# SCREENING OF PARAMETERS OF METABOLIC SYNDROME AND C-REACTIVE PROTEIN (CRP) IN CIGARETTE SMOKERS IN ADAMA, ETHIOPIA

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# SCREENING OF PARAMETERS OF METABOLIC SYNDROME AND C- REACTIVE PROTEIN (CRP) IN CIGARETTE SMOKERS IN ADAMA, ETHIOPIA

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This is to certify that the thesis prepared by **Abinet Teshome Argaw** entitled: **"Screening of parameters of Metabolic Syndrome and CRP in cigarette smokers in Adama, Ethiopia"** and submitted in partial fulfillment of the requirements for the Degree of Master of Science (Medical Biochemistry) complies with regulations of the University and meets the accepted standards with respect to originality and quality.

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# Abbreviations an Acronyms

4- AAP- 4 – aminoantipyrine

ATP- Adult Treatment Panel

BC- Before Christ

BMI- Body mass index

CE- Cholesterol Esterase

CS- Cigarette Smoke

CHD- Coronary heart disease

CHOD- Cholesterol Oxidase

COHb- Carboxyhaemoglobin

COMb- Carboxymyglobin

COPD- Chronic obstructive pulmonary disease

CRP – C reactive protein

dl- Decilitre

DNA- Deoxyribonucleic acid

FFA- Free Fatty Acids

HDAC- Histone deacetylase

HDL- High density lipoproteins

hs-CRP- High Sensitivity C Reactive Protein

HUVECs- Human umbilical vein endothelial cells

HR- Heart rate

IDF- International Diabetes Federation

IGT- Impaired Glucose Tolerance

IFG- Impaired Fasting Glucose

Interleukin 1 RA- Interleukin 1receptor agonist

IL-6 – Interleukin 6

IL- ß- Interleukin beta

IR- Insulin Resistance

kg- Kilogram

LDL- Low density lipoproteins

MAO- Monoamine oxidase

mg- milligram

mmHg- Millimetres of mercury

NEFA- Non esterified fatty acid

nAChRs- Nicotinic Acetylcholine Receptors

OR- Odds Ratio

PIPES = Piperazine-1,4-bis(2-ethanesulfonic acid)

POD- Peroxidase

RAS- Renin Angiotensin System

ROS- Reactive oxygen species

T2DM- Type 2 Diabetes Mellitus

TC- Total cholesterol

TG- Triglyceride

TF- Tissue factor

TNF $\alpha$- Tumor Necrosis Factor alpha

$VO_2$max- Maximum $O_2$ uptake

VTA- Ventral tegmental area

WHO- World Health Organization

WHR- Waist hip ratio

**Abstract**

**Background** – Several previous studies indicated conflicting findings on parameters of Metabolic Syndrome and CRP in cigarette smokers. The aim of this study was to determine whether there is difference in levels of parameters of Metabolic Syndrome and CRP in cigarette smokers and non- smokers in Adama, Ethiopia.

**Methods** – subjects were 99 healthy male individuals from age 15 to 60 years. The subjects were divided into two groups of 50 smokers and 49 non- smokers. BMI, blood pressure plasma levels of total cholesterol, triglycerides, HDL, and CRP were measured and LDL cholesterol was estimated using the Friedewald formula.

**Results** – Overall subjects' plasma levels of total cholesterol, triglyceride, LDL, HDL and serum CRP were significantly elevated in smokers. No significant difference was found in BMI, systolic and diastolic blood pressure between cigarette smokers and non-smokers. In smokers alcohol drinking was significantly correlated with increased plasma HDL, systolic and diastolic blood pressure while khat chewing was correlated with increased plasma total cholesterol.

**Conclusions** – The results showed no difference in BMI between smokers and non smokers and also there was no significant difference in blood pressure in smokers and non smokers. Cigarette smoking is correlated with increased total cholesterol, TG, LDL, HDL and CRP. Among smokers blood pressure, total cholesterol and HDL were significantly higher in those whose alcohol consumption was higher.

# 1. INTRODUCTION

## 1.1 Metabolic Syndrome

The combination of metabolic disturbances known as the Metabolic Syndrome was first described by Kylin in the 1920s as the clustering of hypertension, hyperglycaemia and gout. Two decades later, Vague noted that upper body adiposity (android or male-type obesity) was the type most often associated with the metabolic abnormalities seen with diabetes and cardiovascular disease (CVD) (Alberti *et.al.*,2005). During the 1988 Banting Lecture, Reaven used the term ‗Syndrome X' and firmly established the clinical importance of this syndrome, although obesity was not included (Reaven, 1988). In 1989, Kaplan renamed it ‗The Deadly Quartet' and others then coined the term ‗The Insulin Resistance Syndrome' (Haffner *et al.*, 2009). It is now agreed that the well established term ‗Metabolic Syndrome' remains the most useful and widely accepted description of this cluster of metabolically related cardiovascular risk factors which also predict a high risk of developing diabetes (Alberti *et al.*, 2005).

A number of expert groups have attempted to develop a unifying definition for the Metabolic Syndrome. The most widely accepted of these definitions have been produced by the World Health Organization (WHO), The European Group for the Study of Insulin Resistance (EGIR) and the National Cholesterol Education Program—Third Adult Treatment Panel (NCEP ATP III). All groups agree on the core components of the Metabolic Syndrome: obesity, insulin resistance, dyslipidaemia and hypertension. However, they provide different clinical criteria to identify such a cluster (see Table 1). For example, unlike the other two definitions, the ATP III definition does not obligatorily require impaired glucose regulation or insulin resistance as an essential component. In addition, the levels set for each component and the combination of components required to diagnose the Metabolic Syndrome are slightly different in these three recommendations. The IDF has produced a new set of criteria for use both epidemiologically and in clinical practice world-wide with the aim of identifying people with the Metabolic Syndrome to clarify the nature of the syndrome and to focus therapeutic strategies to reduce the long-term risk of cardiovascular disease. Guidance is included on how to compensate for differences in waist circumference and in regional adipose tissue distribution between different populations (Alberti *et al.*, 2005). The IDF has also produced recommendations for additional criteria that should be included when studying the Metabolic Syndrome for research purposes. Finally, the IDF has identified areas where more studies are

currently needed; these include research into the aetiology of the syndrome (Alberti *et al*., 2005).

Table 1 Classification of Metabolic Syndrome (Alberti *et al*., 2005)

| | **NCEP ATP III(2005 revision)** | **WHO (1998)** | **EGIR (1999)** | **IDF (2005)** |
|---|---|---|---|---|
| Absolutely required | None | Insulin resistance (IGT, IFG, T2D or other evidence of IR) | Hyperinsulinemia (plasma insulin >75th percentile) | Central obesity [waist circumference: >94 cm (M), >80 cm (F)] |
| Criteria | Any three of the five criteria below | Insulin resistance or diabetes, plus two of the five criteria below | Hyperinsulinemia, plus two of the four criteria below | Obesity, plus two of the four criteria below |
| Obesity | Waist circumference: >40 inches (M), >35 inches (F) | Waist/hip ratio: >0.90 (M), >0.85 (F); or BMI >30 kg/$m^2$ | Waist circumference: 94 cm (M), 80cm (F) | Central obesity already required |
| Hyperglycemia | Fasting glucose over100 mg/dl or Rx | Insulin resistance already required | Insulin resistance already required | Fasting glucose over100 mg/dl |
| Dyslipidemia | TG over 150 mg/dl or Rx | TG over150 mg/dl or HDL-C: <35 mg/dl (M), <39 mg/dl (F) | TG over177 mg/dl or HDL-C <39 mg/dl | TG over150 mg/dl or Rx |
| Dyslipidemia (second, separate criteria) | HDL cholesterol: <40 mg/dl (M), <50 mg/dl (F); or Rx | | | HDL cholesterol: <40 mg/dl (M), <50 mg/dl (F); or Rx |
| Hypertension | >130 mmHg systolic or >85 mmHg diastolic or Rx | ≥140/90 mmHg | ≥140/90 mmHg or Rx | >130 mmHg systolic or >85 mmHg diastolic or Rx |
| Other criteria | | Microalbuminuria | | |

## 1.2 PATHOPHYSIOLOGY OF METABOLIC SYNDROME

### 1.2.1 Insulin resistance and Glucose Intolerance

Metabolic Syndrome is also known as Insulin Resistance Syndrome. This syndrome is a cluster of disorders that include insulin resistance, impaired glucose intolerance and hyperinsulinemia. Insulin resistance appears to be the primary mediator of Metabolic Syndrome (Lann and LeRoith, 2007). Insulin promotes glucose uptake in muscle, fat, and liver cells and can influence lipolysis and the production of glucose by hepatocytes. The linked concepts of Metabolic Syndrome/insulin resistance syndrome have served a highly useful purpose by providing a simple construct to characterize many types of patients who clinicians see daily, and to help identify people at risk (Handelsman, 2009).

Insulin is an antiatherogenic hormone and this metabolic effect involves the activation of phosphatidylinositol (PI) 3-kinase. In case of Insulin resistance, PI 3- kinase path is impaired and insulin is no longer antiatherogenic (Wang *et al.,* 2004). Obesity, and in particular abdominal adiposity, is one of the main reasons for Insulin resistance. Non-esterified fatty acids (NEFA) are released from excess adipose tissues, which increase insulin resistance. In case of insulin resistance there is increased lipolysis from the adipose tissue which increases the free fatty acids, further inhibiting the anti-lipolytic effect of insulin (Eckel *et al.,* 2005). Visceral or omental fat appears to be the most detrimental and contributes most to the development of lipotoxicity in peripheral tissues by the secretion of adipocytokines (Gill *et al*., 2005). Metabolic Syndrome is associated with a high amount of intra-abdominal fat, low adiponectin levels, and elevated levels of cytokines (interleukin 1RA and interleukin 1beta) (Salmenniemi *et al.,* 2005). Hyperinsulinemia may increase the production of very low-density lipoprotein triglycerides and thus raise triglycerides. Insulin resistance can raise blood pressure (Grundy, 2004).

Additional contributors to insulin resistance include abnormalities in insulin secretion and insulin receptor signaling, impaired glucose disposal, and proinflammatory cytokines. The relation of impaired glucose tolerance and Insulin resistance is well documented. To compensate for defects in insulin activity, insulin secretion or clearance needs to be modified to sustain normal glucose levels. Hyperglycemia is the end result if these mechanisms fail

(Eckel *et al*., 2005). Since insulin resistance increases a person's risk for developing cardiovascular disease and Type 2 diabetes, several researchers have proposed measures of insulin resistance in obese individuals with and without Metabolic Syndrome. Reilly *et al*., 2004 believe that insulin assays or alternative biomarkers of insulin resistance may facilitate cardiovascular risk prediction in individuals with Metabolic Syndrome.

### 1.2.2 Central Obesity

According to the new criteria of IDF, Metabolic Syndrome can also be called –central obesity syndrome" (Gary, 2006). The importance of the term Metabolic Syndrome is that it helps identify people at risk of cardiovascular disease and type 2 diabetes (Ford, 2005). Central obesity is a high CVD risk factor. Central obesity is more metabolically active than peripheral fat. Recently, studies have suggested that central adiposity precedes the development of the other components of Metabolic Syndrome and that weight reduction at that point could be the best way to prevent it (Thaman and Arora, 2013). Steele, *et al*., 2005 and Plandevall *et al.,* 2006 recommends that waist circumference be routinely measured to assess individuals for increased risk for insulin resistance related cardiovascular disease, Metabolic Syndrome and type 2 diabetes and to target individuals for health promotion interventions. Though insulin resistance is known to be the major factor for the development of Metabolic Syndrome, it is suggested that obesity provides the connection between the insulin-resistant, dyslipidemic and hypertensive factors (Wingard *et al.,* 1996).

Visceral fat releases its metabolic products directly into portal circulation, which carries blood straight to the liver. Therefore free fatty acids are sent to the liver. Free fatty acids also accumulate in the pancreas, heart and other organs. This leads to organ dysfunction, producing impaired regulation of insulin, blood sugar and cholesterol as well as abnormal heart functions. This is known as lipotoxicity (Harvard College, 2006).

Abdominal obesity can be evaluated using computed tomography (CT) or magnetic resonance imaging (MRI) to measure the amount of visceral fat. The National Cholesterol Education Programme Adult Treatment Panel III suggested cut off of 102 cm (40 in) and 88 cm (35 in) for males and females as a marker of central obesity. Parikh *et al.* 2007 proposed that the index of central obesity, which is the ratio of waist circumference and height, was a

better substitute than the widely used waist circumference. Central obesity is correlated with both insulin resistance and T2DM itself (Gabriely *et al.,* 2002).

### 1.2.3 Hypertension

One of the key symptoms of Metabolic Syndrome is hypertension. It is a symptomatically ―silent," and can therefore remain undetected for long periods of time. It is an important risk factor for development of cardiovascular disease. All the hemodynamic and metabolic disorders of essential hypertension and insulin resistance are closely related. Essential hypertension is frequently associated with several metabolic abnormalities, of which obesity, glucose intolerance, and dyslipidemia are the most common (Ferranini and Natali, 1991). Obesity may be the strongest risk factor for uncontrolled hypertension. Studies have shown that obesity provides a connection between hypertension, insulin resistance and dyslipidemia (Wingard *et al*., 1996). In another study three factors were found in the clustering of metabolic variables. These three factors were insulin resistance, hypertension and dyslipidemia. Both general and central obesity was associated with insulin resistance and hypertension and only weakly linked to dyslipidemia (Anderson *et al.,* 2001).

Studies also suggest that both hyperglycemia and insulin activate the RAS (Renin-Angiotensin System) by increasing the expression of angiotensinogen, AII, and the AT1 receptor, which, in concert, may contribute to the development of hypertension in patients with insulin resistance (Malhotra *et al*., 2001). There is cross talk between the RAS and insulin signaling at multiple levels, and the RAS appears to be important in atherogenesis, Activation of RAS may inhibit the action of Insulin via the PI3 pathway (Prasad and Quyyumi, 2004). There is also evidence which supports a strong relation between hypertension and obesity, which may involve insulin and leptin as well as sympathetic nervous system. Leptin and insulin are considered to be compensatory mechanisms required to restore energy balance with sympathetic nervous system as one of the effector arms (Landsberg, 2001).

#### 1.2.4 Dyslipidaemia

In general, with increases in free fatty acid flux to the liver, increased production of very low-density lipoproteins (VLDL) occurs. Under physiological conditions, insulin inhibits the secretion of VLDL into the systemic circulation. In the setting of insulin resistance, increased

flux of free fatty acids to the liver increases hepatic triglyceride synthesis. Thus, hypertriglyceridaemia is an excellent reflection of the insulin resistant condition and is one of the important criteria for diagnosis of the Metabolic Syndrome (Aganović and Dušek, 2014).

The other major lipoprotein disturbance in the Metabolic Syndrome is a reduction in HDL cholesterol. This reduction is a consequence of changes in HDL composition and metabolism. In the presence of hypertriglyceridaemia, a decrease in the cholesterol content of HDL results from decreases in the cholesteryl ester content of the lipoprotein core with variable increases in triglyceride. In addition to HDL, the composition of LDL is also modified in a similar way. In fact, with fasting serum triglycerides > 2.0 mmol/L, almost all patients have a predominance of small dense LDL. This change in LDL composition is attributable to relative depletion of unesterified and esterified cholesterol, and phospholipids, with either no change or an increase in LDL triglyceride. In some studies, this alteration in LDL composition is an independent risk factor for cardiovascular disease. However, more often this association is not independent, but related to the concomitant changes in other lipoproteins and other risk factors (Aganović and Dušek, 2014).

### 1.2.5 Proinflammatory state

Yudkin *et al.* (1999) noted that low-grade inflammation is associated with insulin resistance and endothelial dysfunction and that adipose tissue generates inflammatory cytokines that may link insulin resistance with vascular disease. The origin of the inflammatory state and of endothelial dysfunction was adipocyte-generated inflammatory cytokines, which correlate strongly with insulin resistance. Circulating signal molecules from fat could include FFAs, adiponectin, IL-6 (particularly at the liver, where IL-6 increases CRP production), resistin, leptin, and TNF-α. Levels of C-reactive protein and interleukin-6 were shown to be related to markers of the insulin resistance syndrome and of endothelial dysfunction. Metabolic Syndrome and obesity are a kind of stress that leads to activation of inflammatory pathways. The causation of inflammation is multifactorial. The inflammation in Metabolic Syndrome is not accompanied by infection, autoimmunity or massive tissue injury. In fact the inflammation is low grade chronic inflammation. Researchers have attempted to name this inflammatory state as ―metaflammation" meaning metabolically triggered inflammation. A few studies have confirmed the positive association between obesity indices and

inflammatory markers, mainly CRP (C - reactive protein) in women (Shemesh *et al.*, 2007), but also other inflammatory markers, both in women and men (Mortensen *et al.*, 2009).

Increased concentrations of inflammatory mediators, such as, C-reactive protein, tumor necrosis factor-alpha, interleukin-6 and others have been found in the obese. Adipose tissue has been found to express most of these inflammatory markers. Obesity was the most important feature associated with C-reactive protein (Dandona *et al.,* 2005).

### 1.2.6 Prothrombotic state

The prothrombotic state is characterized by increased plasma plasminogen activator inhibitor (PAI)-1 and fibrinogen, and also associates with the Metabolic Syndrome. Fibrinogen, an acute-phase reactant like CRP, rises in response to a high-cytokine state. Thus, prothrombotic and proinflammatory states may be metabolically interconnected (Grundy *et al*., 2004).

The study of plasminogen activator inhibitor-1 helps in better understanding of association between hemostatic markers and Metabolic Syndrome. A study was conducted by Aso *et al*. ,2005 to determine whether plasma concentrations of thrombin-activatable fibrinolysis inhibitor (TAFI) in patients with type 2 diabetes were associated with components of Metabolic Syndrome , including high-sensitivity C-reactive protein (hs-CRP), plasminogen activator inhibitor (PAI)-1, and LDL cholesterol. The result indicated positive correlation between LDL cholesterol and plasma TAFI with type 2 diabetes mellitus. Co-existence of Metabolic Syndrome and hypercholesterolemia accelerates inflammation and elevated TAFI and PAI-1, inhibits fibrinolysis. PAI-1 is an important risk factor for Metabolic Syndrome. Three other biomarkers, CRP, IL6, and fibrinogen associate also importantly with the Metabolic Syndrome cluster. These 4 biomarkers can contribute in the Metabolic Syndrome risk assessment (Kraja *et al.,* 2007).

