present result is consistence with the findings of Balhara *et al.*, 2012 done using 131 study subjects that showed significant concordance between the self-reported recent tobacco use and urine cotinine levels for both smoking and smokeless tobacco forms irrespective of a higher (550 ng/mL) or a lower (50 ng/mL) cut off score for a urine cotinine level. Another study done by Gorber *et al.*, 2009 shows varying levels of sensitivity for self-reported estimates of smoking, depending on the population studied, the type of biological specimen used in the measurement of cotinine, and the cut-points used to identify smokers. The present study shows substantial agreement (kappa= 0.63) between self-reported smoking status and urine cotinine measurement, compared with a study done by Jhun *et al.*, 2010 using a cutoff point of >100 ng/ mL (kappa 0.20) this may be due to participants in the study reported in this thesis being more reliable in reporting their smoking history.

Taking the questionnaire as gold standard, the present study indicates a prevalence of smoking based on urine cotinine result was 39/46 (84.78%) and 7/46 (15.22) of active smokers were classified as non-smokers. The finding of the study by Wong *et al.*, 2012 shows differences between prevalence based on self-report 18.8% versus 19.1% cotinine concentration were not significant. The present finding show lower cotinine result than finding by Obianuju *et al.*, 2014 done on 81 drivers in whom urine cotinine assessment was performed, the prevalence of smoking based on self-report was 34 (42%) compared to 41 (50.6%) when based on urine cotinine, this may be due to more reliability response of participants.

This study also used urine cotinine as objective way of educating people by taking four families with children to assess passive smoking. The results indicate that a family with an active smoking male, his wife and their 9 year old son were all positive for urine cotinine. In another family, in which a male smoked inside his home, his 6 year old son was positive for urine cotinine while his wife was negative, but in other two family children's and wife's were negative. Detection of these values in urine of two out of five children (40%) examined is

worrying, especially in comparison with the other results from literature data. Mostly, urine cotinine levels in passive smokers can be 5 to 10 ng/mL, but it is also established that these values are often much higher in persons with high exposure to smoking environment (Hoffmann and Wynder, 2002). Another study done by Dostál *et al.,* 2008 determined urine cotinine using a radioimmunoassay of different cutting points 500 ng cotinine per mg creatinine (for active smoking) was detected (38%) and 20 ng/mg as the cut-off, for ETS detected 48.2% of children exposed. Corresponding with the findings of Yilmaz *et al.,* 2010 different urine cotinine levels were seen in infants of smoking mothers than levels in infants of non-smoking mothers.

In the present study 5/24 (20.8%) of passive smokers were positive for urine cotinine, while 19/24 (79.2%) were negative. From this positive number two children and two wives exposed at home due to husband smoking habit at home while one 35 year old man was positive for urine cotinine as a result of socializing with active smoker friends. Most of the passive smokers were negative for urine cotinine, which may be due to very light exposure of passive smokers to cigarette smoke, genetics, sex, age, sensitivity of the cotinine test kit used in this study, or other factors which affects cotinine metabolism. Study by Pacheco *et al.,* 2012 in Lisbon indicates urine cotinine concentrations were significantly higher in nonsmoker employees working in smoking designated areas, confirming exposure to environmental tobacco smokes. Another study by Karabela *et al.,* 2011 semi-open-air café employees indicates cotinine levels of nonsmoking employees increased with indoor particulate matter concentrations, and also with the density of smoking. Open windows and doors do not protect workers from exposure to second-hand smoke.

The result of present study indicates all heavy active smokers 15/15 (100 %) were positive for urine cotinine level at cutoff value 200ng/mL. The finding agreed with Nagano *et al.,* 2010, which found that urine concentrations of cotinine and the sum of nicotine metabolite concentrations also showed significant correlations with degree of cigarette consumption. In another study, stepwise multiple regression analysis showed the total smoking period and urine cotinine levels were positively associated with nicotine dependence (Jung S *et al.,* 2012).

