Chapter One

1.1 Introduction

In many parts of the world, drinking alcoholic beverages is a common feature of social gatherings. Nevertheless, the consumption of alcohol carries a risk of adverse health. The harmful use of alcohol is a global problem which compromises both individual and social development. It results in 2.5 million deaths each year. It also causes harm far beyond the physical and psychological health of the drinker [1]. It harms the well-being and health of people around the drinker. An intoxicated person can harm others or put them at risk of traffic accidents or violent behavior, or negatively affect co-workers, relatives, friends or strangers. Thus, the impact of the harmful use of alcohol reaches deep into society [2]. Harmful drinking is a major determinant for neuropsychiatric disorders, such as alcohol use disorders and epilepsy and other noncommunicable diseases such as cardiovascular diseases, cirrhosis of the liver and various cancers. The harmful use of alcohol is also associated with several infectious diseases like acquired immune-deficiency syndrome, tuberculosis and sexually transmitted infections. This is because alcohol consumption weakens the immune system and has a negative effect on patients’ adherence to antiretroviral treatment. A significant proportion of the disease burden attributable to harmful drinking arises from unintentional and intentional injuries, including those due to road traffic accidents, violence, and suicides [3]. Fatal injuries attributable to alcohol consumption tend to occur in relatively younger age groups. The degree of risk for harmful use of alcohol varies with age, sex and other biological characteristics of the consumer. In addition the level of exposure to alcoholic beverages and the setting and context in which the drinking takes place also play a role. For example, alcohol is the world’s third largest risk factor for disease burden; it is the leading risk factor in the Western Pacific and the Americans and the second largest in Europe [3]. Furthermore, 320 000 young people between the age of 15 and 29 die from alcohol-related causes, resulting in 9% of all deaths in that age group. Alcohol consumption by an expectant mother may cause fetal alcohol syndrome and pre-term birth complications, which are detrimental to the health and development of neonates [1].
1.2 Rationale

The harmful use of alcohol is a global problem which compromises both individual and social development. In Sudan, the high prevalence of homemade alcoholic beverages use might be associated with high risk of developing cardiovascular diseases. Nevertheless, most consumers believe that homemade alcoholic beverages are safe and consequently consume huge quantities. For some tribe, particularly, in Southern and Western Sudan people considered these beverages particularly Marisa, as a nutritional source in daily base life. No published data have been found regarding alcohol consumption in Sudan. Therefore, the aim of this study is to identify and highlight the effect of alcohol on lipids profiles in Sudanese population and help to prevent of alcohol consumption among Sudanese people specially the young people.
1.3 Objectives

1.3.1 General Objective
- To assess plasma lipids profile among alcohol consumer.

1.3.2 Specific Objectives
- To determine the plasma lipids profile (Cholesterol, Triglycerides, HDL-Cholesterol and LDL-Cholesterol) among alcohol consumer.
- To compare plasma lipids level between cases and controls.
- To correlate the duration of alcohol consumption and plasma lipids.
- To identify the effect of alcohol type, regularity of alcohol intake and alcohol use on plasma lipids.

Chapter Two

2. Review of literature

2.1 Lipids and Lipoproteins

2.1.1 Classification of plasma lipids
Lipids are soluble in organic solvent, but nearly water insoluble. Major lipids present in the plasma from endogenous or exogenous sources are fatty acids, Phospholipids, Triglycerides and Cholesterol\(^4\).

Cholesterol
Cholesterol is primarily composed of C-H bonds, and hence it is fairly water insoluble. It does, however, contain a polar hydroxyl (OH) group. Thus it is both a polar and nonpolar molecule (Amphipathic) \[\text{\cite{5}}\]. It’s the major constituent of the cell membrane of animal cells. It would be possible for the body to provide its full daily cholesterol requirement by synthesizing it itself. However, with mixed diet, only about half of the cholesterol is derived from endogenous biosynthesis, which takes place in the intestine, skin and mainly in the liver (about 50%). The rest is taken up from food. Most of cholesterol is incorporated into the lipid layer of plasma membranes or converted into bile acid. A very small amount of cholesterol is used for biosynthesis of the steroid hormones. In addition; up to 1 g cholesterol per day is released into the bile and thus excreted \[\text{\cite{5}}\].

**Fatty Acids**

RCOOH is the general chemical formula for fatty acid, where “R” is an alkyl chain. Fatty acid chain length vary and are commonly classify as; short chain(2 to 4 carbon atoms), medium chain (6 to 10 carbon atoms), or long chain (12 to 26 carbon atoms) fatty acid. Those of important in human nutrition and metabolism are the long chain class. Fatty acids are further classified according to their degree of saturation. Saturated fatty acids have no double bonds between their carbon atoms; monounsaturated fatty acids contain one double bond; and polyunsaturated fatty acids contain multiple double bonds. Fatty acids exist in circulation in either un-esterified or free, the later primarily bound to albumin, or in various esterified forms, such as triglycerides, phospholipids, or cholesterol esters \[\text{\cite{6}}\].

**Triglycerides**

Is made up of fatty acids and glycerol and is partly synthesized in the liver hepatocyte. It is transported through the bloodstream by chylomicrons and very low density lipoproteins (VLDL). Triglycerides provides energy to the cells as it loses its fatty acid and form ATP, thus acting as an energy store in the form of fat, and it insulates organs through fat deposits \[\text{\cite{7}}\].