## 1.3 CIGARETTE SMOKING

Christopher Columbus was a great explorer and probably the first European to see the tobacco plant. In 1492 he arrived in ‗San Salvador' where the natives thought that he and his men were divine beings sent by the Gods. They presented Columbus with gifts including wooden spears, wild fruits and dried leaves. Columbus did not smoke; indeed he threw the leaves away! (http://www.gasp.org.gg/history-of-smoking.htm).

For approximately eight thousand years tobacco has grown on earth. Around 6000BC – tobacco started growing in Central America (Borio, 2011).

1000 BC – People of the Mayan civilization began to smoke and chew the leaves of the tobacco plant. They also mixed the leaves with herbs and other plants to make medicines for the sick and wounded. Ancient carvings show a priest smoking a tube pipe so smoking was an important part of their religious rites; it was used to communicate with the spirits (Borio, 2011).

Tobacco is a green, leafy plant that is grown in warm climates. After it is picked, it is dried, ground up, and used in different ways. It can be smoked in a cigarette, pipe, or cigar. It can be chewed (called smokeless tobacco or chewing tobacco) or sniffed through the nose (called snuff) (http://www.healthliteracy.worlded.org/docs/tobacco).

Cigarette smoke is a complex mixture of over 4000, and possibly as many as 7000 chemicals. Some smoke components, such as carbon monoxide (CO), hydrogen cyanide (HCN), and nitrogen oxides, are gases. Others, such as formaldehyde, acrolein, benzene, and certain N-nitrosamines, are volatile chemicals contained in the liquid- vapor portion of the smoke aerosol. Still others, such as nicotine, phenol, polyaromatic hydrocarbons (PAHs), and certain tobacco-specific nitrosamines (TSNAs), are contained in the submicron-sized solid particles that are suspended in cigarette smoke (Jeffrey, 2005).

In view of this chemical complexity, cigarette smoke has multiple, highly diverse effects on human health. It is not unexpected that multiple chemicals in cigarette smoke can contribute to any single adverse health effect (Jeffrey, 2005).

Conventionally, cigarette smoke is divided into two phases: a tar phase and a gas phase. The tar or particulate phase is defined as the material that is trapped when the smoke stream is passed through the Cambridge glass-fiber filter that retains 99.9% of all particulate material with a size >0.1 μm. The gas phase is the material that passes through the filter. The particulate (tar) phase of cigarette smoke contains $>10^{17}$ free radicals/g, and the gas phase contains $>10^{15}$ free radicals/puff. The radicals associated with the tar phase are long-lived (hours to months), whereas the radicals associated with the gas phase have a shorter life span (seconds) (Ambrose and Barua, 2004).

Cigarette smoke that is drawn through the tobacco into an active smoker's mouth is known as mainstream smoke. Sidestream cigarette smoke is the smoke emitted from the burning ends of a cigarette. Mainstream cigarette smoke comprises 8% of tar and 92% of gaseous components. Environmental tobacco smoke results from the combination of sidestream smoke (85%) and a small fraction of exhaled mainstream smoke (15%) from smokers. Sidestream cigarette smoke contains a relatively higher concentration of the toxic gaseous component than mainstream cigarette smoke. Of all the known constituents, nicotine, a component of the tar phase, is the addictive substance of cigarette smoke (Ambrose and Barua, 2004).

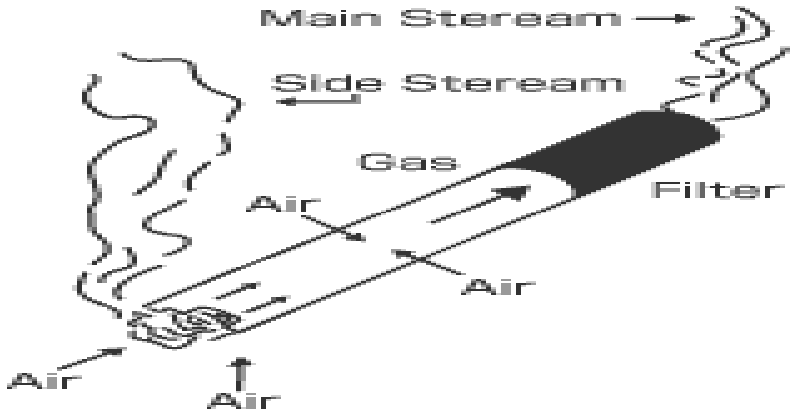


Figure 1 Smoked forms of cigarette (Geiss and Kotzias, 2007)

Discovered in the early 1800s and named ‗nicotianine', the oily essence now called nicotine is the main active ingredient of tobacco. Nicotine, however, is only a relatively minor component of cigarette smoke, which contains about 70 known cancer-causing substances (Ambrose and Barua, 2004).

The effects of smoking on human health are serious and in many cases, deadly. Of the thousands of chemicals in cigarettes hundreds are toxic. The ingredients in cigarettes affect everything from the internal functioning of organs to the efficiency of the body's immune system. The effects of cigarette smoking are destructive and widespread (Martin, 2008).

- Toxic ingredients in cigarette smoke travel throughout the body, causing damage in several different ways.
- Nicotine reaches the brain within 10 seconds after smoke is inhaled. It has been found in every part of the body and in breast milk.
- Carbon monoxide binds to hemoglobin in red blood cells, preventing affected cells from carrying a full load of oxygen.
- Cancer-causing agents (carcinogens) in tobacco smoke damage important genes that control the growth of cells, causing them to grow abnormally or to reproduce too rapidly.
- The carcinogen benzo (a) pyrene is carcinogenic to cells in the airways and major organs of smokers.
- Smoking affects the function of the immune system and may increase the risk for respiratory and other infections.
- There are several likely ways that cigarette smoke does its damage. One is oxidative stress that mutates DNA, promotes atherosclerosis, and leads to chronic lung injury. Oxidative stress is thought to be the general mechanism behind the aging process, contributing to the development of cancer, cardiovascular disease, and COPD.
- The body produces antioxidants to help repair damaged cells. Smokers have lower levels of antioxidants in their blood than do non smokers.
- Smoking is associated with higher levels of chronic inflammation, another damaging process that may result in oxidative stress (Martin, 2008).

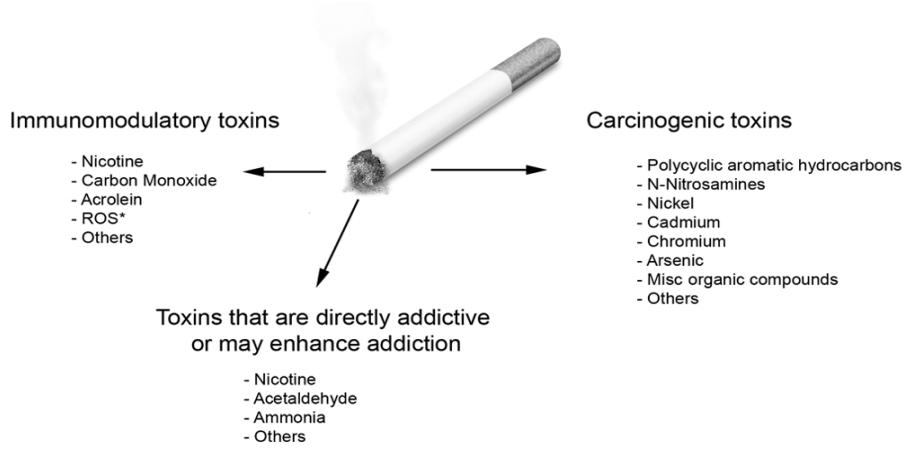


Figure 2 Some harmful components of cigarette (Lee *et al.*, 2012)

### 1.3.1 Cigarette Addiction

Nicotine is one of the thousands of chemicals found in tobacco smoke; when inhaled, the nicotine freely diffuses into the pulmonary blood and enters the systemic circulation, from where it quickly reaches the brain. In the brain, nicotine binds to nicotinic acetylcholine receptors (nAChRs), which are allosteric membrane-bound protein channels that, when opened, allow the passage of cations like sodium, potassium, and calcium (Taly *et al*., 2009). The flux of these cations depolarizes the cell, opening voltage-gated calcium channels; one role of calcium is to mediate neurotransmitter release from the presynaptic terminal (Dajas – Bailador and Wonnacott, 2004). Nicotinic acetylcholine receptors are composed of 5 subunits encoded by 17 genes; in the brain, 9 genes encode for α subunits and 3 genes code for ß subunits (Changeux, 2010). Different combinations of nAChRs have different pharmacological properties and are differentially located in different regions of the brain; the most common types, the α 7 homo-oligomer and the α4 ß 2 hetero-oligomer, are ubiquitous throughout the brain (Changeux and Edelstein, 2005). Studies show that the α4 subunit is a major determinant of nicotine sensitivity (Tapper *et al*., 2004). It has been shown in mice that the disruption of the ß 2 subunit of the nAChR eliminates the behavioral effects of nicotine, and reinserting the ß 2 subunit into the ventral tegmental area (VTA) of the brain reinstates nicotine-mediated behavior (Mineur and Picciotto , 2008). Combinations with other subunits can be formed into a functional nAChR. For example, if a α4 ß2 nAChR is combined with aα5 subunit, calcium conductance is increased seven times (Tapia *et al.*, 2007). Activation of the α3 ß4 subtype of nAChR in rats mediates the cardiovascular effects of nicotine (Aberger *et al*., 2001).

The binding of nicotine or a nicotine agonist to a nAChR mediates the release of neurotransmitters, either by promoting membrane depolarization and opening of voltage-activated calcium channels (which causes exocytosis of neurotransmitters), or by the intrinsic calcium permeability of the nAChR (Benowitz,2010). One neurotransmitter of importance to nicotine addiction is dopamine, which is critical in the acute reward pathways associated with abuse of nicotine and other drugs (Nestler, 2005). Nicotine stimulates dopaminergic transmission in the VTA of the midbrain, which in turn activates the nucleus accumbens, an area critical for nicotine-mediated physiological effects such as addiction, pleasure, and reward (Nestler, 2005). In addition to the nucleus accumbens, dopaminergic neurons of the

VTA project to the prefrontal cortex (PFC), amygdala, habenulo-interpeduncular system, and hippocampus (Davis and Gould, 2009).

Another function of nicotine is to facilitate the release of glutamate from the amygdala, which further activates the dopaminergic neurons of the VTA (Nestler, 2005).

In addition, nicotine activates GABA-ergic neurons, which inhibit dopamine release from the VTA. Chronic stimulation of nAChRs during nicotine addiction will desensitize the GABA-ergic neurons, which lose their inhibitory effect on dopamine release (Dajas – Bailador and Wonnacott, 2004). Through time, as the GABA-ergic response decreases, the dopaminergic response is heightened, further potentiating the addictive effects associated with nicotine (David *et al*., 2011).

Other than nicotine, there are other chemicals in cigarette smoke that are implicated in tobacco addiction. The enzyme monoamine oxidase (MAO), located in catecholaminergic and other neurons, catalyzes the degradation of biogenic amines, including dopamine, noradrenalin, and serotonin, by oxidative deamination (Van Amsterdam *et al.*, 2006). Norharman, a beta-carboline compound found in cigarette smoke, has been identified as a main candidate that inhibits both MAO-A and MAO-B. Other chemicals with MAO inhibitory activities include 2-naphthylamine, cyano-adducts of 1,2,3,4-tetrahydroisoquinoline and 2,3,6-trimethyl-1,4-naphto-quinone, and nitric mono-oxide. Evidence suggests that inhibition of monoamine oxidase plays an important role in nicotine addiction via its effect of increasing dopamine levels; the effects of MAO inhibitors are far reaching, affecting movement, mood, arousal, and memory (Fowler *et al.*, 2003).

### 1.3.2 CIGARETTE SMOKING AND OTHER DRUGS

Many surveys have shown that there is a statistical and dramatic association between the use of alcohol or cigarettes and other drugs. Cigarette smoking acts as a precursor of later illicit drug use (Torabi *et al*., 1993).Nicotine exerts its priming effect on cocaine by means of HDAC inhibition and provides a molecular explanation of the unidirectional sequence of drug use observed in mice and in human populations. Nicotine acts as a gateway drug and exerts a priming effect on cocaine in the sequence of drug use through global acetylation in the striatum, creating an environment primed for the induction of gene expression. Long-term

potentiation in the nucleus accumbens is blocked when long-term exposure to nicotine is followed by cocaine use, which presumably lessens constraints on dopaminergic neurons in the ventral tegmental area and leads to the enhanced release of dopamine. For all the measures studied — locomotor sensitization, conditioned place preference, long-term potentiation, and *FosB* expression — reversing the order of nicotine and cocaine exposure was ineffective: cocaine did not enhance the effect of nicotine. The priming effect of nicotine depended on its being given for 7 days before cocaine. Priming did not occur when nicotine was given for only 24 hours before cocaine. These results provide a biologic basis and a molecular mechanism for the sequence of drug use observed in people. One drug affects the circuitry of the brain in a manner that potentiates the effects of a subsequent drug. Moreover, it was observed the priming effect of nicotine only when mice were given cocaine at the same time as nicotine, which suggests that HDAC inhibition by nicotine depends on the continuous intake of nicotine. This observation is consistent with epidemiologic data that show that most people start using cocaine while using nicotine, a state that may enhance the physiological effects of cocaine. An alternative to the Gateway Hypothesis has been proposed on the basis of the idea that the use of multiple drugs reflects a common liability for drug use and that addiction, rather than the use of a particular drug, increases the risk of progressing to the use of another drug. Population studies have shown both generalized risk across substances and substance-specific risk — in particular, risk attributable to tobacco use (Kandel and Kandel, 2014).

### 1.3.3 CIGARETTE SMOKING AND METABOLIC SYNDROME

Non-communicable diseases (NCDs) are the leading cause of mortality and morbidity globally. In 2008, of the 57 million death occurred worldwide, 36 million were due to NCDs, namely cardiovascular diseases as the leading cause, followed by cancers, diabetes and chronic respiratory diseases. Cardiovascular disease (CVD) as the major contributor towards this NCDs epidemic is of global health concern (Raihan and Azmawati, 2013).

Smoking is widely accepted as a major risk factor for cardiovascular disease. Previous studies have shown that smoking reduces insulin sensitivity, induces insulin resistance, and enhances cardiovascular risk factors such as elevated plasma triglycerides, reduced high density lipoprotein–cholesterol, and hyperglycaemia. Several studies have also shown that smoking is

associated with metabolic abnormalities and increases the risk of Metabolic Syndrome (Hellas *et al.,* 2011).

The main clinical component of Metabolic Syndrome is insulin resistance. Metabolic Syndrome and glucose intolerance are regarded as disturbances with a common background and strong interrelationship (Beaser and Levy, 2007). Cigarette smoking may directly reduce insulin sensitivity by increasing circulating levels of insulin-antagonistic hormones (i.e., catecholamines, cortisol, and growth hormone) and increasing lipolysis, resulting in high circulating levels of free fatty acids (Nakanishi *et al*., 2005). Nicotine, carbon monoxide, and other metabolites derived from smoking also play important roles in insulin resistance. Furthermore, several mechanisms by which cigarette smoking promotes dyslipidemia have been proposed, including reduced lipoprotein lipase activity, increased 3-hydroxy-3-methylglutaryl-CoA reductase activity, increased glucose-6-phosphatase dehydrogenase activity, and increased central obesity (Razay and Heaton , 1995). Other studies may have lent some insight into this process, in that recent work has indicated that epigenetic mechanisms may be involved in Metabolic Syndrome and type 2 diabetes (Gallou-Kabani and Junien, 2005).

Smoking 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. Smoking also causes an increase in the heart rate. A higher heart rate has also been found to be an independent risk factor for hypertension (Yatan, 2012).

Cigarette smoking is a potent source of free radicals and these radicals reduce the amount of reactive oxygen species (ROS) scavengers and induce oxidative damage. Free radicals also oxidize low density lipoprotein (LDL) cholesterol. Oxidized LDL-cholesterol increases the risk of atherosclerosis, one of the compliments of Metabolic Syndrome. Moderate and short term duration of cigarette smoking have been observed to change lipoprotein profiles in a manner that may induce dysfunction and metabolic disorders (Onyesom *et al.*, 2012).