The finding of this study indicates that peer pressure was the major factor for initiation of smoking 34/46 (73.9 %) followed by for relaxing 7/46 (15.2%) and other reasons, which account for 5/46 (10.9%), including that smoking helps to people to escape from snacking on food,

especially when they go to hot areas for farming; it also protect them from a cold environment when they wake early morning for farming (Table 4.3). The present finding were among factors stated by Lasebikan and Ojediran, 2012, factors such as peer pressure, availability, accessibility and affordability of cigarettes, as well as high stress levels associated with the job and the perceived need for stimulants use may contribute to the high prevalence of smoking among commercial drivers. The present study shows that 12/19 (63.1 %) of passive smokers were exposed to smoking environment at home because of smoking habits of the male adult (husband or father) inside house. In one study done by Chen *et al.,* 2011, urine cotinine levels were correlated with reported environmental tobacco smoke exposure and 71% of children with urine cotinine levels > 10 ng/mL. Smoking parents, grandparents, and non-family members were the major ETS sources. After getting detailed advice on harmful effects of tobacco smoking on health of active and passive smokers many of the smokers promised to stop smoking beside this they also promised to taken care of not to expose family and friends to tobacco smoking though we were not able to check the progress.

Smoking and Metabolic syndrome

The present study was conducted to investigate urine cotinine levels as indicators of active and passive smoking, with parameters of metabolic syndrome among adult. As defined by 2009 consensus criteria for metabolic syndrome, overall prevalence of metabolic syndrome was 7/65 (10.8%) with at least three components of metabolic syndrome in 5/46 (10.8 %) of active smokers and 2/19 (10.5 %) among passive smokers. According to ATP III and IDF definitions, the overall prevalence of metabolic syndrome was 12.5% and 17.9%, respectively in working adult of Ethiopia (Tran *et al.,* 2011). Except significant different observed for body mass index ($p = 0.017$) for different levels of smoking other variables TC, TG and HDL, cholesterol is not coinciding with other findings in the literature and this may be due to low number of participants for blood withdrawal in this study, limiting the number of individuals whose cholesterol levels could be determined, as a result of failure of many of the individuals studied to turn up for blood withdrawal and testing.

A study done by Slagter *et al.,* 2013 showed that smoking is associated with an increased prevalence of metabolic syndrome, mainly related to lower HDL cholesterol, and higher

triglycerides and waist circumference. But smoking had no consistent association with blood pressure or fasting blood glucose. Another study by Cena *et al.,* 2013 indicated that there increasing trend from light to heavy smokers, except for HDL cholesterol and there is positive association between heavy smoking and metabolic syndrome by Wakabayashi 2014 in Japanese diabetic men. The findings of many scholars support the conclusion that cigarette smoking is associated with manifestations of metabolic syndrome (Fan, 2009). There is a positive dose-response relationship between smoking level and metabolic syndrome in men (Hwang *et al.,* 2014), increased risk for developing metabolic syndrome, with longer duration of smoking (Rodrigues *et al.,* 2013). Metabolic syndrome was directly correlated with smoking pack-years by Parchwani *et al.,* 2013 and by Suriyaprom *et al.,* 2010, and after adjusting for possible confounders, current smokers who used more than 20 cigarettes per day were 2.24 times more likely to have metabolic syndrome as compared to never smokers (Calo *et al.,* 2013). It also exacerbates the adverse effects of age and metabolic syndrome (Li S *et al.,* 2014).

The present study shows the percentage occurrences of each component of metabolic syndrome in studied participants: abnormal body mass index value is the 27/65 (41.5%) followed by elevated fasting blood sugar 26/65 (40 %), elevated DBP 24/65 (36.9 %), low HDL cholesterol 6/18 (33.33%), elevated TG 4/18 (22.22%), elevated SBP 10/65 (15.38%) and high waist circumference 3/65 (4.62 %), both in active and passive smokers. This findings shows lower value for some parameters but higher for fasting blood sugar when compared with 29.4% for abdominal obesity, 40.7% for high TG level, 40.2% for low HDL, 15.4% for hypertension and 37.8% for abnormal FBS by (Shahbazian *et al.,* 2013).