**Phospholipids**
Phospholipids formed by conjugation of two fatty acids and phosphorylated glycerol. Phospholipids make up the bilayer of cell membranes and also form a coating that surrounds cholesterol and triglycerides and glues them to lipoprotein core\(^7\).

2.1.2 Lipoproteins

Because lipids are not soluble they transported in the plasma associated with proteins. Albumin is the principle carrier of free fatty acids while other lipids circulate in complex known as lipoproteins. These complex of non-polar of triglycerides and cholesterol esters surrounded surface layer of phospholipids, cholesterol and protein known apolipoproteins\(^4\). Lipoproteins are classified into five groups, in order of decreasing size and increasing density, these are: chylomicrons, VLDL, low density lipoproteins (LDL) and high density lipoproteins (HDL). The proportion of apoproteins range from 1% in chylomicrons to over 50% in HDL. These proteins serve less for solubility purpose, but rather function as recognition molecules for the membrane receptors and enzymes that are involved in lipid exchange\(^5\).

Chylomicrons

Chylomicrons are the largest of the lipoproteins particles. They are the major carries of exogenous triglycerides. Chylomicrons comprise 90 to 95% (by weight) triglycerides, 2 to 6% phospholipids, 2 to 4% cholesterol ester, 1% free cholesterol and 1 to 2% Apo-lipoproteins( Apo) C and B. Chylomicrons are responsible for transporting dietary triglyceride and some cholesterol to the rest of the body. The clearance times from the formation of chylomicrons after meal and the removal the remnants of the liver in about six hours. Normally, chylomicrons are not found in 12 to 14 hours fasting blood specimen\(^8\).

Very low density lipoproteins

VLDL, like chylomicrons, are also rich in triglycerides and are major carrier of endogenous triglycerides. VLDLs comprise 50 to 65% (by weight) triglycerides, 8 to 14% cholesterol ester, 12 to 16% phospholipids, 4 to 7% free cholesterol and 5 to 10% Apo( B,C and E). Excess dietary intake of carbohydrate enhances hepatic synthesis of triglycerides, which in turn increase VLDL production\(^8\).
Intermediate density lipoproteins

Which are usually undetectable in normal plasma, it is normally transient intermediate lipoprotein formed during the conversion of VLDL to LDL. It contains both cholesterol and endogenous triglycerides\[8\].

Low density lipoproteins

LDL contains 50% cholesterol by weight and is most cholesterol rich of the lipoproteins. They are synthesized in the liver and are responsible for transporting cholesterol from the liver to peripheral tissue\[5\]. LDL is the most atherogenic lipoproteins and high serum is regard as major Coronary Heart Disease (CHD) risk factor. Lipoproteins of smaller size do not scatter light; even very high concentrations in plasma do not produce lipemia. Another lipoproteins, lipoprotein (a), similar in composition to LDL but has high protein content (contain one molecule of Apo (a) linked to Apo B-100 by a disulfide bond). Lipoprotein (a) is normally present in low plasma concentration, increase level lead to increased risk for premature coronary heart disorders and stroke by promoting clotting that lead to MI and stroke\[8,9\].

High density lipoproteins

HDLs are smallest lipoproteins. HDL particles are synthesis by both the liver and intestine. HDL typically carries 20 to 35 % of total plasma cholesterol, but unlike LDL, which carry cholesterol to the tissue, HDL take excess cholesterol from tissue to the liver (reverse transport) and sometimes referred to as the good cholesterol. Based on density differences there are two major groups of HDL substances: HDL2 and HDL3. HDL2 is larger in size and richer in lipid than HDL3 and may be the efficient vehicle for transfer of cholesterol from the peripheral tissue to the liver\[8\].

2.1.3 Lipids and Lipoproteins Metabolism

Exogenous pathway

Dietary triglyceride and cholesterol are absorbed in the intestine mucosa and incorporated to form the core of nascent chylomicrons, which are then transported to plasma. In peripheral tissue, chylomicrons interact with lipoprotein lipase, which removes most of core triglyceride from the lipoprotein particle. The resulting glycerol and fatty acids are taken up by adipose and other tissues, re-formed into triglyceride and stored. Redundant surface material (Apo C, phospholipids and cholesterol ester) joins the HDL particles. The remnant chylomicron particles, which are now smaller and enriched in their core with cholesterol ester and some remaining triglycerides, are
taken up by the liver. This dietary cholesterol can then be used for bile acid formation, incorporated into membranes, re-secreted back into the circulation as lipoprotein cholesterol or excreted into bile as cholesterol [10].

**Endogenous pathway**

Triglyceride and cholesterol are also synthesized in the liver. This endogenous system, which conveys these lipids from liver to peripheral tissue and back to liver, is divided into two sub-system: the Apo B-100 lipoprotein system (VLDL-c, IDL and LDL-c) and Apo A-1 lipoprotein system (HDL-c)[10].

**Apo B-100 lipoprotein system**

In the liver triglycerides and cholesterol are packed with apo B-100 and phospholipids to form VLDL. Once released into plasma, VLDL undergoes triglycerides removal by means of lipoprotein lipase; the resulting cholesterol ester-rich remnants are the LDL. Unlike the chylomicrons remnants are the IDL. Can be converted by further triglycerides removal to even smaller and denser LDL. During this process the lipoprotein loses all its surface apo except apoB-100[10].