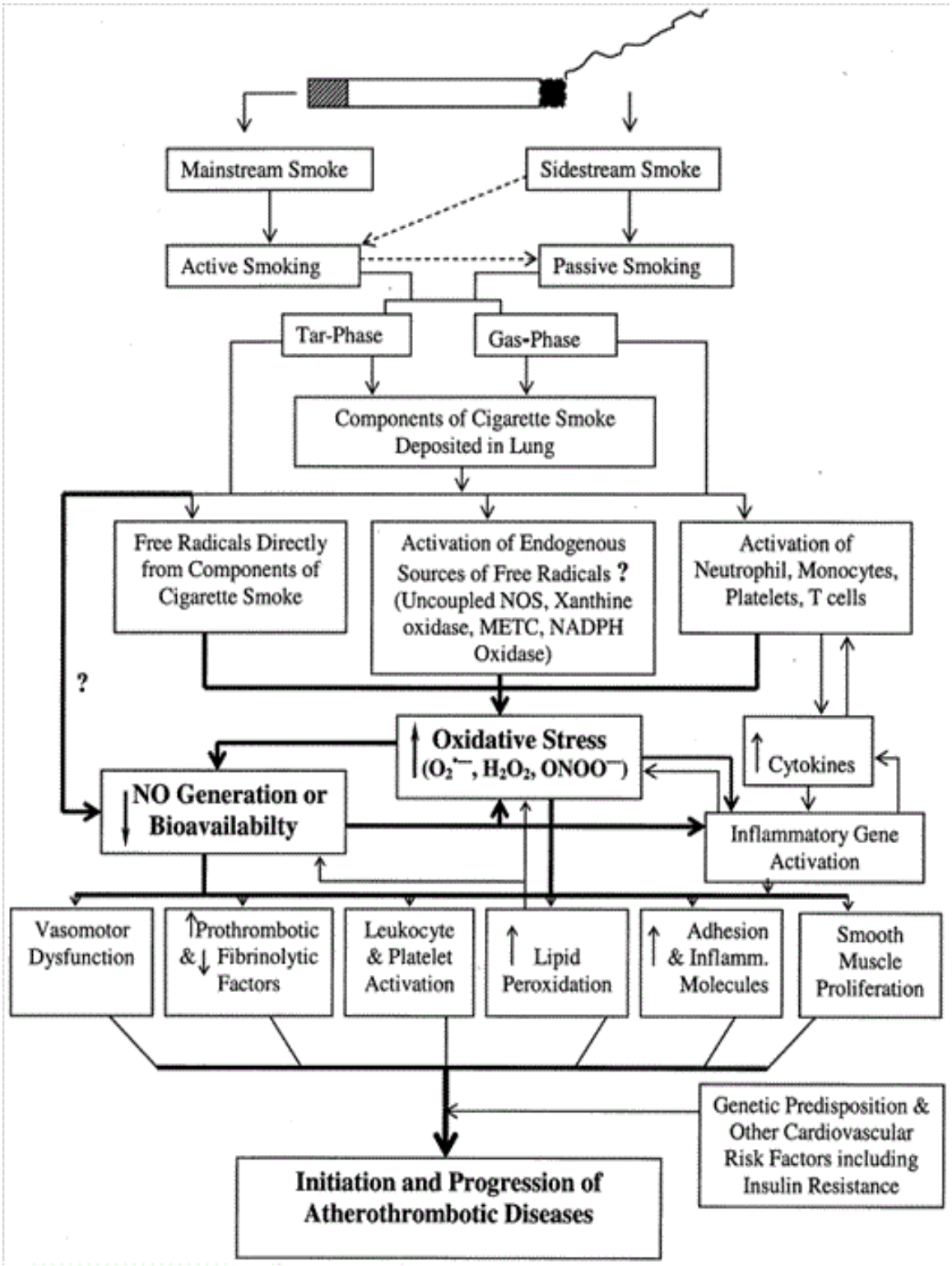


Fig 3 Potential pathways and mechanisms for cigarette smoking-mediated cardiovascular dysfunction (Ambrose and Barua, 2004)

Cigarette smoking (CS) continues to be a major health hazard, and it contributes significantly to cardiovascular morbidity and mortality. Cigarette smoking impacts all phases of atherosclerosis from endothelial dysfunction to acute clinical events, the latter being largely thrombotic. Both active and passive (environmental) cigarette smoke exposure predispose to cardiovascular events. Whether there is a distinct direct dose-dependent correlation between cigarette smoke exposure and risk is debatable, as some recent experimental clinical studies have shown a non linear relation to cigarette smoke exposure. The exact toxic components of cigarette smoke and the mechanisms involved in CS-related cardiovascular dysfunction are largely unknown, but CS increases inflammation, thrombosis, and oxidation of low-density lipoprotein cholesterol. Recent experimental and clinical data support the hypothesis that cigarette smoke exposure increases oxidative stress as a potential mechanism for initiating cardiovascular dysfunction (Ambrose and Barua, 2004).

Smoking is a major risk factor for cardiovascular morbidity and mortality, and is considered to be the leading preventable cause of death in the world. The nicotine and carbon monoxide (CO), among other cigarette components, can have harmful effects on cardiovascular function. These basic ingredients of tobacco smoke cause an increase in oxidative stress, endothelial damage and dysfunction, and are associated with significantly higher serum concentrations of total cholesterol and triglycerides, and lower levels of the cardio-protective high-density lipoprotein. By causing intravascular inflammation, smoking promotes the development of atherosclerosis and cardiovascular disease (Papathanasiou, 2014).

Nicotine deregulates cardiac autonomic function, boosts sympathetic activity and increases heart rate (HR) at rest, while blunting HR elevation during progressive exercise and lowering the maximum HR that can be achieved. At the same time, the smoking-generated CO binds with haemoglobin and myoglobin, reduces arterial $O_2$ blood saturation, compromises the efficiency of respiratory enzymes, and causes dysfunction of the $O_2$ production, transportation and delivery system, especially during exercise, substantially reducing the functional capacity and the performance of the circulatory system (Papathanasiou, 2014).

## 1.4 Nicotine

Nicotine is classed as an alkaloid (like morphine and cocaine) and meets the criteria of a highly addictive drug. One cigarette delivers 1.2 to 2.9 mg of nicotine, and the typical one pack-per-day smoker absorbs 20 to 40 mg of nicotine each day (Lande, 2012).

As an addictive drug, nicotine has 2 very potent effects: it is a stimulant and it is also a depressant (Robertson *et al.,* 1988). Nicotine deregulates cardiac autonomic function, boosts sympathetic activation, raises heart rate, causes coronary and peripheral vasoconstriction, increases myocardial workload, and stimulates adrenal and neuronal catecholamine release. In addition, nicotine is associated with insulin resistance, increased serum lipid levels, and intravascular inflammation that contribute to the development of atherosclerosis (Benowitz and Gourlay, 1997).

### 1.4.1Vascular Function

There are ample published data documenting that chronic exposure to tobacco smoke leads to a pathological alteration of endothelial function. Endothelial dysfunction may be caused by metabolic (dyslipidaemia), environmental (smoking), and physical (arterial hypertension) factors, or by inflammation that provokes pathological conditions. It is characterised by an imbalance between vasodilatory and vasoconstrictive substances originating from the endothelium, anticoagulant and procoagulant mechanisms, growth factors and growth inhibitors (Endemann and Schiffrin, 2004).

Under normal conditions, the free radicals circulating in the human body are neutralised by defensive mechanisms. However, if their concentrations within the blood should increase greatly because of excessive exposure to harmful factors such as smoking, then they cannot be regulated and may cause dangerous mutations that destroy cells. In these circumstances, oxidative stress is seen to arise (Bullen, 2008). The term ―oxidative stress" refers to the total of the intracellular and extracellular conditions that lead to chemical or metabolic production of reactive oxygen species (ROS) (USA Institute of Medicine of the National Academies, 2009). Smoke exists mainly in two states: the gaseous (which includes CO) and the solid (tar). In both these states, it contains a large quantity of free radicals (Ambrose and Barua, 2004). Pryor and Stone determined that 1 g of tar from cigarette smoke contains more than

$10^{17}$ long-lived free radicals (hours to months), while 1 g volatile fraction of smoke contains $10^{15}$ short-lived free radicals (seconds) (Pryor and Stone, 1993). Chronic exposure to tobacco also weakens the antioxidant defensive mechanisms that regulate these large numbers of smoking-induced free radicals, leading to a significant increase in oxidative stress (Ambrose and Barua, 2004). Oxidative stress, the oxidation of lipids, proteins, and DNA, is directly associated with atherogenesis (Bullen, 2008). An indicative finding is that when levels of isoprostanes (indexes of lipid peroxidation and oxidative damage) were measured in smokers, their levels were found to be higher than in non-smokers (USA Institute of Medicine of the National Academies, 2009). The reaction of nitric oxide (NO) with the free radicals contained in smoke reduces NO‘s bioavailability, interfering with its vasodilatory, antithrombotic, anti-inflammatory, and antioxidant effects, as well as its influence on endothelium permeability and myocardial function (reducing the diastolic distensibility of the left ventricle) (Gusarov *et.al*., 2009). The alteration in biosynthesis of NO and its decreased activity (Barua *et al*., 2001), in combination with the smoking-induced reduction in prostacyclin production(Reinders *et.al*., 1986) and the direct toxic effect of nicotine on endothelial cells that causes direct structural damage,(Benowitz and Gourlay ,1997) are important factors that may lead to endothelial dysfunction (Figure 5).

Using an extract of cigarette tobacco or its isolated ingredients, such as nicotine, many *in vitro* studies have found that smoking is associated with reduced NO availability. It has been shown that nicotine concentration in smokers‘ blood serum reduces the availability of NO in human umbilical vein endothelial cells (HUVECs), as well as in human coronary artery endothelial cells, leading to a reduction in the brachial artery‘s endothelium-dependent vasodilation (Ambrose and Barua,2004). Using this model, Barua et al. demonstrated that exposure to smokers‘ sera decreased NO availability in both HUVECs and human coronary artery endothelial cells, by altering the expression and activity of the endothelial NO synthase enzyme (Barua *et al*., 2001). In addition, they noted a significant correlation between flow-mediated brachial artery endothelium-dependent vasodilation and NO bioavailability from cultured HUVECs exposed to serum from the same individuals. On the other hand, CO, which is significantly elevated in smokers, inhibits the creation of NO and takes its place in haemoglobin binding (Coceani, 2000). These findings lead to the conclusion that the large quantities of free radicals contained in smoke enhance oxidative stress and, in combination with reduced NO bioavailability, nicotine-induced vasoconstriction and impaired vasodilation, may lead to endothelial dysfunction. The consequent endothelial damage

contributes to the formation and progression of atheromatous plaque, and reduces blood flow via thrombosis and vasospasm, thus causing cardiovascular disease (Widlansky, 2003 and Endemann and Schiffrin, 2004).

### 1.4.2 Lipid Metabolism

Tobacco smoke, and specifically nicotine, has a significant effect on lipid metabolism and the regulation of lipid levels in the blood (Craig *et.al*., 1989). Therefore, cigarette smoke could promote atherosclerosis, in part, via its effects on the lipid profile (Ambrose and Barua, 2004).

Smoking is associated with significantly elevated serum concentrations of total cholesterol and triglycerides (Craig *et.al*., 1989). In addition, several studies have shown a tendency for low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) cholesterol to be slightly higher in smokers (McGill, 1988).These associations are higher in smokers who smoke a greater number of cigarettes daily (Craig *et al*., 1989). On the other hand, some studies show that smoking lowers serum concentrations of high-density lipoprotein (HDL) cholesterol, a powerful protective factor against the development of atherosclerosis (Gnasso *et al.*, 1984 and Mccall *et al*., 1994). The difference is usually small, 5 mg/dl or less, but this difference represents a 10% decrease and would be expected to affect atherogenesis to a significant degree (McGill, 1988).Giving up smoking improves HDL levels, regardless of body weight, contributing to an improvement in cardiovascular health after smoking cessation (Gepner *et al.*, 2011).

It is possible that oxidative damage to protein and lipid constituents may explain the way in which cigarette smoke affects plasma LDL and HDL (Bullen, 2008). Cigarette smoking increases the oxidative modification of LDL (Ambrose and Barua, 2004). Exposure to cigarette smoke extract also decreases the plasma activity of paraoxonase, an enzyme that protects against LDL oxidation (Ambrose and Barua, 2004). There are two potential mechanisms by which reactive smoke components can produce their deleterious effects on essential plasma constituents: 1) indirectly, gas-phase cigarette smoke may activate macrophages and neutrophils in the lung, which may release enzymes and oxidants capable of damaging lipids and proteins; 2) directly, since the lung possesses an extremely large

surface area for gas exchange, it is possible that gas-phase cigarette smoke components interact with plasma constituents in the interstitial fluid (Mccall *et al*., 1994).

Additional mechanisms have been proposed to explain the link between smoking and changes in serum lipid and lipoprotein concentrations. Nicotine stimulates the release of adrenaline by the adrenal cortex, leading to the increased serum concentrations of free fatty acids (FFA) observed in smokers. As a result, lipolysis is increased along with the blood‘s triglyceride levels. FFA are known to stimulate the hepatic secretion of VLDL and hence triglycerides (Craig *et.al*., 1989). In turn, this chronic increase in levels of fatty acids adversely affects insulin sensitivity and insulin secretion through direct effects on the liver, pancreas and muscle (Targher, 2005).

The increased release of FFA in the heart raises myocardial oxygen consumption, adding to the myocardial workload (Papathanasiou, 2014). A complementary finding is that FFA also stimulates the hepatic synthesis and secretion of cholesterol (Craig *et.al*., 1989). The smoking-induced changes in lipid metabolism, the increased LDL/VLDL and decreased HDL levels, in combination with the damage of vascular endothelium, are associated with a greater incidence of atherosclerosis in smokers. Thus, hypercholesterolaemia and smoking are among the most important factors that may lead to coronary artery disease (Friedman, 1989 and Zamir MA *et al*., 2000).The results of the Framingham Heart Study which is ten year coronary heart disease risk estimated that excess weight was the cause of hypertension in 78% of men and 65% of women (Morse *et al*., 2005). The Framingham Heart Study also indicates that for men who smoke with untreated systolic blood pressure of ≥ 160 and TC/HDL of 6 are high risk groups (McPherson, 2006).

Table 2 Framingham ten-year coronary heart disease risk (%) for men smokers (McPherson, 2006)

| BP(systolic) | TC/HDL | Age in years | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 40-44 | 45-49 | 50-54 | 55-59 | 60-64 | 65-69 | 70-74 | 75-79 |
| 120-129 | 4 | 6 | 10 | 12 | 16 | 16 | 16 | 16 | 20 |
| | 5 | 12 | 16 | 20 | 20 | 20 | 20 | 20 | 20 |
| | 6 | 16 | 20 | 25 | 25 | 25 | 25 | 25 | 25 |
| 130-139 | 4 | 8 | 12 | 16 | 20 | 20 | 20 | 20 | 25 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 5 | 16 | 20 | 25 | 25 | 25 | 25 | 25 | 25 |
| | 6 | 20 | 25 | ≥ 30 | ≥ 30 | ≥ 30 | ≥ 30 | ≥ 30 | ≥ 30 |
| 140-159 | 4 | 8 | 12 | 16 | 20 | 20 | 20 | 20 | 25 |
| | 5 | 16 | 20 | 25 | 25 | 25 | 25 | 25 | 25 |
| | 6 | 20 | 25 | ≥ 30 | ≥ 30 | ≥ 30 | ≥ 30 | ≥ 30 | ≥ 30 |
| ≥ 160 | 4 | 10 | 16 | 20 | 25 | 25 | 25 | 25 | ≥ 30 |
| | 5 | 20 | 25 | ≥ 30 | ≥ 30 | ≥ 30 | ≥ 30 | ≥ 30 | ≥ 30 |
| | 6 | 25 | ≥ 30 | ≥ 30 | ≥ 30 | ≥ 30 | ≥ 30 | ≥ 30 | ≥ 30 |

6 to 8- Low risk  10 to 16 - Moderate risk  ≥ 20 High risk

### 1.4.3 Arteriosclerosis

Arteriosclerosis is a general term that includes almost all the arterial disorders that lead to the abnormal thickening and ―hardening" of all kinds of artery. Atherosclerosis is a specific form of arteriosclerosis, whose most characteristic feature is the concentration of lipids in the intima of large elastic arteries (aorta) and medium-sized muscular arteries (coronary, femoral, carotid, etc.) (Papathanasiou, 2014).

Smoking is considered to be a significant risk factor for the development of atherosclerosis. The atherosclerotic effects of cigarette smoke are due to a substantial degree to thrombosis-related events (USA institute of Medicine of the national Academics, 2009). The accumulation of platelets coating the arterial wall at sites where there is turbulent blood flow or endothelial injury may be the prodromal stage for the creation of atheromatous plaques (Levine, 1973). Nicotine is considered to be responsible for an increase in blood viscosity and platelet aggregation, since it prevents the production of prostacyclins that would limit platelet aggregation (Ball and Turner, 1974). Platelet adhesion increases the production of thrombi, splits the coronary artery intima, accelerates the process of atheromatous plaque creation, and is associated with an increased risk of cardiac ischaemia (USA institute of Medicine of the national Academics, 2009). In addition, nicotine affects prostaglandin metabolism, weakening the vessel's defence against platelet deposition (Ross *et al*., 1977). The increase in platelet aggregation, the effect of nicotine on blood coagulation time, and the increase in blood viscosity, in combination with the increase in levels of LDL and VLDL, the reduction in HDL, and inflammatory processes, promote the creation of atheromatous plaque and the

development of atherosclerosis (Figure 5) (Endemann and Schiffrin, 2004). It is thus likely that chronic smoking, by increasing peripheral vascular resistance in this way, may lead to an increase in cardiac afterload and a consequent reduction in stroke volume (Green *et al.*, 1986).

Figure 4 Plaque and blood clots due to cigarette smoking (U.S department of Health and Human Services, 2007)

Circulatory levels of fibrinogen, one of the most powerful predictive markers of coronary events, are elevated in smokers. The increase in fibrinogen levels acts in combination with the increase in red cell mass from long-term exposure to CO, increasing blood viscosity and boosting the activation of platelets, hence increasing the risk of atherogenesis. Increased fibrinogen levels in the blood circulation can also lead to the development of atherosclerosis, with a direct effect on the increase in platelet aggregation (Hunter *et al.*, 2001).

Tissue factor (TF)—otherwise known as tissue platelet factor, or factor III, or thrombokinase, or CD 142—is a protein found in endothelial tissue, platelets, and leucocytes, and is essential for the initiation of thrombus formation by zymogen prothrombin. TF is expressed by cells that are normally not exposed to blood flow, such as sub-endothelial cells (e.g. smooth-muscle cells) and the cells that surround blood vessels (e.g. fibroblasts) (Chu, 2011).This can change when blood vessels are damaged—for example by physical injury, or rupture, or atherosclerotic plaque. TF is present in atherosclerotic plaque and can promote thrombogenesis and possibly propagation of the thrombus to the already existing atherosclerosis. Sambola *et al*. found that smoking increased plasma TF levels in smokers who smoked 10 or more cigarettes per day, with a smoking history of 10 or more years (Sambola *et al*., 2003).

Atherogenesis and coronary artery disease are the result of inflammatory processes. The fact that smoking is associated with inflammation implies that inflammation may be one of the mechanisms via which cigarette smoking leads to cardiovascular dysfunction. C-reactive protein (CRP) and levels of white blood cells are markers of inflammation, and are thus associated with atherosclerosis and an increased risk of cardiovascular disease (Asthana *et al.*, 2010). Levels of CRP and white blood cells appear to be higher in smokers than in non-smokers. Furthermore, there appears to be a relation between the extent of smoking and the white blood cell count (Dietrich *et al*., 2007). Dietrich *et al.,* 2007 claimed that the increase in CRP observed in smokers is proportional to both the quantity and the years of smoking.

Overall, nicotine boosts sympathetic activity, stimulates the release of neurotransmitters, causes coronary and peripheral vasoconstriction, and elevates blood pressure. Furthermore, nicotine increases lipolysis, leads to increased levels of free fatty acids, increases oxidative stress, endothelial damage and dysfunction, and promotes vessel inflammation, contributing significantly to the development of atherosclerosis and cardiovascular disease (Papathanasiou, 2014).