Smoking and Dyslipidemia: Prevalence of each component of metabolic syndrome in study participants shows normal total cholesterol for all participants 18/18 (100%), higher TG (22.22%), low HDL cholesterol (33.33%). Study by Lima *et al.,* 2011 indicated low high-density lipoprotein cholesterol (HDL-C) was the most prevalent dyslipidemia. Another study shows cigarette smoking increases and lowers the TG and HDL concentrations, respectively but no difference among the lipid profiles of passive smokers and nonsmokers (Danch *et al.,* 2008). But the study done in Chinese nonagenarians/centenarians, cigarette smoking habits were not associated with increased risk for dyslipidemia (Ling *et al.,* 2012).

But the result of present study not coinciding with findings of different scholars which may be due to low response rate of participants for blood sampling that used for the assessment of lipid profile, study done in Southern India by Meenakshisundaram *et al.,* 2010 shows number of pack per years was directly proportional to abnormal lipid profile. Another study shows there is a positive association between heavy smoking and metabolic syndrome, which is mainly due to higher risks of central obesity and dyslipidemia, such as hypertriglyceridemia and low HDL cholesterolemia, in heavy smokers than in nonsmokers Japanese diabetic men (Wakabayashi, 2014). Smokers were having higher rate of low HDL-C than nonsmokers by (Khazale and Haddad, 2011).

Smoking and Blood Pressure: The present study indicated that 10.8% of participants had elevated blood pressure with elevated DBP in 24/65 (36.9 %) and elevated SBP in 10/65 (15.4 %) but did not show statistically significant association with different levels of smoking $p>0.05$. The finding is in agreement with different studies; for example, Abtahi *et al.,* 2011 found that a relation between the smoking status and blood pressure is not yet obvious. However, it seems that smoking cessation or at least reduction in the amount of smoking would significantly decrease blood pressure. A study by Li *et al.,* 2010 did not provide support that smoking was a risk factor of hypertension and elevated blood pressure.

A study done in Iran revealed that prevalence of hypertension was higher among smokers than nonsmokers (Abtahi *et al.,* 2011, Balhara, 2012). Another study by Virdis *et al.,* 2010, showed that smoking increased arterial hardness and blood pressure and it promoted visceral fat accumulation (Defina *et al.,* 2012). A study done in Nigeria indicates that smokers' blood pressure was significantly elevated compared with those of the non-smokers (Onyesom *et al.,* 2012). Pathophysiology of smoking that showed smoking causes endothelial injury thus lead to vasoconstriction of the blood vessels and high cardiac output thus favors the development of hypertension (Linsay *et al.,* 2011), study done by Makris, *et al.,* 2009 in normotensive passive smokers found that self-reported passive smoking was associated with increased levels of BP in a dose-related manner. In another study of 30 healthy nonsmoking women, systolic and diastolic

BP were found to be acutely elevated following the exposure to passive smoking (Yarlioglues *et al.,* 2010).

Smoking and Body mass index*:* The present study indicates that 26/65 (40 %) of participants were underweight, 19/46 (41.30 %) of active smokers and 7/19 (36.84%) and passive tobacco smoking individuals. The result also show BMI decreases (more underweight) proportionally with increase in the number of cigarettes smoked (cigarette pack-years). Taking mild to moderate smokers as reference, heavy smokers were statistically more likely to be underweight (OR: 7.90, p =0.004). Passive smokers, however, were not significantly lower in weight than mild-to-moderate smokers (OR: 2.09, p= 0.234). The majority of smokers 61/65 (93.8 %) were farmers doing work which requires high energy expenditure and this may contribute to their low body mass index. The finding is not coinciding with Kim *et al.,* 2012, who report smokers did not show significant difference in mean body mass index than those who never smoked, they showed more metabolically adverse fat distributions with increasing smoking amounts. Another study done by Gasperin *et al.,* 2014 in Vienna reports, contrary to the beliefs of many smokers, heavy smoking is associated with higher body weight, the number of cigarettes smoked per day was significantly associated with higher body weight (p=0.001) and BMI (p=0.009).