**Apo A-1 lipoprotein system**

HDL, rich in apo A-1 which transport cholesterol from peripheral tissue to the liver. Cholesterol-poor HDL3 particles first form in plasma from coalescence of phospholipids-Apo complexes. Free cholesterol then transfer from cell membranes to HDL3, where it converts into cholesterol ester and enters the HDL core. The HDL3 can then accept more free cholesterol and become the larger more cholesterol-rich HDL2 particles. HDL2 is then metabolized by one of two main pathways: transfer to apo B lipoproteins (which are subsequently removed by liver) by mean of cholesterol ester transfer protein or direct hepatic metabolism with removal of the HDL2 apoproteins from plasma[10].

### 2.1.4 Lipids and Lipoproteins Disorders

Diseases associated with abnormal lipid concentration are referred to as dyslipidemia. They can be cause directly by genetic abnormalities or through environmental/life
style imbalances or they can develop secondarily, as a consequence of other diseases\textsuperscript{[8]}.

**Primary Hyperlipidemia**

Common genetic polymorphisms of the many enzymes, structure proteins, and receptors involved in lipoprotein metabolism, collectively are thought to have a major impact on any individual’s tendency for developing dyslipidemia\textsuperscript{[11]}.

**Secondary Dyslipidemia**

In many patients hyperlipidemia is caused by some underlying "non-lipid" etiology rather than a primary disorder of lipid metabolism. The secondary causes of dyslipidemia are: type 2 diabetes mellitus, excessive alcohol consumption, obstructive liver diseases, nephroticsyndrome, chronic renal failure, hypothyroidism, cigarette smoking, obesity, and drugs (corticosteroids therapy, orally administered estrogens and oral contraceptives pregnancy)\textsuperscript{[12]}.

**Causes follow Hypercholesterolemia:**

Acute intermittent porphyries (also associated with hypertriglyceridemia). High saturated fat intake in patients with hyper absorption (increase total cholesterol and LDL-c). Anorexia nervosa (isolated hypercholesterolemia occur as a result of mobilization of cholesterol from tissue)\textsuperscript{[10]}.

**Causes follow Hypertriglyceridemia**

Cushing’s syndrome (is also associated with hypercholesterolemia). Lipodystrophy and type1 glycogen storage disease. Consumption of simple carbohydrate including fructose (increase VLDL secretion in some patient), systemic lupus erythematosus, and retinoid therapy (also associated with low HDL-c), bile acid sequestrants (can exacerbate hypertriglyceridemia in patient with preexisting triglyceride elevation)\textsuperscript{[10]}.

**Causes follow decrease HDL-c**

Secondary to hypertriglyceridemia regardless of cause (except alcohol and estrogen-induced hyperlipidemia). Anabolic steroids and probucol (can decrease HDL-c without increasing triglyceride), cigarette smoking, sedentary lifestyle, very low fat diet and MI or a major surgical procedure (can temporarily lower HDL-c)\textsuperscript{[13]}.

**Hypolipidemia**
Hypolipidemia is a decrease plasma lipoprotein caused by primary (genetic) or secondary factors. It is usually asymptomatic and diagnosed incidentally on routine lipid screening\[10\].

**Abetalipoproteinemia**

This is autosomal recessive condition caused by mutations in the gene for microtunol triglyceride transfer protein, a protein critical to chylomicron and VLDL (Apo-B) formation. Dietary fat cannot be absorbed and lipoprotein in both metabolic pathways are virtually absent from serum; total cholesterol (TC) is typically less than 45 mg/dl, triglycerides (TG) are less than 20 mg/dl and LDL are undetectable\[10\].

**Hypolipoproteinemia**

Is caused by genetic defect leading to absent or decreased LDL and HDL level. Absent LDL and low serum cholesterol leads to a failure to thrive, steatorrhea, central nervous system degeneration, and malabsorption of fats and vitamins. Decrease LDL lead to an increased life expectancy and decreased risk of MI. Reduced HDL lead to an increased risk of atherosclerosis. Absent HDL (tangier disease) lead to an accumulation of cholesterol ester in tonsils, adenoids and spleen. It is considered a benign disease\[7\].

**2.1.5 Diagnosis**

Dyslipidemia is suspected in patient with characteristic physical findings or complications of dyslipidemia, as an example atherosclerotic disease. Primary lipid disorder is suspected when patient have physical signs of dyslipidemia, onset of premature atherosclerotic disease (at < 60 years), a family history of atherosclerotic disease, or serum cholesterol > 240 mg/dl. Dyslipidemia is diagnosed by measuring serum lipids. Routine measurements of lipid profiles include TC, TGs, HDL-C and LDL-C. Tests for secondary causes of dyslipidemia- including measurements of fasting glucose, liver enzyme, creatinine, and urinary protein- should be done in most patients with newly diagnosed dyslipidemia and when a component of the lipids profile has inexplicably change for the worse\[10\].

**Screening**

Fasting lipid profiles should be obtained in all adults > 20 years and should be repeated every 5 years. Lipids measurements should be accompanied by assessment of other cardiovascular risk factors, defined as: DM, cigarettes use, hypertension and family history of CHD in a male 1st degree relative before age 55 or female 1st degree
relatives before age 65. A define age after which patients no longer require screening has not been established, but evidence supports screening of patients into their 80 years, especially in the presence of atherosclerotic cardiovascular disease. Indications of screening patients < 20 years are atherosclerotic risk factors such as DM, hypertension, cigarette smoking and obesity; premature CHD in a parent, grandparent, or sibling; or a cholesterol level > 240 mg/dl or known dyslipidemia in a parent. If information of relatives is unavailable, as in the case of adopted children, screening is at the discretion of the health care practitioner. Patients with an extensive family history of heart disease should also be screened by measuring lipoprotein (a) level\textsuperscript{10}.