### 1.4.4 Autonomic Nervous System

There is an established link between abnormal heart rate (HR) responses at rest and during exercise, autonomic dysfunction and cardiovascular health (Perret Guillaume *et al.*, 2009). On the other hand, chronic smoking is associated with dysfunction of the autonomic nervous system (Benowitz, 2003) and the abnormal HR responses to tobacco may be implicated in the link between smoking and cardiovascular disease (Myers *et al*., 2007). Although the precise mechanism of action of smoke ingredients is still under investigation, all proposed hypotheses state that the main effects of smoking on cardiovascular function are associated with the direct or indirect action of nicotine on the neuroregulation of the circulatory system, wherein sympathetic activity is increased and parasympathetic activity is reduced (Figure 5) (U.S Department of Health and Human Services, 1983). The nicotine-induced sympathetic overdrive causes the adrenal medulla to increase the secretion of both epinephrine and norepinephrine into the circulating blood (Guyton and Hall, 2006). In addition, nicotine stimulates the vasomotor centre of the medulla, causing secretion of norepinephrine from local deposits. Subsequently, secretion of catecholamines from the free nerve endings of the sympathetic nerves and the local release of epinephrine and norepinephrine are increased. In

addition, vasoconstriction of coronary vessels occurs, the biosynthetic capacity of prostacyclin is reduced, and endothelial function is impaired (Benowitz *et al.*, 2002). The stimulation of catecholamine secretion, in combination with the depressed production of prostacyclins (potent vasodilators), results in an acute rise in blood pressure, a significant rise in HR, an increase in cardiac contractility, and a significant increase in myocardial work. Nicotine affects cardiovascular function both directly, as described previously, and indirectly, through a series of neurohormonal changes. In particular, nicotine molecules interact with and activate the brain's acetylcholine receptors (nAChRs), whose prolonged activation may desensitize a proportion of them (Piccioto *et al.*, 2002). The activation of nAChRs by nicotine boosts the release of neurotransmitters, while altering the function of some of them—such as norepinephrine, dopamine, serotonin (5-HT), and endogenous opioid peptides—thus modifying the action of the peripheral nervous system and causing cigarette addiction(Jiloha, 2010).

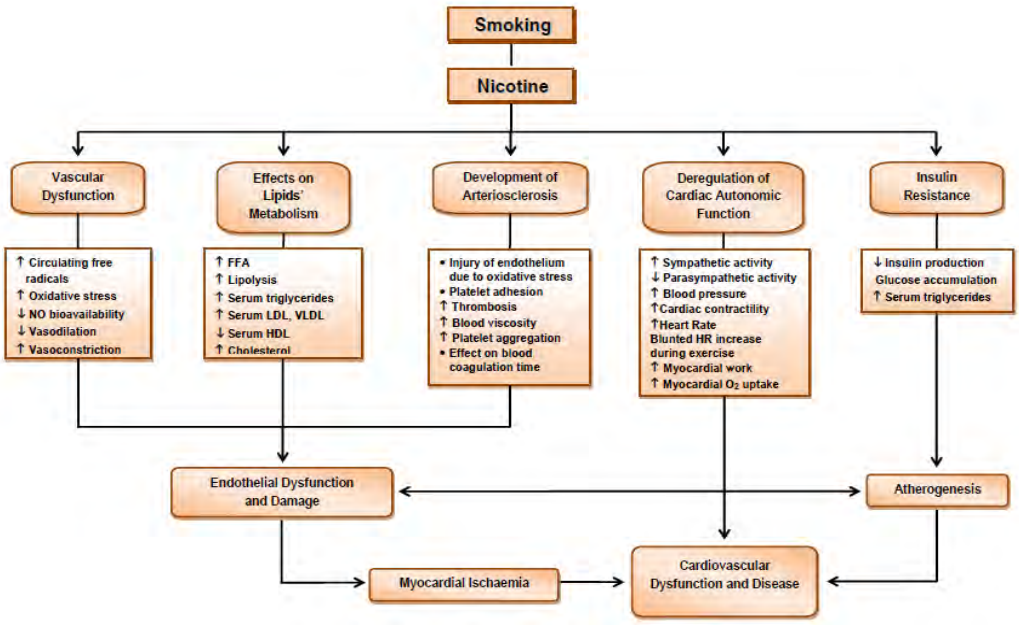


Figure 5 Effects of Smoking on Cardiovascular Function: The Role of Nicotine (Reitbrock *et al.*, 1992)

## 1.5 Carbon Monoxide

Carbon monoxide (CO) is produced from the incomplete combustion of carbon-containing substances, such as gasoline and tobacco (Reitbrock *et al.*, 1992). The background level of CO in the atmosphere is very low and has little effect on humans, while most of the CO produced by natural or technological processes is oxidized to $CO_2$ in the upper atmosphere. Comparatively, then, the 3 to 6% CO in cigarette smoke (and the 2 to 3 times higher concentrations in pipe and cigar smoke) represent considerably higher levels than are normally encountered (Turino, 1981).

Carbon monoxide exposure has been implicated in the process of atherosclerosis, contributing to the accumulation of cholesterol in the aorta and coronary arteries (Astrup *et al.*, 1970). In addition, CO exposure enhances endothelial damage, leading to detrimental effects in the presence of ischaemic heart or peripheral vascular disease (Zevin *et al.*, 2001).

The deleterious effects of CO are more profound in the myocardium than in peripheral tissues, because of the very high oxygen extraction by the myocardium at rest (Zevin *et al.*, 2001). There is epidemiological evidence that workers exposed to high CO concentrations have higher cardiovascular morbidity and mortality compared to the expected rate in the general population (Stern *et al.*, 1981and Koskela, 1994).The main mechanism by which CO causes heart disease is through hypoxia. Inhalation of cigarette smoke, by either active or passive smokers, increases the levels of carboxyhaemoglobin (COHb) in the blood, decreasing the supply of $O_2$ to the tissues. In addition, myoglobin binds with CO so that the heart muscle does not take up the necessary $O_2$ and does not perform optimally. The reduced $O_2$ uptake as a result of smoking, together with an increase in serum lactic acid levels (lactic acidosis), leads to a reduction in peak aerobic capacity and to a significant decrease in maximum $O_2$ uptake ($VO_2max$) (Figure 6).

### 1.5.1 CO and Hemoglobin

The strong chemical affinity between haemoglobin (Hb) and CO is well-known. It has been estimated that the affinity between Hb and CO is 200 times greater than the affinity between Hb and oxygen ($O_2$) (Turino, 1981). A direct consequence of this difference is the widespread binding of Hb by CO in the blood, the creation of COHb, and a great increase in its serum levels, resulting in a significant decrease in oxygen uptake by peripheral tissues. More

specifically, the CO in smoke binds Hb, creating COHb (Zevin *et al.*, 2001) through the following reaction:
$HbO_2 + CO \rightarrow COHb + O_2$ where $HbO_2$ is oxyhaemoglobin (McDonough and Moffatt, 1999).

The presence of COHb in the blood, apart from decreasing its $O_2$ saturation, causes a leftward shift in the $O_2$-Hb dissociation curve, further compounding the state of particulate-induced hypoxaemia. Thus, for the same haemoglobin concentration the $O_2$ supply to the tissues and cells is decreased (Turino, 1981). In smokers, COHb levels are 5% on average and may reach as much as 10% in heavy smokers. In contrast, in non-smokers COHb levels range between 0.5 to 2%, depending on their exposure to automobile exhaust (Bullen, 2008). More specifically, one and a half hours after smoking, COHb levels range on average between 3.9 to 4.1% (Papathanasiou *et al.*, 2014), while elsewhere it has been shown that immediately after smoking COHb levels were around 9% (Reitbrock *et al.*, 1992).

The increase in blood COHb levels and the reduced $O_2$ supply to the tissues affect the vascular permeability (Bullen, 2008). The increase in endothelial permeability, together with the injuries to the intima of the arterial wall associated with exposure to CO, leads to sub-endothelial oedema manifested by early atherosclerotic changes, such as fat deposition in the arterial walls. Finally, the presence of CO in the blood is considered responsible for severe anatomical and morphological changes in the myocardium, such as partial or total necrosis of muscle fibrils, and degenerative processes in the mitochondria (Papathanasiou *et al.*, 2014).

These morphological changes are similar to those found in hypoxia (Zevin *et al.*, 2001). Other observations include extra- and intracellular oedema, capillary wall oedema, an increase in the number of ribosomes, and reparative fibrotic changes (Papathanasiou *et al.*, 2014)

### 1.5.2 CO and Myoglobin

Myoglobin may combine with CO and, like Hb, has a greater affinity (30 to 50 times) with CO than with $O_2$, intensifying the hypoxaemia of peripheral tissues and especially the active muscles. However, myoglobin binds to one molecule of $O_2$, whereas Hb binds to four. Thus, the negative effects of increased COHb levels are much more striking than those of COMb,

effectively reducing both the $O_2$ supply to the tissues and the $O_2$ uptake of working muscles (Papathanasiou *et al*., 2014).

### 1.5.3 CO and lactic acidosis

The term ―lactic acidosis" refers to high levels of lactic acid in the blood. The reduced efficiency of the $O_2$ transportation and supply system in smokers inhibits mitochondrial function. The exposure of mitochondria to smoking-induced oxidative substances results in damage to the mitochondrial DNA, reducing adenosine triphosphate production in heart and muscle cells (King *et al*., 1987). Essentially, smoking disturbs the activity of adenine nucleotide translocator and mitochondrial superoxide dismutase in mitochondria, which are essential for their proliferation, thus reducing their numbers. Because of this damage, the muscles cannot get the energy they need to function (since they no longer have sufficient mitochondria); they therefore seek energy via another route: anaerobic metabolism (McDonough and Moffatt, 1999). The latter process, however, has lactic acid as its final product, so that the quantity of circulating lactic acid increases significantly (lactic acidosis), increasing the blood's acidity, compromising aerobic tolerance, and impairing exercise capacity (Guyton and Hall, 2006).

### 1.5.4 CO and Exercise Capacity

Smoking even one cigarette can immediately affect physical exercise capacity (Barnoya and Glantz, 2005). The effects of CO, such as the widespread binding of Hb and the reduced arterial $O_2$ blood saturation, the insufficiency of respiratory enzymes, in combination with the binding to myoglobin and the effects of CO on aerobic metabolism, result in dysfunction of the $O_2$ production, transportation, and delivery system, especially during exercise (McDonough and Moffatt, 1999). Briefly, the reduced quantities of transported $O_2$ and the decreased $O_2$ supply to and uptake from the active tissues, combined with the binding of myoglobin by CO, significantly decrease maximal oxygen uptake ($VO_2max$) reducing the functional capacity and the performance of the circulatory system (Figure 6).

There is an observable decrease of around 10% in the duration of exercise until exhaustion in smokers, which is attributable to a reduction in $O_2$ production in the metabolically active tissues, as a result of arterial $O_2$ desaturation, and to the insufficiency of the $O_2$ transportation, supply and uptake system (Figure 6) (Barnoya and Glantz, 2005). This impaired exercise

tolerance and the decreased maximal exercise capacity have been recorded even in young healthy smokers (Papathanasiou *et al.*, 2013).

Regardless of the underlying mechanism, the effect of smoking on COHb levels is responsible for a leftward shift in the $O_2$-Hb dissociation curve (McDonough and Moffatt, 1999). Thus, increased COHb levels are able to interfere with the $O_2$ released in the cells in two ways, both of which can reduce $VO_2$max: 1) decreasing the amount of $O_2$ transported in the blood via a reduction in the available binding sites on the surface of Hb, and 2) delaying the unloading of $O_2$ into active muscles. The result is a decrease in the effectiveness of myoglobin in unbinding $O_2$ in muscle cells (Wittenberg and Wittenberg, 1989). During exercise, the hypoxaemia due to smoking becomes more apparent at the lactic acidosis threshold, where the deoxygenation of skeletal muscles increases drastically (McDonough and Moffatt, 1999).

Similar effects of cigarette smoke on the $O_2$ transportation and supply system are seen in individuals who are not active smokers (Papathanasiou *et al*., 2007). Since non-smokers are more vulnerable to CO than smokers, simply being exposed to cigarette smoke may reduce their $VO_2$max. The extent to which $VO_2$max is reduced depends on the amount of CO that smokers inhale (Reitbrock *et al.*, 1992). Horvath *et al*. claimed that no significant reduction in $VO_2$max was observed until levels of COHb reached or exceeded 4.3%, a level exhibited by most smokers (Colberg *et al.*, 1994). From the moment COHb levels reach 4.3%, $VO_2$max decreases in accordance with the following equation:

$VO_2max = 0.91(\%COHb) + 2.2$

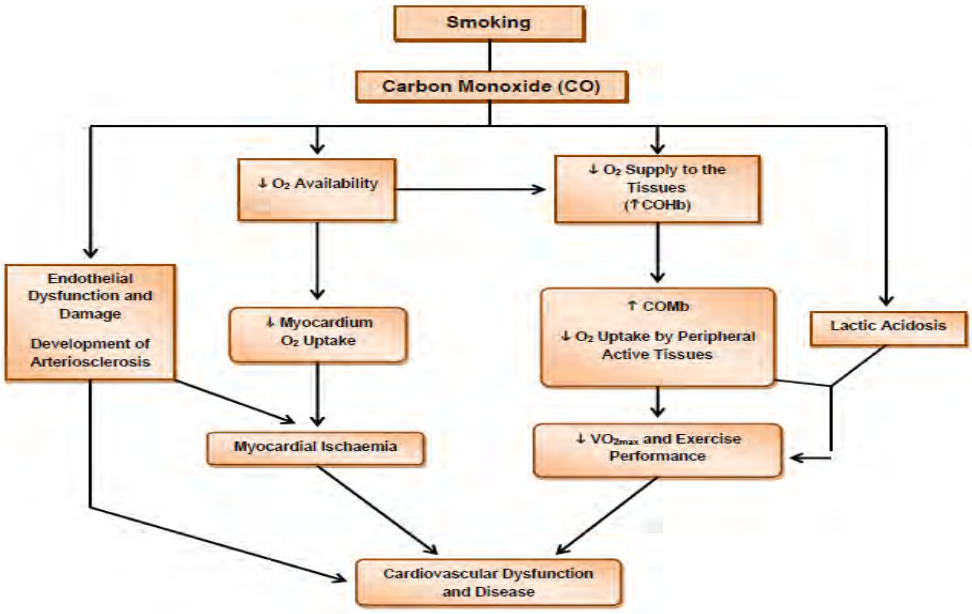


Figure 6 Mechanism of Carbon Monoxide actions in cardiovascular dysfunction (Papathanasiou *et al*., 2014)

### 1.6 Cigarette smoking and CRP

Low-grade inflammation may be part of the ~~"~~common ground" underlying the Metabolic Syndrome, Type 2 diabetes and cardiovascular disease. Low-grade chronic inflammation is associated with cardiovascular disease (Fernandez-Real and Ricart, 2003), diabetes, insulin resistance, features of the Metabolic Syndrome (Laaksonen *et al*., 2004) and the full Metabolic Syndrome itself (Laaksonen *et al*., 2003). IL-6 is a pro-inflammatory cytokine produced by adipose tissue, endothelial cells, macrophages and lymphocytes. C-reactive protein (CRP), an acute-phase reactant, is synthesised in the liver largely in response to IL-6. Recent studies indicate that inflammation, as measured by IL-6 and CRP predicts not only cardiovascular events but also the development of diabetes (Laaksonen *et al*., 2004).

Low-grade inflammation may promote atherosclerosis and insulin resistance and predispose to the development of the Metabolic Syndrome and diabetes by several mechanisms (Laaksonen *et al*., 2004). IL-6 may interfere with insulin signalling through the induction of proteins that bind to the insulin receptor (Senn *et al*., 2003), and it appears to down-regulate corticosteroid-binding globulin, which may lead to increased free cortisol concentrations, insulin resistance and other manifestations of the Metabolic Syndrome (Fernandez-Real *et al*., 2002). IL-6 also inhibits lipoprotein lipase activity and increases concentrations of NEFA, contributing to dyslipidaemia and insulin resistance (Fernandez-Real and Ricart, 2003). In

addition, IL-6 stimulates the secretion of major pro-inflammatory cytokines such as IL- 1. In turn, IL-1 and TNF-α, which are also secreted from adipose tissue, induce IL-6 secretion. TNF-α decreases insulin-mediated glucose uptake and impairs endothelial function (Rask-Madsen *et al*., 2003). Pro-inflammatory cytokines, and possibly CRP itself, may also directly promote atherosclerosis and thrombosis by the induction of nuclear factor-κB and the release of adhesion molecules and plasminogen activator inhibitor-1 (Laaksonen *et al*., 2004).

There is a positive association between smoking status and elevated CRP in adolescents, and in particular among heavier past-month smokers. Damage related to cigarette smoking may begin soon after tobacco use initiation, reinforcing the preventive message that no level of smoking is safe in youth (Tonstad and Cowan, 2009).In older men and women it took several years after smoking cessation for CRP concentrations to return to that of individuals who never smoked (Dietrich *et al.*, 2007).

A great deal of evidence suggests that many smokers with and without diabetes displays the typical features of the Metabolic Syndrome. Interestingly, it was also reported that after people stop smoking they experience improvements in insulin sensitivity and in all the other components of the Metabolic Syndrome (Targher, 2005).

It has been suggested that the increase in resistance to insulin that is experienced by people who smoke is provoked by nicotine and the other chemicals in tobacco smoke. It has been reported that in people with type 2 diabetes, the intake of nicotine acutely reduces insulin sensitivity; and that sensitivity to the action of insulin is reduced in people who use nicotine chewing-gum for extended periods. Nicotine therefore might be a major contributor to the development of the Metabolic Syndrome, including the impairment of a person‘s sensitivity to insulin (Targher, 2005).

Catecholamines, as well as other hormones, such as glucagon and growth hormone, impair the action of insulin and can induce insulin insensitivity. In fact, it has been reported that, at the cellular level, catecholamines impair the pathways that are related to the production of insulin, and the activity and synthesis of the proteins that transport glucose to cells. It is possible then that nicotine – via these and probably other as yet poorly understood brain and tissue-receptor mechanisms – impairs both insulin sensitivity and insulin secretion (Targher, 2005).

It has also been suggested that chronic tobacco smoking may have a direct impact on the distribution of a person‘s body fat. Several studies support this, showing that chronic smokers suffer abnormal function in the area of the brain (the hypothalamus) related to weight gain and obesity. This plays an important role in determining the accumulation of a person‘s fat (visceral fat) around the abdominal organs. In turn, this puts the person at increased risk of developing insensitivity to insulin and impaired glucose tolerance (Targher, 2005).