But the finding of present study is in consistent with previous studies of many scholars; there is a trend toward lower BMI in smokers than non-smokers, total energy expenditure was larger in high smokers than in never smokers among men (Gonseth *et al.,* 2014; Berlin *et al.,* 2012, Jitnarin *et al.,* 2014). There is negative correlation of obesity with smoking (Calole *et al.,* 2011; Yumi *et al.,* 2011). Compared to adult never smokers, men and women current smokers had lower BMI and lower probability of obesity in the U.S from (1999–2012) (Plurphanswat and Rodu, 2014). Another study done in Indian population shows that smoking associated with reduced BMI after adjusting for gender and economic status (Chhabra P and Chhabra S, 2011). Study by Tan *et al.,* 2013 shows as the casual smoking habit evolves into compulsive smoking, overweight or obese likelihoods are lowered as individuals are more likely to be in the underweight or normal weight. Another study done by Travier *et al.,* 2012 on effect smoking cessation on BMI shows quitting smoking is risk of subsequent body weight gain due to increased energy intake, decreased metabolic rate, increased physical inactivity. Among men

without the metabolic syndrome at entry, body weight gain, compared with never smokers, higher in smokers who had quit smoking (Jamshidi *et al.,* 2014).

Smoking and Blood glucose: The present study indicates 26/65 (40%) of active and passive tobacco smoking participant had higher fasting blood sugar levels but it is not actually statistically significant in different levels of smoking ($p>0.05$), the finding of the study not agreed with, tobacco smoking is tightly associated with impairments in glucose metabolism and insulin sensitivity and insulin secretion (Piatti *et al.,* 2014). There is an increased risk of diabetes among nonsmokers who were occasionally or regularly exposed to passive smoke and active smoking is positively and independently associated with the risk of type 2 diabetes (Zhang *et al.,* 2011). There is a positive dose response relationship between environmental tobacco smoke exposure level and diabetic mellitus in never-smokers (Eze *et al.,* 2014). A positive association between smoking and diabetes was found and it was found to be significant (Venkatachalam *et al.,* 2012). Cigarette smoking predicts incident type 2 diabetes, but smoking cessation leads to higher short-term risk (Yeh *et al.,* 2010). Other study show comparable glucose level between smokers and nonsmokers (Ahmed and Memon, 2008). Smoking had no consistent association with blood pressure or fasting blood glucose (Cena *et al.,* 2013).

6. CONCLUSIONS

Based on the urine cotinine result, 39/46 (84.8 %) of the active smokers were positive for the cut off value 200ng/mL. Active smokers classified to mild to moderate and heavy smokers based on pack-years, all heavy smokers were positive for urine cotinine test. The result of urine cotinine test in passive smokers indicated 5/24 (20.84 %) were detected positive. This study also used urine cotinine as objective way of educating people by taking four families with children to assess passive smoking. The results indicate that a family with an active smoking male, 2/5 (40%) children's were positive.

Most of the participants started smoking because they were influenced by peer pressure and attempted to stop smoking because they were worried about health and to spend money reasonably, regarding the knowledge of health effects of smoking most of them aware of tobacco smoking affects health but the source of anti-tobacco education and information not strong for example cigarette packs accounts 9/40 (22.5%). The most frequent site for passive exposure to smoking environment was the home 12/19 (63.2%).

Based on the present study overall prevalence of metabolic syndrome was 7/65 (10.8%) with at least three components of metabolic syndrome, 5/46 (10.8 %) and 2/19 (10.5 %) among active and passive smokers respectively. Prevalence of each component of metabolic syndrome shows abnormal body mass index 27/65 (41.5%) followed by elevated fasting blood sugar 26/65 (40 %), elevated DBP 24/65 (36.9 %), low HDL cholesterol 6/18 (33.33%), elevated TG 4/18 (22.22%), elevated SBP 10/65 (15.38%) and high waist circumference 3/65 (4.62 %), both in active and passive smokers, but except BMI other variable no statistical significant between smoking levels.

The number of each components of metabolic syndrome increase with increased cigarette smoking years indicating the more years smoked there is more chance to develop metabolic syndromes.