2.2 Alcohols
Several alcohols are toxic and medically important; they include ethanol, methanol and isopropanol\textsuperscript{[6]}.

2.2.1 Alcohols of Toxicological Interest
Ethanol is widely used and often abused chemical substance. The measurement of ethanol is one of the more frequently performed tests in the toxicology laboratory. Although less frequently encountered, it is important to include methanol, isopropanol, and acetone (a metabolite of isopropanol) in a test battery for alcohols for proper evaluation of acutely intoxicated patient\textsuperscript{[6]}.

Ethanol
The principle pharmacological action of ethanol is depression of the Central Nervous System (CNS). The CNS effect vary, depending on the blood ethanol concentration, from euphoria and decreased inhibitions (less than or equal to 50 mg/dl), increase disorientation and loss of voluntary muscle control resulting in irregular movement (100 to 300 mg/dl) and then to coma and death (greater than 400 mg/dl). Ethanol is metabolized principally by liver Alcohol Dehydrogenase (ADH) to acetaldehyde, which is subsequently oxidized to acetic acid by aldehyde dehydrogenase\textsuperscript{[6]}.

Methanol
Methanol is used as a solvent in number of commercial products, constituent of antifreeze and window cleaning fluids, and component of canned fuel. It may be consumed by alcoholics intentionally as an ethanol substitute or accidentally when present as a contaminant in illegal whiskey. Accidental ingestion occurs in children. The CNS effects of methanol are substantially less severe than those of ethanol. Methanol is oxidized by liver ADH (at about one tenth the rate of ethanol) to
formaldehyde. Formaldehyde in turn is rapidly oxidized by Aldehyde Dehydrogenase to formic acid, which may cause serious acidosis and optic neuropathy, resulting in blindness or death[^6].

**Isopropanol**

Isopropanol is readily available to the general population as a 70% aqueous solution for use as rubbing alcohol. It has about twice the CNS depressant action as ethanol, but it is not as toxic as methanol. Rapidly metabolized by ADH to acetone which is eliminated primarily in alveolar air and urine. Acetone has CNS depressant activity similar to that of ethanol. Severe isopropanol intoxication has been known to result in coma or death[^6].

### 2.2.2 Type of Alcoholic Beverages

An alcoholic beverage is a drink containing ethanol, commonly known as alcohol. Alcoholic beverages are divided into three general classes: beers, wines, and spirits (or distilled beverage). They are legally consumed in most countries, and over 100 countries have laws regulating their production, sale, and consumption. In particular, such laws specify the minimum age at which a person may legally buy or drink them. This minimum age varies between 16 and 25 years, depending upon the country and the type of drink. Most nations set it at 18 years of age[^14].

**Distilled and Undistilled Beverages**

A distilled beverage, spirit, hard liquor, or liquor is an alcoholic beverage produced by distillation of a mixture produced from alcoholic fermentation, such as wine. This process purifies it and removes diluting components like water, for the purpose of increasing its proportion of alcohol content (commonly known as alcohol by volume, ABV). As distilled beverages contain more alcohol they are considered "harder" - in North America, the term hard liquor is used to distinguish distilled beverages from undistilled ones, which are implicitly weaker. As examples, this does not include beverages such as beer, wine, and cider, as they are fermented but not distilled. These all have relatively low alcohol content, typically less than 10%. However, brandy is a spirit, is distinct as a drink from wine (due to distillation), and has an ABV over 35%. Other examples of common distilled beverages in the West include vodka, gin, tequila, rum, whisky[^15].
Homemade Sudanese drink

The possession or the consumption of alcohol is prohibited by law in the country of Sudan. When it comes to morality, there are strict laws in place. Despite these laws, there are still people in the country making and sell it to the locals. The spirit is called “araqi” and it is made out of fermented dates, of which there are many, available in Sudan. The fermented dates are mixed with yeast and some water and then left to ferment over a three day time span, the liquid is then distilled. Many type of undistilled, the most popular in Sudan known as, “Marisa” and “Assalia”. Marisa is produced by fermented maize or fume. Assalia is prepared as the same as marisa, but with very low alcoholic content, because it is fermented for a very short period.

2.2.3 A standard drink

Moderate drinking

Depend to, National Institute on Alcohol Abuse and Alcoholism (NIAAA), a standard drink is equal to: 12 oz. (355 ml) beer with 5% alcohol, 5 oz. (150 ml.) glass of wine (12.5% alcohol), 1.5 oz. (45 ml.) of 80 proof liquor (40% alcohol). For men, no more than 4 drinks on any single day AND no more than 14 drinks per week. For women, no more than 3 drinks on any single day AND no more than 7 drinks per week [16]. In Sudan as alcohol is prohibited, so no standard for drink and percent of alcohol is not estimated. In this study moderate alcohol consumption is chosen for people who drink less than 1.6 liter per day and no more than 5.6 liter per week for undistilled alcohol. For distilled type less than 200 ml per day and no more than 0.7 liter per week.