Finally, we know that smoking increases oxidative stress, causes inflammation, and reduces the flow of blood to muscle, further contributing to the development and progression of insulin insensitivity and type 2 diabetes (Targher, 2005).

Given the metabolic effect of smoking, it is expected that the greater the number of cigarettes smoked, the lower the smoker's body weight. However, cross-sectional studies indicate that heavy smoking could be associated with a greater risk of obesity. In the Cancer Prevention Study, whereas lighter smokers had lower body weight than did never or former smokers, heavy smokers (≥2 packs cigarettes/d) were more likely to be overweight than were other smokers (Arnaud *et al*., 2008).

Over the past decade, several studies have demonstrated that smoking can severely reduce insulin sensitivity both in people with type 2 diabetes and in those without the condition (Targher, 2005). The association of tobacco use with the onset of Metabolic Syndrome has been recognized in the past decade. Cigarette smoking has been proven to play a role in emergence of various components of Metabolic Syndrome and hence could lead to occurrence and progression of the disease through multiple mechanisms. However, available data from epidemiological studies on this issue are inconsistent and controversial. The positive correlation between smoking and Metabolic Syndrome is significant in some but not all studies, and many studies are confounded by other factors, such as alcohol use and low exercise levels, which might contribute to the observed correlations with Metabolic Syndrome (Sun *et al*., 2012).

It is well known that cigarette smokers generally weigh less than non-smokers and their age and gender adjusted body mass index (BMI) is on average 1 $kg/m^2$ less than that that of non-smokers. Nicotine is a component of tobacco that may contribute to weight loss. The average weight gain after quitting smoking is about 10 lb, and patients should be advised to control their calorie intake and increase their exercise after quitting. Despite this, compared to non-smokers, current smokers are more likely to have abdominal type obesity. The lower BMI of smokers compared to non-smokers raises questions regarding the impact of smoking on cardiovascular disease (CVD) risk factors such as the Metabolic Syndrome, its components and inflammatory markers such as CRP **(**Ivan *et al.*, 2012).

Numerous prospective investigations have demonstrated a substantial decrease in CHD mortality for former smokers compared with continuing smokers. This diminution in risk occurs relatively soon after cessation of smoking and increasing intervals since the last cigarette smoked are associated with progressively lower mortality rates from CHD. There is overwhelming evidence demonstrating both the cardiovascular hazards of smoking and the prompt benefit that occurs with smoking cessation (Ira and Nancy, 2013).

Smoking is a strong risk factor for atherosclerosis and CVD, with a dose-dependent relationship (the heavier the smoking, the higher the risk). Smokers have abnormalities in lipoprotein metabolism and endothelial function. Moreover, there is some evidence that smokers are at greater risk than nonsmokers of becoming insulin resistant and hyperinsulinemic (Sang Woo *et al.*, 2005).

In the first 3 years following smoking cessation, there may be a higher risk for incident Metabolic Syndrome compared with sustained smoking. Smokers often gain weight after smoking cessation, so weight control after quitting smoking is critical to attenuate the additional risk for incident Metabolic Syndrome (Byungjinkim *et al.,* 2009).

There is a positive and dose response relationship between the daily number of cigarettes smoked and the risk of developing Metabolic Syndrome. The risk of developing Metabolic Syndrome was significantly higher in those who smoke 21 to 30 cigarettes per day (Noriyuki *et al*., 2005).

Smoking cessation is beneficial to Metabolic Syndrome and its individual components. Individuals who currently smoked had a higher prevalence of Metabolic Syndrome than those who had never smoked. Current smokers who smoke ≥20 pack-years have a significantly increased risk of developing Metabolic Syndrome, high triglyceride level, and low HDL-C level (Ching-Chu *et al.*, 2009).

Adipose tissue inflammation appears to down-regulate leptin expression in adipose tissues. Nicotine further suppresses leptin expression. Thus, both smoking and inflammation may diminish leptin effect in obese subjects. Therefore, obese, but not normal weight, smokers might be more resistant to weight loss than non-smokers (Shintaro *et al.*, 2012).

Many clinical and experimental studies have found significant associations between cigarette smoking and development of diabetes, impaired glycaemic control, and diabetic complications (microvascular and macrovascular). A different lifestyle of smokers, in contrast to that maintained by non-smokers, may also contribute to these effects. The development of type 2 diabetes is yet another harmful consequence of cigarette smoking, and one that adds to the heightened risks of CVD; smoking cessation is crucial to facilitating glycaemic control and limiting development of complications (http:/www.surgeongeneral. gov/library/tobaccosmoke/report/index.html)

Significantly higher ORs were found in currently smoking men and women than in their non smoking counterparts. The association between smoking and the Metabolic Syndrome remained even after adjusting for other covariates, possibly a reflection of the effect of cigarette smoking on insulin resistance (Yong-Woo *et al.*, 2003).

Moderate and heavy smokers significantly elevate their total cholesterol, LDL-C and triglycerides and reduce HDL-C, and this develops the greater chance for serious blood vessel problems and heart disease in the future (Babiker and Elsayir, 2012).

Cigarette smoking is a classical and a major risk factor in the development of several diseases with an inflammatory component, including cardiovascular disease and chronic obstructive pulmonary disease. Improvements in assays for protein markers of inflammation have led to many studies on these factors and their roles in disease (Tonstad and Cowan, 2009).

Smokers have increased numbers of white blood cells, mainly because of a particular increase in polymorphonuclear neutrophils, which are released from the bone marrow and recruited to inflamed tissue. IL-β and IL-6, which are increased in response to lung inflammation and are implicated in the induction of CRP gene expression, may mediate the stimulation of bone marrow cells (Tonstad and Cowan, 2009). In older men and women it took several years after smoking cessation for CRP concentrations to return to that of individuals who never smoked (Dietrich *et al*., 2007).

CRP levels in current smokers are elevated but unrelated to the number of cigarettes smoked per day. In past smokers, long-term smoking cessation may contribute to the reduction in risk of development of cardiovascular diseases through inflammatory mechanisms (Oshawa *et al*., 2005).

Levels of CRP in the healthy elderly are tightly regulated and reflect lifetime exposure to smoking as well as level of obesity, ongoing level of fibrinolysis, diabetes status, and level of subclinical atherothrombotic disease. Moreover, exposure to smoking affects the relation of CRP to these other factors (Tracy *et al*., 1997).

Smoking has been associated with an increased waist circumference (and increased waist–hip ratio, WHR). WHR is positively associated with the number of pack-years of smoking, and there is a dose–response relation between WHR and the number of cigarettes smoked (Yatan, 2012).

Serum total cholesterol level is considered a risk factor in arteriosclerosis and is used as a common clinical diagnostic tool. A low level of HDL-cholesterol relative to total cholesterol is thought to lead to arteriosclerosis. Cigarette smoking and elevations of low-density lipoprotein (LDL) are independent major risk factors for atherosclerosis. Cigarette smoking and obesity are associated with increased risk of cardiovascular disease and are known to have a negative impact on lipid and lipoprotein metabolism (Miyao *et al.*, 1993).

## 1.7 Statement of the problem

Cigarette smoking is the second cause of death in the world. It is also responsible for 1 in 10 adult deaths or more that 4.9 million deaths each year. A number of adverse effects have been reported to be associated with smoking, affecting several of our physiological systems including cardiovascular system, immunological system, and others (Yuvrajsing, 2008).

Smoking is reportedly associated with the development of Metabolic Syndrome, presumably via a pathway leading to the development of cardiovascular disease (Kazuhiko *et al*., 2012). World Health Organization (WHO) estimated in 2011 that 34% of Ethiopian population is dying from non-communicable diseases, with a national cardiovascular disease prevalence of 15%, cancer and chronic obstructive pulmonary disease prevalence of 4% each, and diabetes mellitus prevalence of 2% (Awoke *et al*., 2014).Other studies have indicated that the use or misuse of addictive substances, such as cigarettes, alcohol, and khat (*Catha edulis* Forsk) is increasingly prevalent in Ethiopia (Fikru *et al*., 2008), although Ethiopia is among the nations with a relatively low prevalence of cigarette smokers (Marie *et al*.,2014).

## 1.8 Significance of the study

This study should expand the present knowledge of the health impacts of cigarette smoking and specifically add to the paucity of documented data correlating cigarette smoking and Metabolic Syndrome in Ethiopia.

This research will provide a documented file which can be used as a reference for further study on the same area, try to suggest strategies to circumvent the health effects of cigarette smoking and also can give information for policy makers.

## 1.9 Hypothesis

Null Hypothesis- There is no difference in levels of parameters of Metabolic Syndrome in smokers and non smokers

Alternate Hypothesis- There is difference in levels of parameters of Metabolic Syndrome between smokers and non smokers

# 2. Objective

## 2.1 General Objective

- To evaluate the relationship between smoking cigarette and levels of parameters of Metabolic Syndrome and CRP

## 2.2 Specific Objectives

- To explore difference of body mass index between smokers and non smokers
- To compare lipid profiles of smokers with non smokers
- To compare level of CRP in smokers and non smokers
- To investigate a possible link between age and duration of smoking with risk of developing Metabolic Syndrome
- To evaluate the association between combinatorial effects of chewing khat and alcohol consumption with cigarette smoking and parameters of Metabolic Syndrome
- To assess the combinatorial effects of using marijuana, sniffing glue and other stimulants with cigarette smoking on levels of markers of Metabolic Syndrome

# 3. Materials and Methods

## 3.1 Study area

The study was conducted in Adama, Ethiopia and Adama Hospital Medical College Laboratory found in the city. The city is situated along the road that connects Addis Ababa with Dire Dawa. It's located at an elevation of 1712 meters, 99 km southeast of Addis Ababa. The city sits between the base of an escarpment to the west, and the Great Rift Valley to the east. Following World War II, Emperor Haile Selassie renamed the town after Biblical Nazareth, and this name was used for the remainder of the twentieth century. In 2000, the city officially reverted to its original Oromo language name, Adama, though "Nazareth" is still widely used. Based on the 2007 Census conducted by the Central Statistical Agency of Ethiopia (CSA), this city has a total population of 220,212.

## 3.2 Study design and period

Community based cross sectional study design and questionnaire filling was conducted from July 1, 2014- November 1, 2014 to determine levels of parameters of Metabolic Syndrome in smoker and non smoker residents in Adama, Oromia region.

**3.3 Source population-** Adult residents living in Adama, Oromia, Ethiopia

**3.4 Study population-** Adult resident volunteers found in the city during the study period

## 3.5 Sample size

Convenient sampling method was used and 99 individuals were taken and randomly divided into two groups, smokers and non smokers each group having 50 and 49 individuals respectively.

## 3.6 Data collection techniques and procedures

Data was collected by preparing standardized questionnaire and the participants respond to trained data collectors. Sample from participants was collected and tested by laboratory technologists who are trained to operate clinical chemistry machine.

### 3.7 Anthropometric and Clinical Measurement

Arterial blood pressure was measured after the sampled individuals have 5 minute rest and comfortably sat on a chair. The measurement was taken using standard mercury sphygmomanometer by professional nurse. Weight and height was measured using

weight scale with height scale attached and Body mass index was calculated using the formula (weight in kilogram divided by height in metre squared).

### 3.8 Laboratory Analysis

For preparation of plasma, 10 ml of blood was collected by trained laboratory technologist into a tube containing sodium heparin anticoagulant, then centrifuged with 3500 revolution per minute for 10 minutes, and the plasma separated from the whole blood was then transferred to nunc tubes and stored in a deep freezer of -30 degree centigrade and transferred to EPHI for analysis using sample transporter.

For serum preparation, 10 ml of blood were collected into a tube without anticoagulant and left to form a blood clot at room temperature for 30 minute then centrifuged with 3500 revolution per minute for 10 minutes, and then the serum was transferred to nunc tubes and stored in a deep freezer of -30 degree centigrade and transferred to EPHI for analysis using sample transporter.

The sample was analyzed for plasma Total Cholesterol, Triglycerides, HDL and serum CRP using clinical chemistry machine (Cobas Integra 400 plus) and LDL was calculated using the Friedewald formula (Total Cholestrol-HDL-Triglycerides/5). Different micro litres of plasma and serum sample was inserted into the Cobas Integra 400 Plus clinical Chemistry machine based on the analyte and the result was taken from the computer screen attached to the machine.

## 3.9 Variables

### 3.9.1 Dependent variable

- Level of parameters of Metabolic Syndrome and CRP in smokers

### 3.9.2 Independent variables

- Socio demographic status
  - Sex
  - Age
  - Income
- Number of cigarette smoked per day
- Use of other stimulants other than cigarette
- Consumption of alcohol along with cigarette smoking
- Chewing khat
- Literacy
- Number of years of smoking cigarettes

## 4. Inclusion and Exclusion criteria

### 4.1 Inclusion

Respondents who read (read to them) the invitation letter and were voluntary to give sample and participate in the research and where there was absence of disease based on clinical signs and symptoms and function.

### 4.2 Exclusion

Individuals who were sick or those who were unwilling to sign the consent form were excluded from the research.

## 5. Statistical Analysis

The data was analyzed using spss version 20 and the values were analyzed using correlations and cross tabulation and confidence interval of 95% were taken with p-value less than 0.05 considered to be statistically significant.

### 6. Ethical Consideration

The research proposal was reviewed and ethically approved by Ethical Review committee of the Department of Medical Biochemistry, College of Medicine and Health Sciences, Addis Ababa University with Ref. No. SOM/BCHM/012/2006. A letter of invitation was written and distributed for residents of Town of Adama and those willing to partake in the research signed letter of consent. The confidentiality of both the information provided in the questionnaire, anthropometric measurements and laboratory assay results were strictly maintained.

# 7. Results

## 7.1 Relationship between literacy and cigarette smoking

From the sample population of smokers 82% were literate and 18% were illiterate, while in non smokers 87.8% were literate and 12.2% were illiterate but the difference was not statistically significant(p= 0.577)

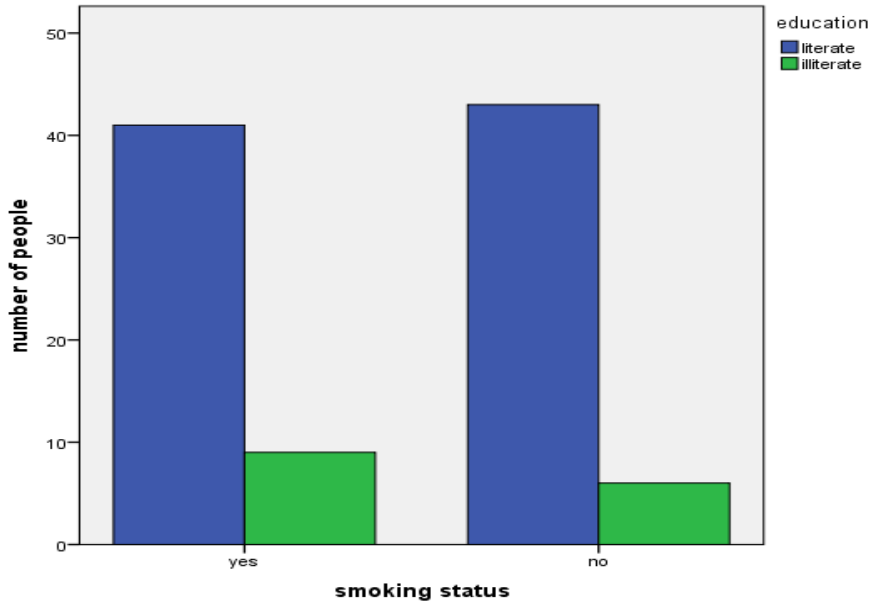


Figure 7 Education and cigarette smoking status

## 7.2 Marital status of respondents in relation to smoking status

In the sample of smokers 14% were married, 70% single, 14% divorced and 2% widowed and of non smokers 16.3% were married, 77.6% single and 6.1% divorced

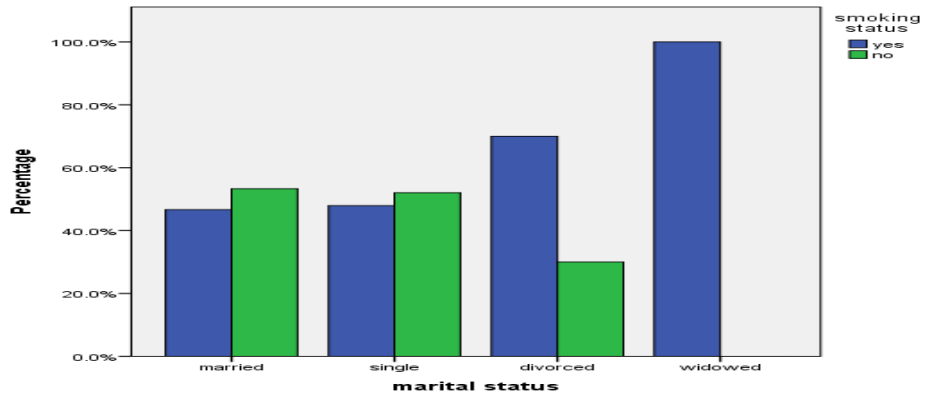


Figure 8 Distribution of smoking status defined by marital status

## 7.3 Income status of respondents in relation to smoking status

In this research from the non smokers 24.5% of respondents earn <500 birr per month, 34.7% earn 500- 1000 birr/month, 14.3% earn 1001-2000birr/month and 26.5% earn more than 2000 birr per month. 16% of smokers earn <500 birr per month, 40% of smokers earn 500- 1000 birr/ month, 20% earn 1001-2000 birr/month and 24% earn more than 2000 birr/ month.