After getting detailed advice on harmful effects of tobacco smoking on health of active and passive smokers, many of the smokers promised to stop smoking and besides this they also promised to take care not to expose family and friends to tobacco smoking, though we were not able to check the progress.

7. RECOMMENDATIONS

- Assessment of active and passive smoking should be carried out based on evaluation of the questionnaire and measurement of the nicotine metabolite in the urine (cotinine) which helps to effectively convey the recommendations for quitting tobacco smoking and to protect passive smokers from being exposed to smoking environment.

- Home smoking precautions adopted by active smokers. Educational interventions on parents are essential to increase their awareness about environmental tobacco smoke exposure and to teach correct behaviors to protect health of families, especially in household environment.

- It is strongly recommended to avoid smoking for the benefit of metabolic syndrome and the study underscores the need for the implementation of smoking prevention and health promotion programs especially anti-tobacco education and information should be made strong by government, National Tobacco Enterprise (NTE) need to improve labeling for anti-cigarette information in cigarette packs.

- This study is cross sectional and further investigation should be done in larger studies using different cut-off points to validate urine cotinine as a biomarker for active smoking and passive exposure to smoking environment and clarify the mechanisms between cigarette smoking and the metabolic syndrome.

8. STRENGTHS AND LIMITATION

To start with the strength of the study it adopted rigid criteria to select the subjects; other possible strength is the use of anthropometric measurements instead of self-reported weight and height, as well as waist circumference. Sometimes people tend to over report their height and under report their weight, resulting in an underestimation of BMI.

The limitation of study includes participants with a similar socioeconomic background and belonging to the same geographical area and small cross sectional study. Other limitation may be linked to the fact that we considered the answers to the questionnaires as the gold standard for assessing the smoking status of individuals, assuming that all the answers were sincere and other limitation is many participants were not happy to give blood sample.

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10. ANNEXES

Annex I: Questionnaire

1. Section on Socio-demographic factors

1. Code:_____ Age_____ Sex_____ Ethnicity_____Marital status_____

2. Please fill the table below.

Occupation	Private ☐	Government ☐	Other _____	
Education	Illiterate ☐	Primary ☐	Secondary ☐	Above secondary ☐
Income	Per day_____	Per month_____		

2. Section on smoking

Only to be answered by tobacco user

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? _____

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

4. How many days a week do you smoke tobacco? _____

5. Did you smoke inside your house, near/or in front of your families or friends a. Yes b. No

6. If yes how often does you smoke in your house, near/or in front of your families or friends

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

7. Which were the important reasons for starting smoking?

a.Smoking is enjoyable b. peer pressure c. Smoking helps people relax

d.Smoking makes people look more grown-up e. Smoking helps people forget their worries

8. 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

9. 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. Better role model for children e. Worried about own health d. Others_____

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

11. Did you notice any anti cigarette information? a.Yes b. No

12. If yes where did you gate the information? a.Television b.Radio c.Health warning on cigarette packs d. New papers e. Other _____

13. Did you visit nurse/health worker to discuss tobacco use or smoking? a. Yes b. No

Only to be answered by passive smokers

14. Did your family, relatives or friends smoke tobacco? a. Yes b.No If so

15. Where do you expose to tobacco smoking? a.At home b. At work place

c. Public recreation areas d. Others_____

16. For how many hours, on average each day, are you closely subjected to other people's tobacco smokes?

17. Do you think the smoke from other people's cigarettes is harmful to you? a.Yes b.No

To be answer by both active and passive smokers

18. Do you participate in any physical activity? If so which one

a. walking b. doing any sport regularly c. doing hard work which require high energy d. other

19. Which types of diet you use every day eat?_____

20. Do you use other substance? If so which one a. Alcohol b.khat c. other_____

21. Do you have any health problems? If so which ones? a. cancer b. Breathing Problems c. Ear Infections d. Other _____

Annex II: Consent form

i. Information sheet

I am Anteneh Wondimu (DVM), from Addis Ababa University Graduate Studies, Faculty of Medicine, who is conducting my study, on Investigation of Urine Cotinine Levels as indicators of Tobacco Use and Passive Smoking, in conjunction with Parameters of Metabolic Syndrome, in chenna wacha for my partial fulfillment of the requirements for the degree of Masters in Medical Biochemistry.