Heavy drinking

For healthy adults in general, heavy drinking means consuming more than the single-day or the weekly amounts listed above [14]. For homemade alcohol in this study heavy intake for people who consume more than the single-day or the weekly amount listed above.
Chapter Three

3. Materials and Methods

3.1 Study Design
Analytical case control study.

3.2 Study Area
North Darfur state, Elfasher town (Capital of the state).

3.3 Study Population
The study was involving 150 participants (male) randomly selected, 100 alcohol consumers (cases); and 50 non-alcohol consumers (controls) with the same criteria as case and free from chronic diseases or medication therapy in the last month before sample collection.

3.3.1 Inclusion Criteria
- Adult male alcohol abuse.
- Age group >18 years old.
- Free from disease affect lipids metabolism (DM, Hypertension, and Hypothyroidism, Clinical history of renal disease, cardiovascular disease and hyperlipidemia.)
- Not use cholesterol lowering drugs.
- Nonsmokers.
- People who are not Obese or Underweight (Body mass index [BMI] less than 30 and more than 18.5.

3.3.2 Exclusion Criteria
- Female.
- With disease affect lipids metabolism.
- Cigarette smokers.
- Obesity and underweight (BMI more than 30 and less than 18.5.

3.4 Sampling Technique
Fasting venous blood (3.5 ml) taken slowly using disposable plastic syringe from antecubital vein, this was done after cleaning the puncture site with 70% alcohol, then the blood was slowly poured in EDTA container (1.5 mg in 2.5 ml blood) and mixed
well, the rest of the blood (1 ml) was slowly poured in a covered plain test tube (without anticoagulant) for serum preparation[17,18].

3.5 Data Collection
Questionnaire and laboratory investigation (TC, TGs, LDL-C and HDL-C) used to collect data. (Appendix-1).

3.6 Data Analysis
Data management was done using Statistical Package for Social Sciences (SPSS version 11.5), to get if there is association or correlation between variables, the statistical significance (P value) < 0.05.

3.7 Study variables
- TC, TGs, LDL-C and HDL-C(all this variable measured by using Reflotron Plus System.)
- Age
- Type, Period and use of alcohol.

3.8 Ethical consent:
All study subjects consented to participation by completing the self-administered questionnaire.(Appendix-2).

3.9 The Reflotron® System
The Reflotron Plus is an in vitro diagnostic device designed for the quantitative determination of 17 clinical chemistry parameters from whole blood, plasma or serum by using Reflotron test strips which works on the principle of reflectance photometry and ensures rapid and reliable results while being simple to use[19].(Appendix-3).

Measuring Principle
For measurement, the test strips is fixed to movable carriage, the transporter. When the flap of the measuring chamber is closed this transporter moves the test strip into the measuring position with the help of motor. After a certain individual waiting time depending on the test, the so-called Ulbricht sphere is lowered onto the test strip. This brings the reagents contained in the test strips into contact with the sample (if blood is used the erythrocytes have been separated from the plasma during the waiting time) and start reaction. Depending to the test, the colour reaction is measured in different ways (end point or kinetic analysis) and at different times. The Ulbricht sphere
contains leds of different wavelength and detectors and the opto-electric
determination is performed as follow:

- A light emitting diode flashes light of defined wavelength onto the surface of
  the test area.
- The light that strikes the strips is reflected by the surface with varying
  intensity depending on the colour of the test area.
- Two photodiodes (sample& Reference) symmetrically positioned in the
  Ulbricht sphere serve as light detectors: the reference detector measure the
  intensity of this diffuse light, while the sample detector measure the intensity
  of light diffusely reflected by the test area and weakened through absorption
  by the indicator. The ratio of the two intensity is proportional to the
  reflectance. The electrical signals from the detector are carried to an analog-to-
  digital converter, which convert analog signals into digital values. The
  reflectance is calculated in the microprocessor.

The concentration or the activity of the test performed is then determined
mathematically from the measured reflectance. This is done using a test and lot-
specific function stored on a magnetic strip on the back of each test strip and
transmitted to the instrument automatically when the test strip is inserted[19].

**Reagents and Materials**

Commercial close system reagents were provided by Roche Diagnostics operators and
Consist of:

- Reflotron®Reagent strips.
- Reflotron® precinorm U &H (control material for checking the performance
  of the reflotron system).
- Reflotron® Clean and check (used for cleaning and checking the optical
  system).

**3.10 Methods for Analysis**

Methods used for analysis of all parameters are shown in the appendices.

**Cholesterol method**

Cholesterol using CHOD-PAP method (appendix-4).

**Triglycerides method**

Triglycerides using GPO-PAP method (appendix-5).

**HDL Cholesterol method**
HDL-C using Homogeneous HDL Cholesterol plus method (appendix-6)

**LDL Cholesterol method**

A program integrated in the Reflotron® photometers can calculate LDL cholesterol easily from the measured cholesterol, triglycerides and HDL cholesterol values (indirect method by using freidewald equation). Freidewald equation as follow:

\[
LDL-C = TC - HDL-C - ([TG]/5). \tag{6}
\]

The Friedewald equation should not be used when plasma triglyceride concentration exceeds 400 mg/dl (4.52 mmol/L) \[^{[6]}\]. Due to the limitation of the equation, for all results exceed their T.G 400 mg/dl, can used direct method for measuring LDL-C. Direct method for measuring LDL-C by using precipitating method (appendix-7).