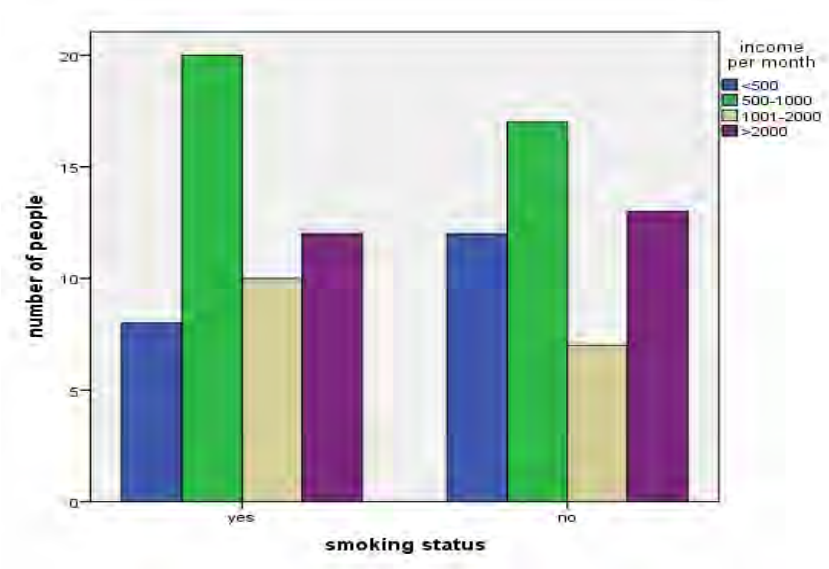


Fig 9 Income distribution between smokers and non smokers

## 7.4 Smoking status and parental smoking

From those who responded that their parents do smoke 50% were smokers and 50% were non smokers and from those who responded that their parents do not smoke 50.6% were smokers and 49.4% were non smokers.

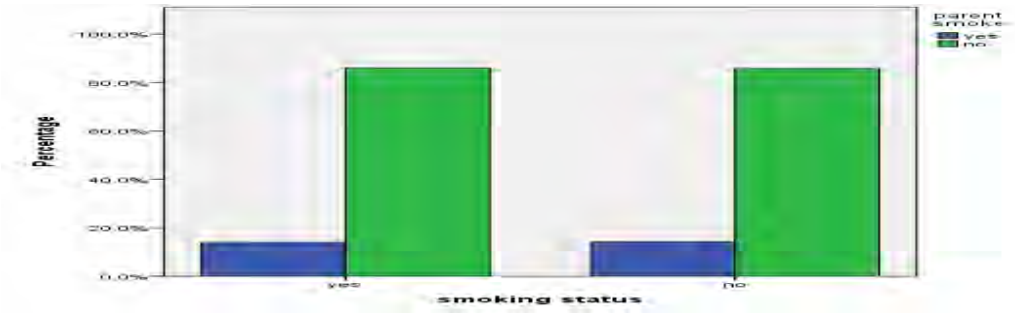


Fig 10 Parental smoking and respondents` smoking status

## 7.5 Knowledge about Risks of Cigarette Smoking

83.8 % of respondents know about risks of cigarette smoking while 16.2% responded that they do not know about risks of cigarette smoking. From those who responded of knowing the risk 27.7% describe cancer, 55.4% lung disease, 3.6% heart disease, 1.2% lung cancer and 12.1% describe others (Loss of Appetite, Gastritis, Depression, Immunosuppression, Skin disease, Neurological disorder, Halitosis, Oral disease and Decrease life expectancy)

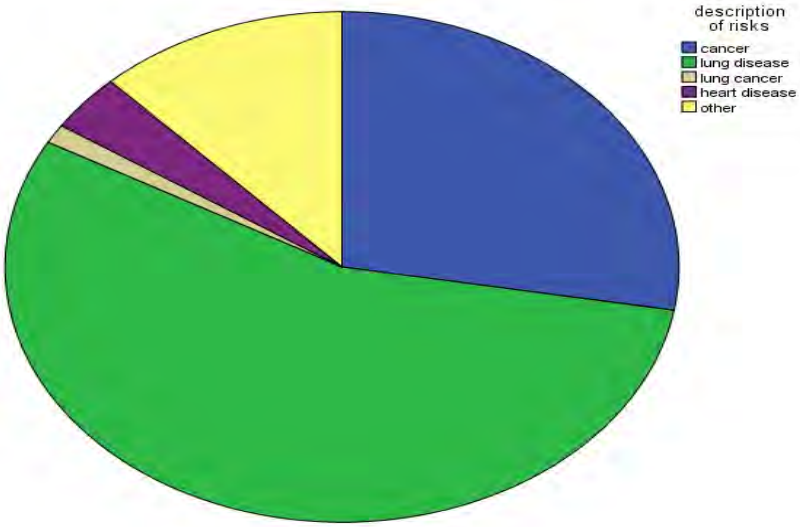


Fig 11 Risks of cigarette smoking described by respondents

## 7.6 Cigarette smoking and other drugs of abuse

The distribution of smoking status with khat chewing and alcohol drinking and alcohol consumption is shown in Figure 12. All smokers drink alcohol and 80% of smokers also chew khat.

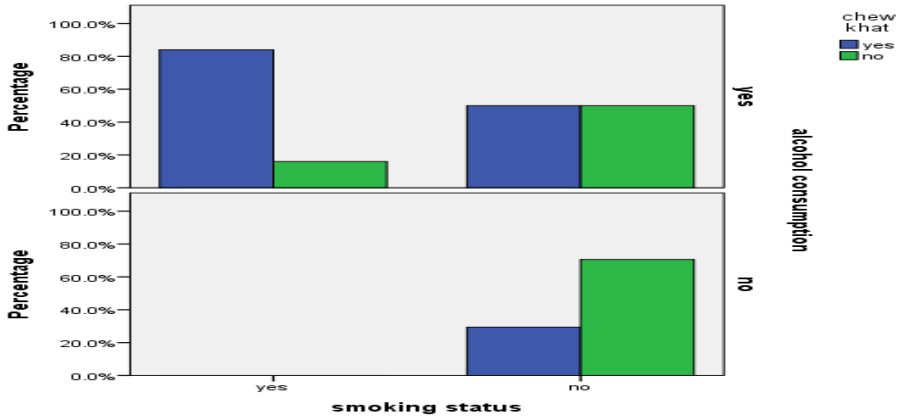


Figure 12 Difference in chewing khat and alcohol drinking between cigarette smokers and non smokers

In respondents there was statistically significant difference in usage of marijuana between cigarette smokers and non smokers (p= 0.006)

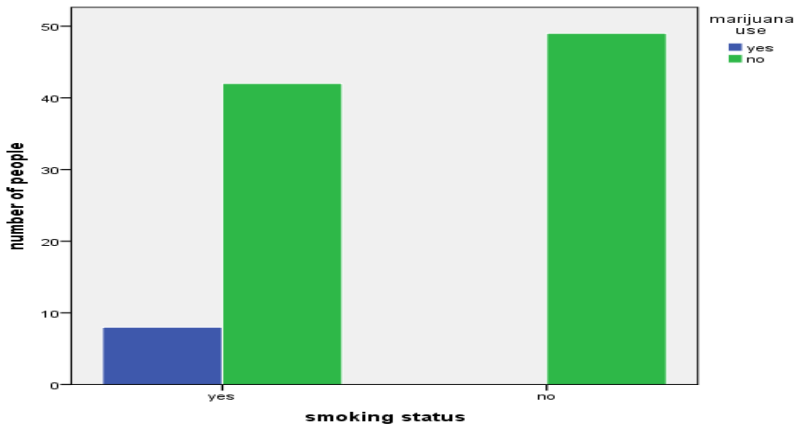


Figure 13 Difference in marijuana use between smokers and non smokers

## 7.7 Relationship between age and parameters of Metabolic Syndrome

In the overall sample population of smokers and non smokers there was statistically significant correlation between age and triglyceride (r= 0.257, p= 0.010), age and total cholesterol (r=0.365, p=0.000) and age and CRP (r=0.397, p=0.000). But there was no statistically significant correlation between age and systolic blood pressure (r=0.090, p= 0.375), age and diastolic blood pressure (r=0.046, p= 0.655), age and HDL (r=0.165, p=0.103), age and LDL (r=0.102, p=0.341).

In smokers there was statistically significant correlation between age and total cholesterol (r= 0.361, p= 0.010), age and CRP (r=0.428, p= 0.002). But there was no statistically significant correlation between age and HDL (r=0.075, p=0.605), age and LDL (r=-0.021 p=0.895), age and systolic blood pressure (r=0.112, p=0.438), age and diastolic blood pressure (r=-0.046, p=0.753) and age and triglycerides (r=0.194, p=0.178).

## 7.8 Blood Pressure difference between smokers and non smokers

The frequency distribution of systolic and diastolic blood pressure in all of the sample population is shown in Figure 14 and Figure 15. 24.2% of the sample population have systolic blood pressure ≥135 and 24.3% have diastolic blood pressure ≥90. There appears to be a difference in systolic blood pressure between smokers whose mean blood pressure was 119.3 mm Hg when compared to 114.8 mm Hg of non smokers but the difference was not

statistically significant(p= 0.107). Also there was higher mean of diastolic blood pressure of smokers (79.8 mm Hg) than non smokers ( 77.35 mm Hg) but the difference was not statistically significant(p= 0.256)

Table 3 Systolic and diastolic blood pressure difference between smokers and non smokers

| Blood Pressure | Smokers | Non smokers | Significance(p-value) |
|---|---|---|---|
| Systolic | 119.3 ± 16.22 | 114.8 ± 10.7 | 0.107 |
| Diastolic | 79.8 ± 12.2 | 77.35 ± 8.84 | 0.256 |

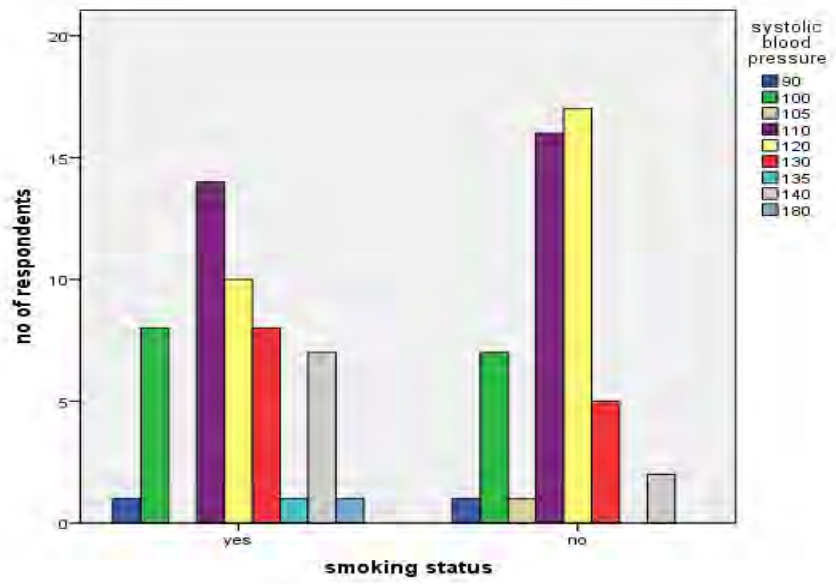


Figure 14 Frequency distribution of systolic blood pressure in respondents

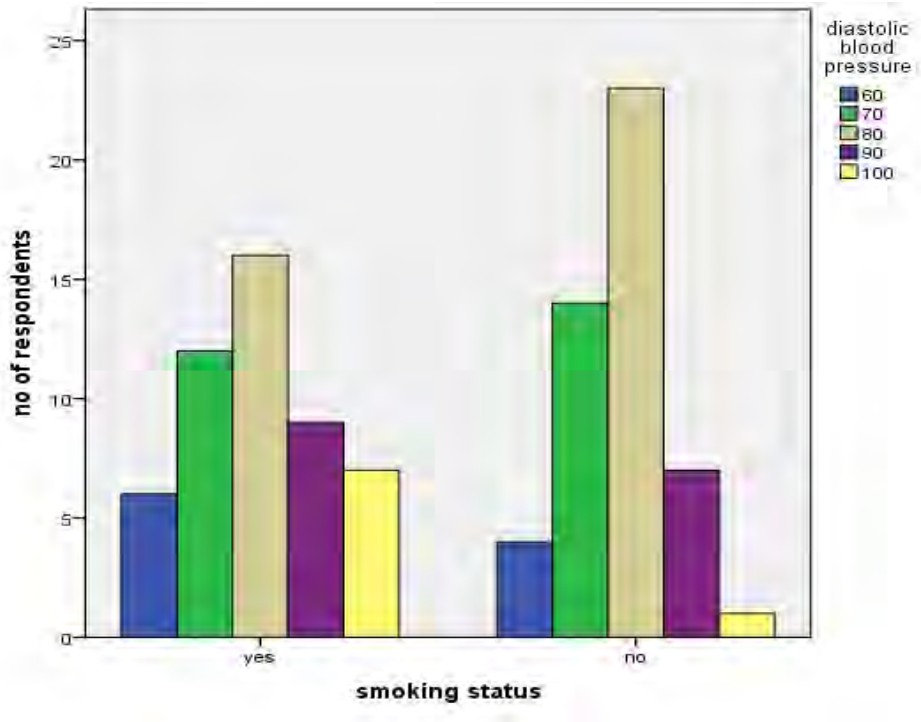


Figure 15 Frequency distribution of diastolic blood pressure in respondents

## 7.9 Cigarette smoking and BMI

There was no statistically significant correlation (p=0.667) between BMI in smokers (20.71±2.43) and non smokers (20.94 ± 2.94)

## 7.10 Relationship between cigarette smoking, plasma cholesterol levels and serum CRP

Levels of Total cholesterol, HDL, LDL and CRP in smokers and non smokers are given in table 4. Of the smokers 16.8% had LDL levels ≥ 130 mg/dl, 62% had triglycerides level ≥ 150 mg/dl, 24 % had total cholesterol ≥ 200 mg/dl, among these 5 individuals had triglycerides level > 600 mg/dl and one individual had 858 mg/dl of triglycerides. In all these laboratory tests levels were higher among cigarette smokers. There was significantly higher total cholesterol (p= 0.000), Triglycerides (p= 0.000), HDL (P=0.025), LDL (P=0.002) and CRP (p=0.001) among smokers than non smokers. Among smokers 23 individuals have met one of the criteria of the Metabolic Syndrome, 20 individuals met two criterias of the Metabolic Syndrome while 2 individuals met the three criterias of the Metabolic Syndrome. Among non smokers 30 individuals met one criteria of the Metabolic Syndrome, 5 individuals met two criterias of the Metabolic Syndrome and 2 individuals met the three criterias of the Metabolic Syndrome.

Table 4 Lipid profiles and CRP in smokers and non-smokers

| Blood levels | Smokers | Non smokers | Significance(p-value) |
|---|---|---|---|
| Total Cholesterol(mg/dl) | 179.56 ±48.8 | 134.05 ± 40.25 | 0.000 |
| Triglycerides (mg/dl) | 254.39 ± 192.21 | 118.3 ± 86.66 | 0.000 |
| HDL-C(mg/dl) | 47.76 ± 16.09 | 39.97 ± 17.91 | 0.025 |
| LDL-C(mg/dl) | 89.92 ± 34.70 | 70.31 ± 23.78 | 0.002 |
| CRP(mg/l) | 3.02 ± 2.04 | 1.07 ± 0.89 | 0.001 |

## 7.11 Relationship between pack-year smoking history and biomarkers of Metabolic Syndrome

There was statistically significant correlation between pack years and C-reactive protein (r=0.293,p-value=0.039),triglycerides(r=0.501, p-value=0.000) and total cholesterol (r=0.419, p-value=0.002). But there was no statistically significant correlation between pack years and HDL(r= 0.062,p-value=0.669), systolic(r=0.89,p-value=0.540) and diastolic blood pressure(r=0.043, p-value=0.764)

## 7.12 Combinatorial effects of cigarette smoking with other drugs of abuse

There was no statistically significant difference in smokers who chew khat and who do not in levels of lipids except total cholesterol, no statistically significant difference in systolic and diastolic blood pressure and C - reactive protein between those who chew khat and those who do not in smokers.

Table 5 Combinatorial effects of khat chewing and cigarette smoking

| | Khat chewing | | |
|---|---|---|---|
| | Yes | No | Significance(p-value) |
| SBP | 120.4± 16.67 | 113.13 ± 12.8 | 0.244 |
| DBP | 80.48 ± 12.48 | 76.25 ± 10.61 | 0.375 |
| TG | 250.37±191.41 | 275.53 ± 208.36 | 0.738 |
| TC | 173.53 ± 36.7 | 211.25 ± 86.1 | 0.044 |
| HDL | 48.32 ± 16.36 | 44.84 ± 15.33 | 0.58 |
| LDL | 86.39 ± 32.47 | 115.31 ± 43.6 | 0.081 |
| CRP | 2.58 ± 2.53 | 5.35 ± 4.32 | 0.076 |

In smokers amounts of alcohol consumed per day was significantly correlated with levels of systolic and diastolic blood pressure, high density lipoprotein. But amount of alcohol consumed per day was not significantly correlated with levels of total cholesterol, LDL, triglycerides and CRP.