1. Objective of the study

The overall objective is Investigation of Urine Cotinine Levels as indicators of Tobacco Use and Passive Smoking, in conjunction with Parameters of Metabolic Syndrome.

2. About participation

2.1 Procedures

If you decide to participate you will be asked to respond to the questioner that will take you about 10 minutes followed by testing for parameters of metabolic syndrome and urine cotinine level. Blood sample will be taken for the assessment of parameters of metabolic syndrome and urine sample used for determination of the levels of cotinine for active and passive tobacco use.

2.2 Inclusion/exclusion criteria

Active tobacco user and passive smokers will be involved in the research. Passive smokers that will be included in the research are individuals whose families or friends are tobacco users and have frequent contact with the smokes of tobacco. Exclusion criteria are significant clinically overt disease and treatment that might disturb the measurements performed in the study or unwillingness to participate.

3. Possible risks

This study has no health risk except minimum pain associated with blood with drawl procedure.

This is just the result of the routine work which experienced by all the individuals that will involve in the research or the assessment of metabolic syndrome by experienced health practitioner.

4. Benefits

Being the member of the research will give you the following benefits from this research; you will have the chance to look the status of your passive and active tobacco use and metabolic syndrome profile and may take measure like need to visit clinic for further test to control before worse. You also get necessary advice regarding active and passive tobacco smoking and its health effects. In addition to this the information you provide is helpful for the health programme planners in the chenna wacha to improve future smoking cessation or prevention interventions.

5. Confidentiality

The information you provide is confidential and will only be used for the objective mentioned above. You have the right to refuse participation or quit participation at any point of the interview process if you are not comfortable.

6. Right to get information

Ethical clearance will be obtained from Addis Ababa University College of Health Sciences Department of Biochemistry and Department of Medicine. A letter of cooperation will be written from department authorities. The main objective of this committee is to protect the participant from any risk and discomfort which may result due to the procedure of the study.

ii. Agreement form

I volunteer to participate in a research project conducted by Anteneh Wondimu from Addis Ababa University.

I understand that the project is designed to gather information about Investigation of Urine Cotinine Levels as indicators of Tobacco Use and Passive Smoking, in conjunction with Parameters of Metabolic Syndrome. I will be one of approximately 70 people being participated for this research.

I understand that I will not be paid for my participation. I may withdraw and discontinue participation from the study at any time without penalty.

I understand that the researcher will not identify me by name in any reports using information obtained from this interview and that my confidentiality as a participant in this study will remain secure. Subsequent uses of records and data will be subject to standard data use policies which protect the anonymity of individuals and institutions.

I have been given a copy of this consent form and I have read and understand the explanation provided to me. I have had all my questions answered to my satisfaction, and I voluntarily agree to participate in this study.

Signature of subject_____ Signature of investigator _____

(Participant)

Date_____

Annex III: Tobacco advice sheet

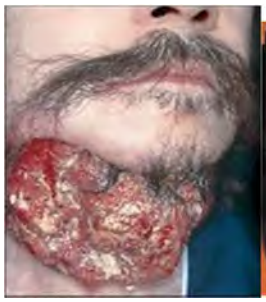
1. Smoking kills millions of people every year. In fact, one in every ten deaths in the world is caused by smoking. One out of every two (one half) of all smokers will die from smoking-related diseases, and many more will suffer for years from smoking-related illness.

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 and happy by advising them about the health dangers of smoking! Help your neighbors, 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.