### 3.11 Quality control

**Reflotron® Check**

By using a Reflotron® Check quality control strip the user can check the function of the instrument’s optical system easily and quickly. Depending on the laboratory’s standing instructions, this check may be performed in addition to the overall system check with control sera or partly as a replacement for it, making it possible to perform total-system quality controls less frequently. Comparison of the on-screen result with the confidence interval printed on the tube indicates whether the optical system is functioning correctly.

**System Checks**

The well-proven Precinorm® control sera should be used the Reflotron test strips to ensure that the test strips are working correctly and giving accurate and reliable results. Precinorm® is a range of freeze-dried control sera that are reconstituted with distilled water. Precinorm® HDL should be used for HDL strips. Precinorm® U should be used for all others test strips.

### 3.12 Body mass index (BMI)

The simplest expression for this is the body mass index (BMI) calculated as weight (kg) divided by height squared (m\(^2\)). Normal weight ranges:

- Underweight: BMI is less than 18.5
- Normal weight: BMI is 18.5 to 24.9
- Overweight: BMI is 25 to 29.9
- Obese: BMI is 30 or more

The World Health Organization (WHO) guidelines of 1985 defined obesity as a BMI \(>30.0\) for men and \(>28.6\) for women\[^{[20]}\].
Chapter Four

4. Results

4.1 Numbers of cases and controls among participants

Figure (4.1) shows the frequency of cases and controls among participants.

![Pie chart showing 66.7% controls and 33.3% cases.](image)

Figure 4.1 Numbers of cases and controls among participants.
4.2 Age group distribution

Figure (4.2); shows age group of participants.

Figure 4.2: Age distribution among participants (mean age 38.6).
4.3 The duration of alcohol intake per years among alcohol consumers.

Figure (4.3); shows the duration of taking alcohol per years among participants.

Figure 4.3: Alcohol intake per year.
4.4 Alcohol consumers and alcohol type

Figure (4.4); shows the frequency of alcohol type among alcohol consumers.

Figure 4.4: Type of alcohol used.
4.5 Frequency of alcohol intake

Figure (4.5); shows mood of alcohol use among participants.

Figure 4.5 Frequency of alcohol intake.
4.6 Regularity of alcohol intake

Figure (4.6); shows volume of alcohol intake among participants who take alcohol regularly.

Figure 4.6: Regularity of alcohol intake.
4.7 The relationship between plasma lipids level and samples (case and control).

Table (4.1); shows significant differences between the mean of plasma lipids in test and control groups, done by Independent-Samples T Test (P value <0.05, considered significant).

Table 4.1: The relationship between serum lipids level and samples (case and control).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Population</th>
<th>P. value</th>
<th>% of abnormal results</th>
</tr>
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<tr>
<td></td>
<td>Case {n=100} mean ± SD (mg/dl)</td>
<td>Control {n=50} mean ± SD (mg/dl)</td>
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</tr>
<tr>
<td>TC</td>
<td>186.2 ± 37.8</td>
<td>145.5 ± 21.6</td>
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<td>TGs</td>
<td>154.9 ± 50.8</td>
<td>91.5 ± 14.7</td>
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<tr>
<td>HDL</td>
<td>45.9 ± 12.2</td>
<td>25.2 ± 6.1</td>
<td>0.000</td>
</tr>
<tr>
<td>LDL</td>
<td>108.9 ± 23.2</td>
<td>99.8 ± 18.8</td>
<td>0.018</td>
</tr>
</tbody>
</table>
4.8 Correlation between the duration of alcohol intake per year and TC level.
Figure (4.7); shows positive correlation between period of alcohol intake and TC level (mg/dl).

Figure 4.7 Correlation between TC levels and duration of alcohol intake per year. [P value (0.000), r (0.222)].
4.9 Correlation between the duration of alcohol intake per year and TG level.

Figure (4.8); shows positive correlation between period of alcohol intake and TG level (mg/dl).

![Graph showing correlation between TG levels and duration of alcohol intake per year.]

Figure 4.8 Correlation between TG levels and duration of alcohol intake per year.

[P value (0.000), r (0.426)].
4.10 Correlation between the duration of alcohol intake per year and HDL level

Figure (4.9); shows positive correlation between period of alcohol intake and HDL-c level (mg/dl).

![Graph showing correlation between HDL levels and duration of alcohol intake per year.](image)

Figure 4.9 Correlation between HDL levels and duration of alcohol intake per year.

[P value (0.000), r (0.225)].
4.11 Correlation between LDL and the duration of alcohol intake

Figure (4.10); shows positive correlation between period of alcohol intake and LDL-c level (mg/dl).

Figure 4.10 Correlation between LDL levels and duration of alcohol intake per year.

[P value (0.009), r (0.068)].
4.12 The effect of alcohol type on serum lipids

Table (4.2); shows the significant association between plasma lipids and alcohol type, done by One-Way Anova test.

<table>
<thead>
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<th>Alcohol type</th>
<th>P. value</th>
<th>% of abnormal results</th>
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<td>Distilled (n=40)</td>
<td>Undistillid (n=25)</td>
<td>Mixed (n=35)</td>
</tr>
<tr>
<td>TC</td>
<td>199.1± 44.1</td>
<td>172.6 ±30.6</td>
<td>181 ±30.1</td>
</tr>
<tr>
<td>TGs</td>
<td>164.9± 56.3</td>
<td>123.2± 28.1</td>
<td>166.1±48.3</td>
</tr>
<tr>
<td>HDL</td>
<td>49.7±13.9</td>
<td>41.3 ± 12.3</td>
<td>44.7 ± 8.5</td>
</tr>
<tr>
<td>LDL</td>
<td>116.3± 25.9</td>
<td>105.2 ±18.3</td>
<td>103 ± 21.4</td>
</tr>
</tbody>
</table>
4.13 Relationship between regular intake and serum lipids.