Table 6 Combinatorial effects of alcohol consumption and cigarette smoking

| | Alcohol per day | | | |
|---|---|---|---|---|
| | None | 1-2 drinks | >2 drinks | Significance(p- value) |
| SBP | 110 ± 8.16 | 117.5 ± 12.58 | 122.08 ± 17.46 | 0.035 |
| DPB | 71 ± 8.75 | 77.5 ± 9.57 | 82.5 ± 12.28 | 0.006 |
| HDL | 38.25 ± 11.88 | 49.4 ± 16.52 | 50.22 ± 16.45 | 0.044 |
| TC | 157.65 ± 37.02 | 157.07 ± 19.94 | 188.15 ± 51.74 | 0.057 |
| LDL | 82.17 ± 22.21 | 64.28 ± 28.64 | 94.55 ± 37. 13 | 0.263 |
| TG | 237.49 ± 237.86 | 265.6 ± 167.89 | 257.84 ± 186.49 | 0.792 |
| CRP | 1.87 ± 1.62 | 2.49 ± 1.44 | 3.4 ± 2.63 | 0.279 |

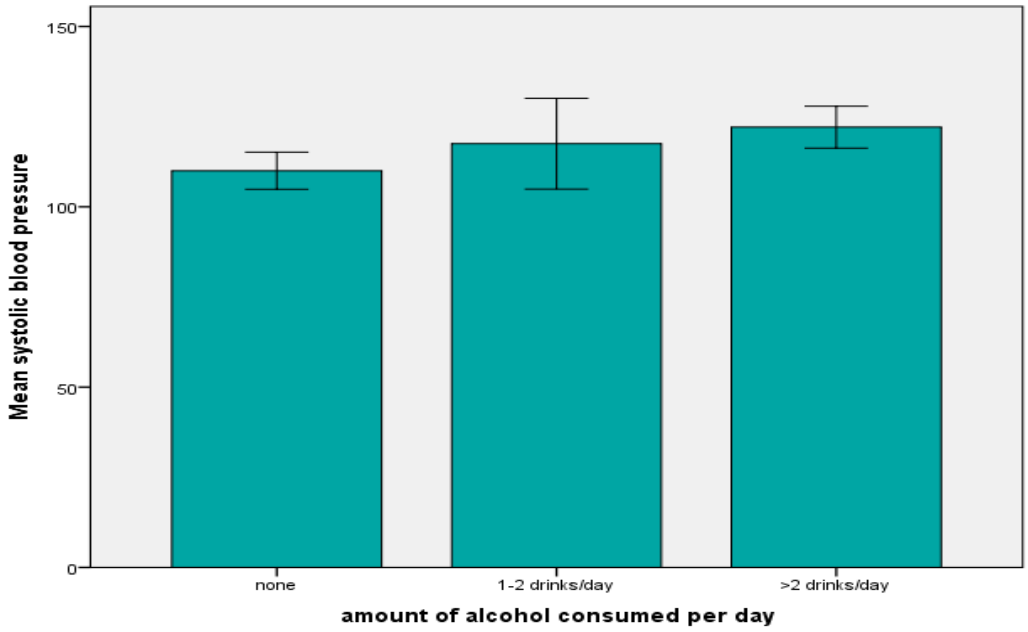


Fig 16 Amounts of alcohol consumed per day and systolic blood pressure in smokers

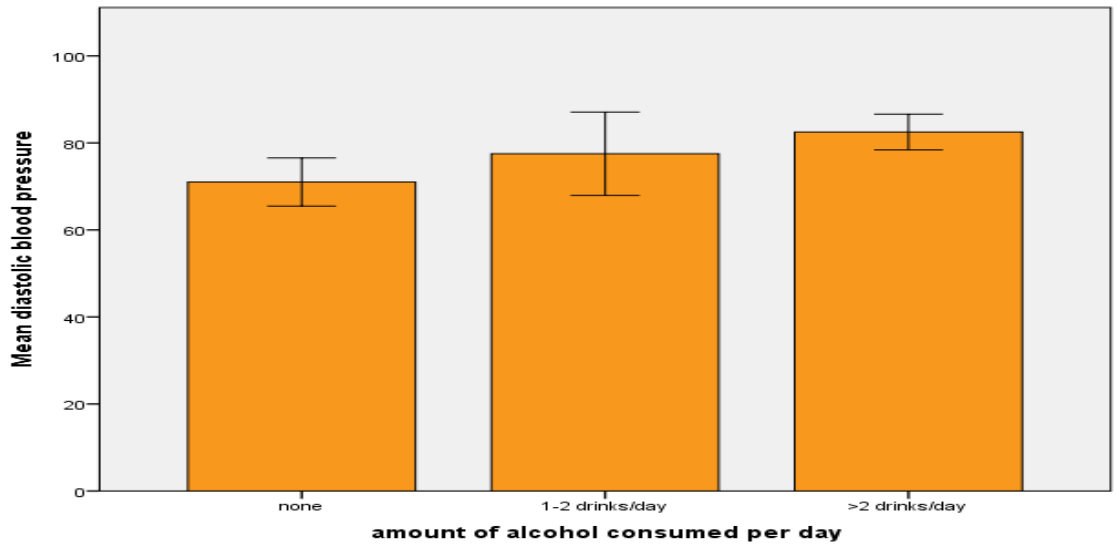


Fig 17 Amounts of alcohol consumed per day and diastolic blood pressure in smokers

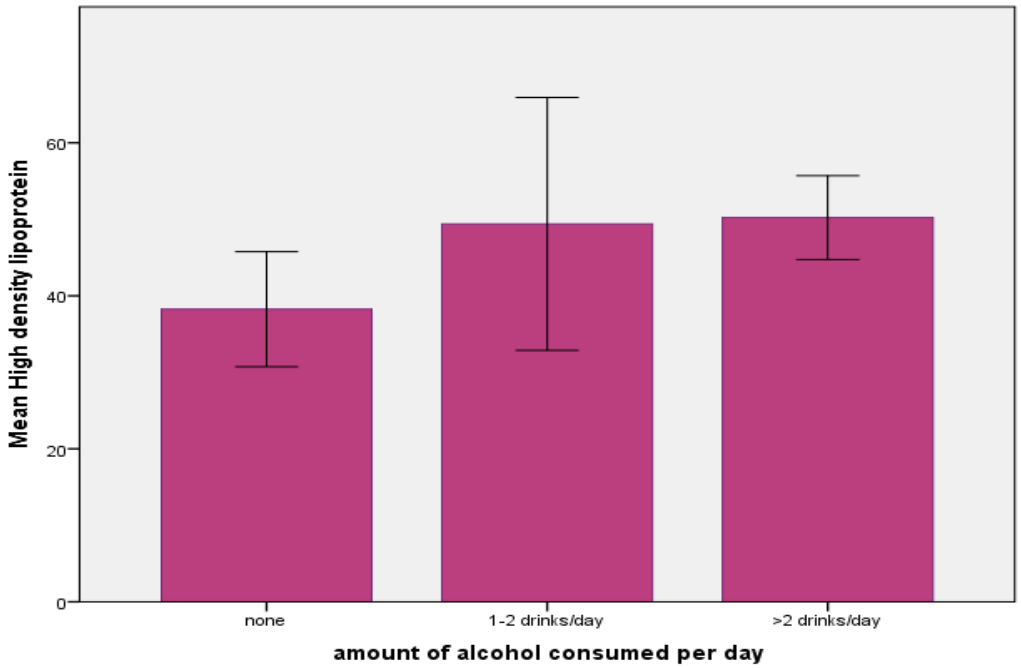


Fig 18 Amounts of alcohol consumed per day and HDL in smokers

Fig 19 shows the levels of total cholesterol in smokers grouped based on their alcohol drinking habits

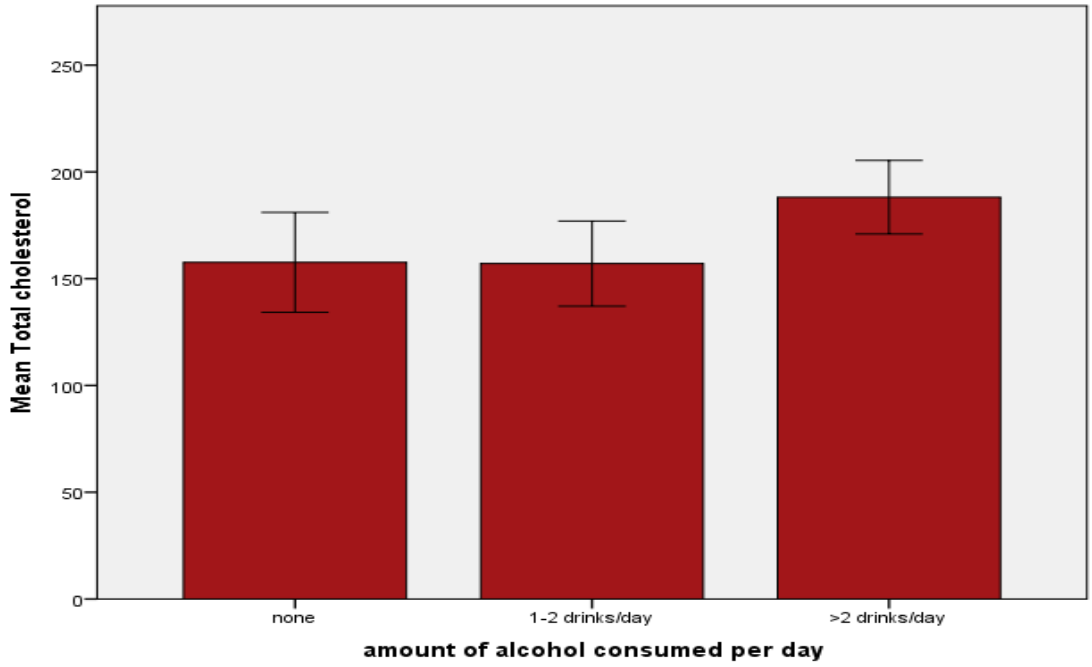


Fig 19 Amounts of alcohol consumed per day and TC in smokers

## 7.13 Framingham risk score for some of the smokers respondents

Table 7 shows the Framingham risk score for some of the smoker respondents and their score if they quit smoking

Table 7 Framingham 10 year coronary heart disease risk score (%) for some smoker respondents

| Age | TC | HDL | SBP | Framingham score(smokers) | Framingham score(if they quit) |
|---|---|---|---|---|---|
| 26 | 198 mg/dl | 61 mg/dl | 140 mmHg | 2% | Less than 1% |
| 27 | 186 mg/dl | 54 mg/dl | 100 mmHg | 1% | Less than 1% |
| 29 | 194 mg/dl | 55 mg/dl | 120 mmHg | 2 % | Less than 1% |
| 30 | 170 mg/dl | 61 mg/dl | 140 mmHg | 1 % | Less than 1% |
| 32 | 241 mg/dl | 57 mg/dl | 130 mmHg | 5 % | 1 % |
| 33 | 168 mg/dl | 56 mg/dl | 110 mmHg | 1 % | Less than 1% |
| 34 | 176 mg/dl | 35 mg/dl | 110 mmHg | 3 % | Less than 1% |
| 35 | 155 mg/dl | 45 mg/dl | 140 mmHg | 2 % | Less than 1% |
| 38 | 164 mg/dl | 100 mg/dl | 130 mmHg | 2 % | Less than 1% |
| 40 | 221 mg/dl | 42 mg/dl | 100 mmHg | 7 % | 2 % |
| 41 | 158 mg/dl | 36 mg/dl | 120 mmHg | 4 % | 1 % |
| 42 | 212 mg/dl | 53 mg/dl | 110 mmHg | 7 % | 2 % |
| 45 | 158 mg/dl | 62 mg/dl | 180 mmHg | 6 % | 2 % |
| 53 | 197 mg/dl | 64 mg/dl | 120 mmHg | 9 % | 4 % |
| 54 | 320 mg/dl | 31 mg/dl | 135 mmHg | Greater than 30 % | 20 % |
| 60 | 200 mg/dl | 48 mg/dl | 100 mmHg | 10 % | 7 % |

## 8. Discussion

This study was undertaken to evaluate the Metabolic Syndrome in cigarette smokers in Adama, Ethiopia. As per our finding the prevalence of smoking was not significant between literate and illiterates (p=0.577). It may be due to small sample size so in the future the research should be done with increasing sample size. Wagenknecht *et al*., 1990 and Gilman *et al.,* 2008 also found that the prevalence of smoking decreased with increasing education. This research generally record the literacy status of the respondents without considering their level of education so further studies incorporating levels of education of respondents is recommended.

We found that the parental smoking was not significantly associated with children smoking. In contrast to our findings, Anna *et al.,* 2008 reported that children of smokers were more likely to smoke and reported more favourable attitudes toward smoking compared to children of non-smokers. Elizabeth *et al.,* 2004 also found that parental smoking was significantly associated with youth smoking. Flay *et al*., 1994 reported that having a parent who smokes affects smoking initiation through imitation of the behaviour and it also influences smoking attitudes, norms, and beliefs.

There was no statistically significant difference in body mass index between smokers and non smokers (P=0.667). These may be due to living style, behavioural patterns between the two groups which were not considered during the study. Additionally, there are other factors associated with both BMI and smoking (e.g. poor dietary choices, poor sleeping habits, etc.) that may mediate or moderate this relationship. These finding do not match with the findings of Jennifer *et al*., 2008 who found smoking to significantly reduce body mass index in regular smokers.

This study indicated that there is no statistically significant difference in blood pressure between the smokers and non smokers and also pack years. This finding is in line with Okubo *et al*., 2002 and contrast to Au *et al*., 2010 finding that longer duration of smoking or pack years of cigarettes had a higher risk of hypertension. Fasting *et al*., 2008 found that blood pressure of smokers was lower than that of non-smokers and other researchers found that smoking would raise blood pressure (Dyer *et al*., 1982). It has been shown that smoking would raise blood pressure and heart rate through its acute vasoconstriction effect (Aronow *et al*., 1974).The relation between long-term smoking and hypertension is still unclear and

controversial (Narkiewicz *et al*., 2005). It is established that smoking could cause acute increase in blood pressure due to release of catecholamines induced by nicotine (Rohleder and Kirschbaum, 2006)

It has long been established that one of the major constituents of tobacco i.e. nicotine has a considerable influence in increasing the lipid levels in blood. Lipid have important roles in virtually all aspect of life, serving as hormones or hormones precursors, aiding in digestion, providing energy storage metabolic fuel, acting as functional and structural component in cell membranes and forming insulation to allow nerve conduction or to prevent heat lost but their excessive concentrations are associated with various metabolic disorders (Nader *et al*., 1999). The result of this work showed a statistically significant increase in the total cholesterol level of smokers ($p<0.05$) when compared with non-smokers. The result of this work is in line with work of Adedeji and Etukudo, where high concentration of cholesterol was recorded in smokers when compared with the nonsmokers (Adedeji and Etukudo, 2006) and Babiker and Elsayir, 2012. In contrast Waheeb and Alharbi in their work, which was on the influence of cigarette smoking on lipid profile in male university students recorded non significant result in total cholesterol in smokers when compared with non-smokers(Waheeb and Alharbi,2011). The increase in the total cholesterol level seen in the smokers was as a result of increase in the activity of hepatic HMG-CoA reductase (Sinha *et al.*, 1995) and Natio HK, 1985 reported that hepatic HMG CoA reductase, the main rate limiting enzyme in cholesterol synthesis is subject to induction and repression by several hormones, dietary factors and drugs one of which is nicotine. Increased cholesterol is a causative factor in the etiology of atherosclerotic disease (Carl and Edward, 1999). The rise in blood cholesterol levels in smokers may be through catecholamine and adenyl cyclase axis including tissue lipolysis (Devaranavadgi *et al.*, 2012). Within the smoking group of people plasma total cholesterol was higher among heavy drinkers than light drinkers and also those who do not drink daily. This finding is not in line with that reported by Wakabayashi, 2008 who found that total cholesterol in smokers to be lower in the drinker subgroups.

The finding of smoking being associated with high TG was consistent with previous studies (Chen *et al.*, 2008 and Nakashita *et al.*, 2010). Contrary report to this has been documented by Nesje and Mjos, 1985 who found no significant difference between smokers and non-smokers concerning triglycerides and total cholesterol**.** One possible explanation for this association is that nicotine may increase sympathetic nerve activity, which stimulates release

of catecholamines and thereby induces lipolysis, with a consequent increase in plasma concentration of TG (Andersson and Arner, 2001).

Significantly increased level of LDL was observed among smokers than non smokers in consonance with reported by Craig *et al*., 1989 and Khurana *et al*., 2000 where it was reported that increase in LDL level in cigarette smokers was due to the down regulation of LDL receptors and failure of receptor mediated endocytosis by metabolite of cigarette. This finding is in line with the work of Brischetto *et al.*, 1983 specifically attributed the down regulation of LDL receptor to inhibiting action of smoke allylamine and nicotine. Adedeji and Etukudo, 2006 also reported high level of LDL in smokers, suggesting that there is increased LDL-Cholesterol synthesis in smokers which is dangerous to their health but is in contrast to findings of Gupta *et al*., 2006. Carl and Edward reported that clinically increase in LDL cholesterol is associated with increased risk of coronary heart disease (Carl and Edward, 1999).

In our finding, the level of HDL was significantly raised in cigarette smokers compared with non smokers and this finding was not consistent with findings of Min Yu *et al*., 2014 and Craig *et al*., 1989 who showed levels of HDL to be significantly lower among cigarette smokers and the investigation in the current report could not interpret the results obtained by Siekmeier *et al*., 1996 where in the HDL-C levels are same for smokers and non-smokers. Moreover, the present results differ from another report where smokers had lower but non-significant HDL cholesterol (HDL-C) contents (Lopes *et al*., 2004). Our finding may be due to casual drinking habits of the smokers as slight and moderate alcohol consumption may have beneficial effect on HDL levels (Bleich and Bleich, 2002). It may also be due to the fact that most of the smokers in our samples are daily labourers who own money by doing physical work. In the present study HDL cholesterol concentrations increased with increasing alcohol intake and this relationship was not different for smokers and non-smokers. This finding agrees with those of previous studies (Whitehead *et al*., 1996; Wu *et al*., 2001). HDL-C has been shown to be higher in drinkers than in nondrinkers and tends to be higher as alcohol intake increases (Sadakane *et al*., 2009). Our present results are consistent with this previous finding. As TC, HDL and LDL were significantly raised in smokers mean LDL/HDL and mean TC/HDL ratios were calculated and the finding showed that both were higher among cigarette smokers compared with non smokers indicating relatively higher risk of cardiovascular disease in cigarette smokers.

Patterson *et al.*, 1988 analyzed factors influencing total cholesterol and HDL-cholesterol using multiple regression analysis. They found that total cholesterol increased with age, while HDL-cholesterol showed little variation with age in both sexes. They also found lower HDL-cholesterol levels among men and women who abstained from alcohol, and indicated that cigarette smoking was associated with significant increases in total cholesterol values and decreases in HDL-cholesterol values. In our research total cholesterol increased with age while there was no statistically significant association between age and HDL.

It is revealed that pack years have statistically significant correlation with Triglycerides and Total Cholesterol. These observations are in tune with the findings of other workers (Rustogi *et al*., 1989), But pack years was not correlated with HDL, LDL, Systolic and Diastolic blood pressure.

In our sample level of C-reactive protein was significantly higher among smokers when compared with never smokers (p=0.000). This finding coincides with Hastie *et al*., 2008 who found that in adults without CHD, CRP levels were significantly higher for current smokers compared with never-smokers. Wannamethee *et al*., 2005 also found the same outcome. In recent years, there has been a large volume of studies, some of which are conflicting, in which serum CRP concentrations have been measured in parallel to smoking status because of the possible link between smoking and the induction of inflammatory pathways (Yanbaeva *et al*., 2007). Smokers have increased numbers of white blood cells, mainly because of a particular increase in polymorphonuclear neutrophils, which are released from the bone marrow and recruited to inflamed tissue (Van Eeden and Hogg, 2000). IL-b and IL-6, which are increased in response to lung inflammation and are implicated in the induction of CRP gene expression, may mediate the stimulation of bone marrow cells (Van Eeden *et al*., 2005).

## 9. Conclusions

In summary this study was undertaken to evaluate variations of lipids, blood pressure, BMI and CRP between cigarette smokers and non smokers. As of our knowledge this is the first research about cigarette smoking and Metabolic Syndrome in Ethiopia.