Died from throat cancer,

Lung cancer kills,

Smoking kills!,

Stop smoking

Echee woreeqato

1. Beemii hinoo

1. Yaare Hado_____Animoo____Enoo_____Shisho _____ Shaagi hinoo _____

2. Hooyochi Ceechibii

Shuuno	Qeliishuuno ☐	Mangisteecho ☐	Baroo_____	
Doyoo	Doyanno ☐	Iikkinee Cyclo ☐	Guutinee Cyclo ☐	Gutiinee Cyclo Damba ☐
Gijoo	Decochi _____	Agenoochi _____		

2. Kuxaa guuto tumbahee uuyo

Tubahooni uchiibeeto bachi hooyon ceechibe

1. Tubaaho uchabeetine? Ne uchabeeti tumbaho amone _____

2. Decochi ambichoo uchehin, ambichee natonii ucheetine? _____ _____

3. Anibiichenaatonanee tumbahee uuyo nee koteeto?_____

4. Kechi dagoochi uuchatiine a. uchoyee b. uyachi

5. Ambichee kaalone kechii dagochi nee uchabeeto a. decochi b. shabatochi c. ageenoch d. ariichiyache

6. Baati baati tumbahee uyo nekooteti nabo amoone?

a. kaayochi b. asho ciinaa c. diiciti asho bishahi beetochi D. mooyo batoochi

7. Tubachii uyo nechochi gabii aribeetine? a. ariiho b. ariiyachi

8. Nechoo ne gabiiti naabo amoone? a. shello shixihe b. godee doyooche keeyochi c. gijooni naboona gachiiyochi d. gawee shuriyooni bekimoochu e. achee iiwitinoochi d. baroo_____

9. Tumbahee uyo ache iwiitinoo mitihee? a. mixihee b. mixaache

10. Tubachii gondiitinooni waaya aribeetine? a.arihoo b. ariyaachi

11. Nee aribeeto gaata amooche wayenee? a. Televisinoche b. Radionoche c. tubahee woraqaatoche d. gazeetoche e. baroo _____

12. Iiwee beerochi haama aribeetine? a. hammete b. haamachi

Hiniyee deshi beetii hooyoni ceechemo tubahooni cufiyaano tunetaana tubachi cufochon gudee gomoona danabeeto

13. Nee nucho woyee nee xiboo tubahoo uchitee? a. uchiyee b. uyaache

14. Abicheeniyee tubachii cufo nee danaabeto? a. kechi daagochi b. shuune xaa'ochi c. ashoo kachiibeeti xaa'ochi d. baroo_____

15. Ambiichee shaatone tubahooni uchibeeti asho tooki nee heechabeeto? a. bulle abooni . b.ikkike abooni

16. Baree ashoo wane waabeti cufo achee iwiitinoni mixeehe ? a.mixiihe b. mixaache

Hiniyee deshi beetii hooyoni ceechemo tubahoo uchibeetona tubahee cufoo bi achochii shagibeetone

17. Giido qaawibeeti shunoo shunaabeetine? a. sha'o b. bulle abooni sporiton shuuno
c. Giido qaawibeeti shunoo shuneehoyee d. baroo_____
18. Bulle abooni nee maabeeti mayoo abiinee?_____
19. Nee gaaachabeeti bare moyoo beete? a. uyoo b. chaato c. baroo_____
20. Achee iiwitiine iiritoo beete? a. canceero b. kaachi shuuye c. waajii biiyo d. baroo

Iibaariyoo

1. Aarichiyoo: Taa anteenehi weendimi addisabii university medicinee faculty doyeecho taa tuneemona, hinniyee deshi beetii herechoni; sheechi daagochi daaneebeti cotinine geetebeti chemicaloni tubahooo uchibeeti na'onaa tubachii cufochnii daaneebeeti asheena'o dagochee, boonoshii achee iiwitinoo hinooni bedaaha cineemi herechooni shuniyeemone

Moochi wullo: Sheechi daagochi daaneebeti cotinine geetebeti chemicaloni tubahooo uchibeeti na'onaa tubachii cufochnii daaneebeeti asheena'o dagochee, boonoshii achee iiwitinoo bedaaha cineemi herechooni shunioo.

Mochi hamadaboo: Hinii heerechochi gimo negabigataa ikkike moyoni 10 daqiqo beedaha echiyemo neene, aroyee gubii gisheche sheyonaa damonaa imeenene ebiyoo bituneto sheechi daagochi daaneebeti cotininonaa, achee iiwitinee hinooni bedaaha cineemi herechooni shunioyeemone.