Table (4.3); shows significant association between plasma lipids and regular alcohol intake, done by Independent-Samples T Test.

Table 4.3: Relationship between regular intake and serum lipids.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Regular alcohol intake</th>
<th>P. value</th>
<th>% of abnormal results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moderate {n=49}</td>
<td>Heavy {n=21}</td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>mean ± SD (mg/dl)</td>
<td>mean ± SD (mg/dl)</td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>183.6 ± 33.4</td>
<td>219.4 ± 31.5</td>
<td>0.000</td>
</tr>
<tr>
<td>TGs</td>
<td>148 ± 47.4</td>
<td>187 ± 37.8</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL</td>
<td>47.9 ± 9.9</td>
<td>57.1 ± 9.1</td>
<td>0.000</td>
</tr>
<tr>
<td>LDL</td>
<td>105.4 ± 22.2</td>
<td>124.7 ± 22.9</td>
<td>0.001</td>
</tr>
</tbody>
</table>
4.14 Relationship between alcohol use and serum lipids

Table (4.4); shows significant association between plasma lipids and alcohol use, done by Independent-Samples T Test.

Table 4.4: Relationship between alcohol use and serum lipids.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Alcohol uses</th>
<th>P. value</th>
<th>% of abnormal results</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>Occasional (n=30) mean ± SD (mg/dl) 167 ± 34 &amp; Regular (n=70) mean ± SD (mg/dl) 194.4 ± 36.6 &amp; 0.001 &amp; 10 % &amp; 42.8 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGs</td>
<td>143.6 ± 56.1 &amp; 159.7 ± 48 &amp; 0.146 &amp; 36.6 % &amp; 24.3 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>34.7 ± 8.0 &amp; 50.6 ± 10.5 &amp; 0.000 &amp; 33.3 % &amp; 15.7 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td>103.5 ± 20.9 &amp; 111.2 ± 23.9 &amp; 0.132 &amp; 0 % &amp; 5.7 %</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Chapter Five

5. Discussion

Independent-samples T test is used to compare the finding of plasma lipids between alcohol drinkers and nondrinkers. The results of the study showed that, the mean of TC levels increase significantly with alcohol consumption (P < 0.00), this result in agreement with previous finding done in British 1992, which found that increase TC levels are significantly associated with alcohol consumption (P<0.01)\textsuperscript{[21]}. The mean of TG levels in this study statistically increase with alcohol consumption (P<0.00), this result in accordance with previous study done in Japan 1994, which found TG levels are higher (P<0.001) with alcohol consumption\textsuperscript{[22]}. The mean of HDL levels are significantly higher with alcohol consumption(P<0.00), this result in agreement with study done in Japan 1994, which found HDL level increase with alcohol intake (P<0.001) \textsuperscript{[22]}. The mean of LDL levels are statistically increase with alcohol consumption (P<0.018), this result in contrast with previous study done in Turkey 2008, in which no increase in LDL level was noted\textsuperscript{[23]}, this difference might be attributed to the dose and mode of alcohol intake, individual susceptibility, genetic variables, and dietary factors.

In this study, there is positively correlation observed between durations of alcohol intake and increase plasma lipids. HDL levels are positively correlated with the duration (P<0.00), this result in contrast with previous study done in Romania 2008, which found no correlation with increase HDL and duration of alcohol intake (P<0.5)\textsuperscript{[24]}, this differences might be due to individual susceptibility, dietary factors and dose of alcohol between community in Romania and Sudan. In the same time other parameters; TC, TG and LDL levels are positively correlated with the duration (P<0.00, 0.00 and 0.009 respectively); whilst in other studies this correlation not often mentioned, since most studies tend to show correlation between alcohol and HDL level than TC,TG and LDL levels.

One-Way Anova test is used to compare means of alcohol types (Distilled, Undistilled and people who drink both types) .The result found significant association of all types of alcohol with increase plasma lipids; TC, TG, HDL and LDL (P<0.013, 0.001,
0.019 and 0.030 respectively), this results in agreement with the previous study done in USA 1999, which found that HDL level is significantly higher in drinkers of any type of alcoholic beverage\textsuperscript{[25]}. At the same time results of HDL and LDL levels are in contrast with the previous study in Japan which found no significant association with the type of alcohol, however TG level found significantly lower in those who drank beer\textsuperscript{[22]}. This differences suggest to be from component of homemade alcohol in Sudan; because no study available regarding component and concentration of ethanol in homemade alcohol, so we depend in our study to volume of alcohol intake only.

Independent-samples T test is used to compare the finding of plasma lipids between participants whose intake moderate or heavy alcohol. Results showed that alcohol intake is statistically associated with increase plasma lipids; TC, TG, HDL and LDL (P<0.00, 0.001, 0.00 and 0.001 respectively), also found all parameters increase with participants who take alcohol heavily. These results are in agreement with other study done in Japan, which found that TG and HDL are higher (P<0.001) with heavy alcohol intake and in contrast with the same study in decrease LDL level (P<0.01) with heavy intake, so in Japan they found that alcohol consumption is positive for decreasing risk of cardiovascular disease\textsuperscript{[22]}. This study found that heavy alcohol had potentially adverse effects by elevating all lipids profile, these differences as explain above, so further study in alcohol consumers is highly recommended.