Our research found that cigarette smoking raises Triglycerides, Total Cholesterol, and LDL and also raise systolic and diastolic blood pressure even if the difference in this two were not statistically significant. Among smokers alcohol consumption was significantly associated with an increase in both systolic and diastolic blood pressure. All the above mentioned analyte and clinical finding differences are risk factors for the development of Metabolic Syndrome. While, HDL was higher in smokers than non smokers probably due to average intake of alcohol being higher in smokers. Therefore we accept the alternate hypothesis stating that there is a difference in parameters of Metabolic Syndrome and CRP between smokers and non smokers. The sample size of this research was small so further studies with larger sample size is recommended.

## 10. Limitations

This cross-sectional study could not establish causal relations, but could generate a hypothesis that can be evaluated by future prospective studies. Therefore, the results are merely reflective of associations observed between smoking and clinical/biochemical parameters. In addition, Amount of Salt intake which could influence the Blood pressure was not considered in the questionnaire. The respondents may not have reported the exact amount of cigarette smoked and alcohol consumed. We did not assess the respondents‘ dietary and exercise habits which may influence the outcome of the measurements. Longitudinal studies with long-term follow-up among smokers are recommended.

## 11. Recommendations

We recommend further studies with larger sample size and in all over the country incorporating other factors that can influence a person chance of developing Metabolic Syndrome like diet, exercise and sleeping habits. Future studies should also include the educational levels of participants.

There appears to be a lack of knowledge on the risk of cigarette smoking on cardiovascular disease so education should be given for the smokers in particular and the public in general about the catastrophic effects of cigarette smoking including the risk of cigarette smoking in development of cardiovascular impairments.

At last we would like to address the budget constraints that we have faced and more budget should be allocated in the future and reagents should be provided for the researchers in collaboration with other institutes.

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12. ANNEX Annex I Letter of Invitation

Research Study: Screening of parameters of Metabolic Syndrome and CRP in cigarette smokers in Adama, Ethiopia

**Dear sir/madam**,

I am currently undertaking a research study as part of my final year in the Msc. Medical Biochemistry. I am a senior medical biochemistry student with an interest in all aspects of health effects of smoking and I want to do further research with your help. This study aims to answer the question: Is there a difference in metabolic syndrome in smokers and non smokers and what other factors predispose smokers for metabolic syndrome? In the hope to identify, highlight and improve any areas of research in this field which may be in need of improvement or complete development.

I would like to invite all volunteer individuals with smoking experience and non smokers in Nazareth to take part. Anyone who chooses to take part will be requested to sign a consent form to partake in the research, which will be held in the city.

The research will not have any harm on the participants who will provide 10 ml of venous blood for the research and if there is any complications the researcher will take full responsibility and make sure the participant get the right treatment.

Any information gathered during this study which is identifiable to you will remain fully confidential and anonymity will be maintained throughout the study. All participants have the right not to take part or to withdraw from the study at any stage without penalty.

Thank you for taking the time to read this letter. Should you wish to take part in the study or have any further questions you would like to ask before making a decision, please feel free to contact me you can ring me on 0912023009 or email me at ableteshe@yahoo.com

If you do decide that you would like to participate in this research study please sign the consent form attached.

Yours sincerely,

Abinet Teshome

Signed: _____

Annex II Invitation letter (Amharic Version)

**<u>የግብዣ ደብዳቤ</u>**

**የጥናቱ ርዕስ** «Screening of parameters of Metabolic Syndrome and CRP cigarette smokers in Adama, Ethiopia»

**ክቡራንና ክቡራት**

**እኔ በዚህ ወቅት በአዲስ አበባ ዩኒቨርሲቲ ህክምና ፋኩልቲ የድህረ ምረቃ የሜዲካል ባዮኬሚስትሪ ተማሪ ስሆን የመመረቂያ ስራዬንም የእናንተ እርዳታ ታክሎበት ሲጋራ በሚያስከትለው የጤና ጉዳት ላይ የመስራት እቅድ አለኝ። ይህ ጥናትም የሜታቦሊክ ሲንድረም አመልካቾች በሚያጨሱ እና በማያጨሱ ሰዎች ውስጥ በምን ያህል ልዩነት ይገኛሉ የሚለውን ጥያቄ ለመመለስ ይሞክራል።**

**ስለዚህም በናዝሬት እና አካባቢው የሚገኙ ፈቃደኛ ግለሰቦች በዚህ ጥናት ላይ እንዲሳተፉ ተጋብዘዋል። በዚህ በናዝሬት ከተማ በሚደረገው ጥናት ላይ ለመሳተፍ ፍቃደኛ የሆኑ ሰዎች የፍቃደኝነት ደብዳቤ መፈረም አለባቸው።**

**ይህ ጥናት 10 ሚሊ ሊትር ደም በሚሰጡ ተሳታፊዎች ላይ ምንም አይነት ጉዳት አያስከትልም። ምንአልባት በጥናቱ መሀል ያልታሰበ ነገር ተፈጥሮ ተሳታፊው ቢጎዳ ጥናቱን የሚያደርገው ሰው ሙሉ ኃላፊነቱን ወስዶ ተሳታፊው ትክክለኛውን ህክምና እንዲያገኝ ይደረጋል።**

**በጥናቱ የሚሳተፍ ማንኛውም ሰው ስም እና የሚገኘው ውጤት ሚስጥራዊነቱ የተጠበቀ ሆኖ ለተሳታፊው የሚገለጽ ይሆናል። ማንኛውም ሰው በጥናቱ ያለ መሳተፍም ሆነ በማንኛውም ጊዜ ያለ ቅጣት ጥናቱን ማቋረጥ ይችላል።**

**የግብዣ ደብዳቤውን ጊዜ ሰጥታቹ ስላነበባቹ አመሰግናለው። በጥናቱ ለመሳተፍ ፍቃደኛ ከሆናቹ በስልክ ቁጥር 0912023009 ወይም በኢሜይል አድራሻ** ableteshe@yahoo.com
**ሊያገኙኝ ይችላሉ።**

**ከሠላምታ ጋር**
**አብነት ተሾመ**

Annex III Consent Form

I _____have read (read to me) and understand the letter of invitation to take part in the research study: Screening of parameters of Metabolic Syndrome and CRP in cigarette smokers in Adama, Ethiopia

I have received adequate information regarding the nature of the study and understand what will be requested of me. I am aware of my right to know the result of the research and withdraw at any point during the study without penalty.

I hereby consent to participate in this research study.

Participants Signature: _____

Date: _____

Researchers Signature: _____

Date: _____

Annex IV Consent form (Amharic Version)

**<u>የፍቃደኝነት ደብዳቤ</u>**

**በአብነት፡ ተሾመ፡ የቀረበውን እናበሲጋራ፡ አጫሾች እና በማያጨሱ፡ ሰዎች፡ ላይ፡ የሚደረግ«Screening of parameters of Metabolic Syndrome and CRP in cigarette smokers in Adama, Ethiopia»በሚባል፡ ጥናት፡ ላይ፡ የተዘጋጀ፡የግጥ፡ ደብዳቤ፡አንብቤ (ተነቦለልኝ) ተረድቻለው።**

**ስለ፡ ጥናቱ፡ በቂ፡ መረጃ እግኝቼ፡ ከእኔ፡ የሚጠበቀውንም፡ ግዴታ፡ በአግባቡ፡ ተረድቻለው።**

**ከጥናቱ፡ የሚገኘውን፡ ውጤት፡ የማወቅና፡ በጥናቱ፡ ማንኛውም፡ ጊዜ፡ ያለምንም፡ ቅጣት ከጥናቱ፡ ውጪ፡ መሆን፡ እንድምችልም፡ አውቄአለው።**
**ስለዚህም በጥናቱ ለመሳተፍ ፍቃደኛ መሆኔን እገልጻለው።**

**የተሳታፊው ፊርማ** ----------

**ቀን** ----------

**የባለጥናቱ ፊርማ** ----------

**ቀን** ----------

Annex V Questionnaire

- **Sex M F**
- **Age _____**
- **Marital status**

  **A. Married B. Single C. Divorced D. Widowed**
- **Educational Background**

  **A. Literate B. Illiterate**
- **Income Per month**

  **A. <500 B. 500- 1000 C. 1001- 2000 D.>2000**
- **Do your parents smoke?**

  **A. Yes B. No**
- **How long have you smoked for (in years, months or weeks) _____**
- **Do you chew tobacco or smoke cigars? Write "Yes" or "No." If "No," state which of these you use.**

_____

- **Do you know the health risks of smoking?**

  **A. Yes B. No**

  - **If "yes," briefly describe what you think are the health risks of smoking.**

_____
_____
_____
_____
_____
_____

- **How many cigarettes do you smoke per day on average? _____**

- **Do you smoke every day or do you smoke intermittently? If intermittently, specify how frequently and how many cigarettes ( such as "10 cigarettes a day for three days out of every week")**

_____
__

- **Do you chew khat?**

  **A. Yes B. No**

- **Do you sniff glue or other substances?**

  **A. Yes B. No**

- **Do you use marijuana?**

  **A. Yes B. No**

- **Do you drink alcohol and if so, how many drinks daily and what kind of alcohol (beer, wine, tej wine, whiskey, gin, etc)?**

  **A. Yes B. No**

- **Do you take any other drugs or stimulants (such as cocaine, heroin or amphetamines)?**

  **A. Yes B. No**

- **If "Yes," state which ones you take:**

_____
_____
____

- **Do any of your siblings smoke cigarette?**

  **A. Yes B. No**

Annex VI Questionnaire (Amharic Version)

**መጠይቅ**

**ጾታ** **ወንድ** **ሴት**

**ዕድሜ** ----------

**የጋብቻ ሁኔታ**

**ሀ. ያገባ** **ለ. ያላገባ** **ሐ. የፈታ** **መ. አግብቶ የሞተበት**

**የትምህርት ሁኔታ**

**ሀ. የተማረ** **ለ. ያልተማረ**

**የወር ገቢ**

**ሀ.** <500 **ለ.** 501- 1000 **ሐ.** 1001- 2000 **መ.** >2000

**ቤተሰቦችዎ ያጨሳሉ**

**ሀ. አዎን** **ለ. አይ**

**ምን ያህ ጊዜ አጭሰዋል(በአመት፣ በወር፣ በሳምንት)**

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**ትምባ አኝከው ወይም በትምባሆ ቅጠል የተጠቀለለ ሲጋራ አጭሰው ያውቃሉ? .መልስዎ አዎን ከሆነ የትኛውን እንደሆነ ይጥቀሱ**

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**ሲጋራ ማጨስ የሚያስከትለውን የጤና ጉዳት ያውቃሉ ?**

**ሀ. አዎን** **ለ. አይ**

**መልስዎ አዎን ከሆነ በአጭሩ ጉዳቶቹን ይጥቀሱ**

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**በአማካይ በቀን ምን ያህል ሲጋራ ያጨሳሉ?----------**

**የሚያጨሱት በየቀኑ ነው ወይስ አልፎ አልፎ ነው ?አልፎ አልፎ ከሆነ በሳምንት ለስንት ቀን ስንት ሲጋራ እንደሚያጨሱ ይጥቀሱ**

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**ጫት ይቅማሉ ?**

**ሀ. አዎን** **ለ. አይ**

ቤንዚን ወይም ሌላ ነገር ያሸታሉ ?

ሀ. አዎን ለ. አይ

ማሪዋና ይጠቀማሉ ?

ሀ. አዎን ለ. አይ

የአልኮል መጠጥ ይጠጣሉ ?

ሀ. አዎን ለ. አይ

መልስዎ አዎን ከሆነ በቀን ምን ያህል ይጠጣሉ?የመጠጡንም አይነት ይግለጹ

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ሌላ እንደ ኮኬይን፣ሄሮይን ወይም አምፌታሚን ያሉ አነቀቂዎችን ይወስዳሉ?

ሀ. አዎን ለ. አይ

መልስዎ አዎን ከሆነ የትኛውን እንደሚጠቀሙ ይጥቀሱ

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ከወንድም ወይም ከእህትዎ መካከል የሚያጨሱ አሉ?

ሀ. አዎን ለ. አይ

## Annex VII Principles and Procedures of tests performed

**Plasma HDL**

**Principle of the test**

Homogeneous enzymatic colorimetric assay

In the presence of magnesium ions and dextran sulfate, water-soluble complexes with LDL, VLDL, and chylomicrons are formed which are resistant to PEG-modified enzymes. The cholesterol concentration of HDL cholesterol is determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups (approximately 40 %). Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase. In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ4-cholestenone and hydrogen peroxide.

PEG-cholesterol esterase

HDL-cholesterol esters + H2O ----------→HDL-cholesterol +RCOOH

PEG-cholesterol oxidase

HDL-cholesterol + O2----------→ Δ4-cholestenone + $H_2O_2$

Peroxidase

$2H_2O_2$ + 4-aminoantipyrine + HSDA + $H^+$ + $H_2O$ ----------→purple blue pigment + $5H_2O$

HSDA- Sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline

The color intensity of the blue quinoneimine dye formed is directly proportional to the HDL cholesterol concentration. It is determined by measuring the increase in absorbance at 583 nm.

Reagents - working solutions

R1-HEPES buffer: 10.07 mmol/L; CHES: 96.95 mmol/L, pH 7.4; dextran sulfate: 1.5 g/L; magnesium nitrate hexahydrate: > 11.7 mmol/L; HSDA: 0.96 mmol/L; ascorbate oxidase (Eupenicillium sp., recombinant): > 50 µkat/L; peroxidase (horseradish): > 16.7 µkat/L; preservative

SR- HEPES buffer: 10.07 mmol/L, pH 7.0; PEG-cholesterol esterase (Pseudomonas spec.): > 3.33 µkat/L; PEG-cholesterol oxidase (Streptomyces sp., recombinant): > 127 µkat/L; peroxidase (horseradish): > 333 µkat/L; 4-amino-antipyrine: 2.46 mmol/L; preservative

(Cobas Integra 400 plus manual, Roche diagnostics, 2011-12)

Pipetting parameters

2.5 µL of plasma sample was mixed with 150 µL of R1 plus 50 µL SR and 7 µL of water by the clinical chemistry analyzer. Then the absorbance was measured automatically at 583 nm using internal HDL standard.

**Plasma Triglyceride**

**Principle of the assay**

Enzymatic colorimetric test

This method is based on the work by Wahlefeld using a lipoprotein lipase from microorganisms for the rapid and complete hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form red dyestuff (Trinder endpoint reaction). The color intensity of the red dye stuff formed measured at 659 nm is directly proportional to the triglyceride concentration and can be measured photometrically.

$$\text{Triglycerides} + 3H_2O \xrightarrow{\text{Lipase}} \text{glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow[MG^{++}]{\text{Glycerokinase}} \text{Glycerol-3-phosphate} + \text{ADP}$$

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

$$H_2O_2 + \text{4-aminophenazone} + \text{4-chlorophenol} \xrightarrow{\text{Peroxidase}} \text{4-(p-benzoquinone-monoimino)-phenazone} + 2H_2O + HCl$$

Reagents

PIPES buffer: 50 mmol/L, pH 6.8; Mg2+: 40 mmol/L; sodium cholate: 0.20 mmol/L; ATP: ≥ 1.4 mmol/L; 4-aminophenazone: ≥ 0.13 mmol/L; 4-chlorophenol: 4.7 mmol/L; LPL (microbial): ≥ 83 µkat/L; GK (microbial): ≥ 3 µkat/L; GPO (microbial): ≥ 41 µkat/L; POD (horseradish): ≥ 1.6 µkat/L; preservative; stabilizers(Cobas Integra 400 plus manual, Roche diagnostics, 2011-12)

Pipetting parameters

2 µL of plasma sample was mixed with 120 µL of R plus 28 µL of diluent ($H_2O$) by the clinical chemistry analyzer. Then the absorbance was measured automatically at 659 nm using internal TG standard

**Total Cholesterol**

**Principle**

Enzymatic, colorimetric method

Cholesterol was measured enzymatically in plasma in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reaction byproducts, $H_2O_2$ is measured quantitatively in a peroxidase catalyzed reaction that produces a color. Absorbance is measured at 512 nm. The color intensity is proportional to cholesterol concentration. The reaction sequence is as follows:

$$\text{Cholesteryl ester} + H_2O \xrightarrow[\text{Hydrolase Cholesteryl ester}]{} \text{cholesterol} + \text{fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow[\text{Cholesterol oxidase}]{} \text{cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow[\text{Peroxidase}]{} \text{4-(p-benzoquinone- monoimino)-phenazone} + 4\ H_2O$$

Reagents

PIPES buffer: 225 mmol/L, pH 6.8; $Mg^{2+}$: 10 mmol/L; sodium cholate: 0.6 mmol/L; 4-aminoantipyrine: ≥ 0.45 mmol/L; phenol: ≥ 12.6 mmol/L; fatty alcohol polyglycol ether: 3 %; cholesterol esterase (Pseudomonas spec.): ≥ 25 µkat/L (≥ 1.5 U/mL); cholesterol oxidase (E. coli): ≥ 7.5 µkat/L(≥ 0.45 U/mL); peroxidase (horseradish): ≥ 12.5 µkat/L (≥ 0.75 U/mL); stabilizers; preservative(Cobas Integra 400 plus manual, Roche diagnostics, 2011-12)

Pipetting parameters

First 47 µL of R was mixed with 73 µL of the diluent ($H_20$) then 2 µL of plasma sample was added followed by 20 µL of the diluent ($H_20$) by the analyzer. Then the absorbance was measured at 512 nm using internal TC standard.

## Serum CRP

## Principle

Test principle

Particle enhanced turbidimetric assay

Human CRP agglutinates with latex particles coated with mono clonal Anti-CRP antibodies. The precipitate is determined turbidimetrically at 552nm.

Reagents - working solutions

R1 TRIS buffer with bovine serum albumin and immunoglobulins (mouse); preservative

R2 = SR Latex particles coated with anti-CRP (mouse) in glycine buffer; preservative (Cobas Integra 400 plus manual, Roche diagnostics, 2011-12)

**Pipetting parameters**

First 82 µL of R1 was mixed with 48 µL of diluent ($H_20$) followed by addition of 2.5 µL of serum sample, then 30 µL of diluent ($H_20$) is added, 28 µL of R2 is then added and finally 14 µL of diluent ($H_20$) is added by the clinical chemistry analyzer. Then the turbidimetry was measured at 552 nm using internal CRP standard.