Herechochi gimoona gimii qayi naboo : Tubahooni uchibeetona tubachii cufoon gudee gomoona dabeetoo gimo hakiyee. Gimii qayi naboo aricheeti biyoonaa dawee atooni damiibeetina'o weeye herechochi gimoochi qawii qayoo
Bihayiboonan bigacoo: Hinii herecho achee iwiitine toomochi bidewiibeeti hayibo alone tunebaani damoo damemonaa gishechooni bijeemone bitunetaana herechochi gimo wedee gaaco beete hiniwo achee iwiitinee hinooni arimooch hiniyee beshon weeta chani dubiyee ache iiwitine gamooni kichochi shahiyoochi tubachii uuyoni nechoo

Herechochi mochii hinoo: Hiniyee danibaa itoshi biriiti moyoo bigaacemo hiniyeedambaa icheeti mochii naabochi baachiye. Ebii hereechochee nee qaawit goroochi, echaa weeche gooroche tunebaa qajii keyoo hakihinee

2. Ibaariyoo

Taa shunoona antenehi wendimi shunaabeeti herechooch gimoochi dagiitanee, bishunaabeeti wullemochi hinoono aribeetotanee ebiyoo; sheechi daagochi daaneebeti cotinine geetebeti chemicaloni tubahooo uchibeeti na'onaa tubachii cufochnii daaneebeeti asheena'o dagochee, boonoshii achee iiwitinoo bedaaha cineemi herechooni shunyoo

Herechoochi tagimoo tachii qociiyeemi birewoo allo bitunoona taa qawitii goorochi qajaa keeyo tahakeemo bitunooni arihoo.
Hinii heerecho taani taa shigonaa taa ariyaani gomoonaa amoomi xaa'ochi bikichaachemo biitunooni digineeto taane

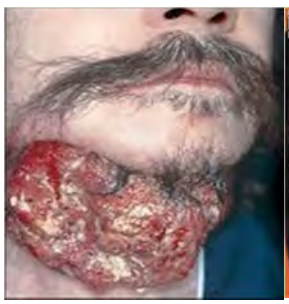
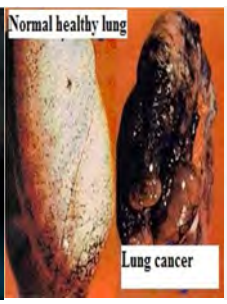
Ibariyee wochoo taachi bii echeeto tunooni bii mochii wulloon sheemano shemiino taa digeenetii qodoochi hinii herechochi taa shunoona gimochii kuxaa tiyeeto tanee.
Heerechebeetochii duko_____Herechonii shunaabeetochi duko _____ Deco_____

Tubachii boyee beshiyoo

Tubaachi uyoo weede ashooni wuxiyee, ashiree ashoche ikoo tubahoona waabeeti biyoona qitiihee
Tubahoo uchiibeeti asho, weede abooni mullee biyoona qitihee. Tubaachi uyoo weede achee qeephena'ochi shikiyee biyoo dewiyee; eboshiwoona shobee shikiyoo, noone shikiyoo, qamee shikiyoo eebiyee beshooni weede achee iiwitinoon shapiihee

Tubahoo uyaane asho tuneebani tunbachi cufooni gudeegomona daneebeto tubahoona waabeeti biyee naboona qitihee. Eboshicheeno bushishoon shobee biyooch dupiiyee, wajii biyoochi dewiyee
Tophiyee gisheeche asho tubahoo uchibeeti showochee ikeene tuneebani tubahonii uchibeeti ashii hadoo dakibeetoone ebichii tubahonii nechonaa, barrena'o boono nechemoomon booyona, no kexooni, no nuchooni tubahonaa waabeti ache iiwitine shaphooche quyoo qawihee.

Tubahee uchii achee iiwee shapoo, ubaa tubachii yareenaona wahee eboshiwoon, shisho, tujaaro. ebichii tunbaahoo uchayoote


Qetee shikiyoo shobee shikiyoo dewiyee tubachi uyoo asho wxiyee, tubahooni neechiboni