Independent-samples T test is used to compare the finding of plasma lipids with participants whose intake alcohol in occasional or regular mood. The results of the study showed that, means of TC and HDL levels are significant increase with regular intake (P<0.001 and 0.00 respectively) while increase means of TG and LDL levels with no significant differences noted (P<0.146 and 0.132 respectively). This finding in line with other study done in British 1992, which found that TC and HDL levels are significantly increase from occasional to regular intake (P<0.01)\textsuperscript{[21]}. The same results in this study for LDL levels in agreement with the previous study done in Turkey, in which no increase of LDL level observed \textsuperscript{[23]}. The results of TG levels in this study in contrast with the study conducted in Poland 2013, which found that TG levels elevated with regular intake \textsuperscript{[26]}, this variance could be attributed to the influence of alcohol on lipid metabolism to be attributed to the transient changes in lipid metabolism.
Chapter Six

6.1 Conclusion

- The mean age of participants is 38.6 years.
- The majority of alcohol consumers being consumed alcohol for more than 4 years, followed by 3-4 years, 1-2 years and less than one year respectively.
- The majority of alcohol consumers are reported consuming distilled alcohol, followed by using both (distilled and undistilled alcohol) and uninstalled alcohol.
- Out of alcohol consumers, 70% are moderately used, whereas heavily consumers are recorded in 30%.

The present study indicates:

- Significant association between the use of alcohol and increase plasma lipids; TC, TG, HDL and LDL levels.
- Positively correlation between increase plasma TC, TG, HDL and LDL levels and duration of alcohol intake per years.
- Significant association noted between type of alcohol and increase plasma TC, TG, HDL and LDL levels.
- Regularity of alcohol is statistically association with increase plasma TC, TG, HDL and LDL levels.
- Significant association observed between the use of alcohol (Occasional or Regular) and increase TC, HDL levels.
- No significant association observed between the use of alcohol (Occasional or Regular) and increase TG, LDL levels.

In conclusion this study suggest that, significantly increase lipids profile is found in short-term, moderate and heavy drinkers compared to abstainers, which indicates that, alcohol consumption increased serum lipid profiles.
6.2 Recommendations

1. Encourage people who choose not to drink alcohol should not be urged to drink to gain any potential health benefit, and should be supported in their decision not to drink. Thus, Non-drinkers can use other strategies, such as regular exercise, giving up smoking, and a healthy diet, to gain protection against heart disease.

2. Reinforcing health education against miss-believes of alcohol benefits and enlightening about the adverse effects of alcohol of serum lipid profiles.

3. Establishing Task Force against alcohol sales are based solely on evidence related to legal and health consequences.

4. Future research is highly needed in serum lipid profiles targeting alcoholic dependence.
References


13. **Paul S. Jellinger, MD.Face.** Medical guidelines for clinical practice for diagnosis and treatment of dyslipidemia and prevention of atherogenesis.the American association of clinical endocrinologist,(2000),177-182

15. **Alan T.** Thomas, Britannica Encyclopedia: distilled spirit/distilled liquor. Last Updated 1-5-2014.

16. **Overview** of alcohol consumption, National institute on Alcohol Abuse and Alcoholism (NIAAAA). USA


Appendix-1

Sudan University of Science and Technology
College of Graduate Studies
Department of Clinical Chemistry
Assessment of Plasma Lipids Profile among Alcohol Consumer in North Darfur state

Questionnaire (No. _____)

I. Age: ___________________________________________________________ __

II. Alcohol intake

   Yes ○
   No ○

III. Period of drinking:

   < 1 year ○
   1-2 year ○
   3-4 year ○
   > 4 year ○

IV. Type of alcohol:

   Distilled ○
   Undistilled ○
   Mixed ○

V. Use of alcohol:

   Occasional ○
   Regular ○
   Moderate (≤ 4 drink/day and ≤ 14 drink/week ) ○
   Heavy (> 4 drink/day and > 14 drink/week) ○

Laboratory examination

<table>
<thead>
<tr>
<th>Test</th>
<th>Result/ mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol level</td>
<td></td>
</tr>
<tr>
<td>Triglyceride level</td>
<td></td>
</tr>
<tr>
<td>HDL-C level</td>
<td></td>
</tr>
<tr>
<td>LDL-C level</td>
<td></td>
</tr>
</tbody>
</table>
بسم الله الرحمن الرحيم

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا برنامج ماجستير- مختبرات طبيه

قسم الكيمياء السريريه

براءه اخلاقيه

اسم/..................................................................

سوف يتم اخذ عينه من الدم (3.5مل) من الوريد بواسطة حقنه وذالك بعد مسح منطقة اخذ العينه بواسطة المطهر كل الادوات المستخدمة لاخذ العينه معقمه ومتبع فيها كل وسائل السلامة المعتمده.

وانا اقر بان هذه العينه يتم تحليلها فقط لطلب البحث.

انا المذكور اعلاه اوافق على اخذ عينه لإجراء الدراسة.

الامضاء/البصمه:
Appendix-3

Reflotron® Plus