ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCES
DEPARTMENT OF BIOCHEMISTRY

EVALUATION AND COMPARISON OF SERUM LIPID PROFILES BETWEEN NEWLY DIAGNOSED AND TAMOXIFEN TREATED BREAST CANCER FEMALE PATIENTS ATTENDING THE ONCOLOGY CLINIC AT TIKUR ANBESA SPECIALIZED HOSPITAL, ADDIS ABABA, ETHIOPIA

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This is to certify that the dissertation prepared by Bihonegn Birhan, entitled: **Evaluation and comparison of serum lipid profiles between newly diagnosed and tamoxifen treated breast cancer female patients attending the oncology clinic at Tikur Anbesa Specialized Hospital, Addis Ababa, Ethiopia,** and submitted in partial fulfillment of the requirements for the degree "Master of Science in Medical Biochemistry" in the department of Biochemistry complies with regulations of the university and meets the accepted standards with respect to originality and quality.

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ABSTRACT

Background: Breast cancer is the most common cancer in women worldwide and the second most common cancer overall. It is the leading cause of cancer death in less developed countries including Ethiopia. Dyslipidemia in breast cancer patients has long been considered as the risk factor for cardiovascular diseases. Estrogen induces hyperlipidemia through its multiple effects on lipid metabolism. Tamoxifen used for breast cancer treatment, is essentially anti-estrogenic, however, currently, it has been suggested that its estrogenic activity is mainly responsible for the changes in lipid parameters. Because marked hyperlipidemia is a potent risk factor for life-threatening acute pancreatitis and arteriosclerosis, plasma lipid levels should be tested periodically in breast cancer patients. However, detection of lipid profiles in newly diagnosed and tamoxifen treated breast cancer women have not been investigated yet in our country, Ethiopia.

Objective: Therefore, the aim of this study was to evaluate and compare serum lipid profiles between newly diagnosed and tamoxifen treated breast cancer women attending the oncology clinic at TASH.

Patients and methods: Comparative cross-sectional study design was used to determine fasting serum lipid profiles in newly diagnosed and tamoxifen treated women with breast cancer in the oncology clinic of TASH. Convenience sampling method was applied to recruit study subjects and 52 breast cancer women treated with tamoxifen for three months and above and 51 new medically diagnosed breast cancer women without treatment were included and serum lipid parameters (TG, TC, HDL-C, and LDL-C) were measured.

Result: In breast cancer women receiving tamoxifen, the mean serum TC and LDL-C levels were significantly reduced but TG levels were insignificantly decreased accompanied by the elevation of HDL-C though it was insignificant as compared to those women with no treatment.

Conclusion: lipid profile is significantly improved upon tamoxifen treatment and this confirms that tamoxifen has cardio protective roles in lipid metabolism.

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ABBREVIATIONS AND ACRONYMS

ApoA-I……. Apo lipoprotein A-1

CE…….….Cholesterol esterase

CHD……...Coronary heart disease

CHOD…….Cholesterol oxidase

CM ……..…Chylomicrons

CVD……Cardiovascular diseases

ER…….Estrogen receptor

ERBB2…….....erb-b2 receptor tyrosine kinase 2

ER+…….…… Estrogen receptor positive

ERs……..Estrogen receptors

GK……..glycerolkinase

G-3-P……..glycerol-3-phosphate

GPO……...glycerphosphate oxidase

HDL……High density lipoprotein

HDL-C……...… High density lipoprotein-cholesterol

HER2 ……..Human epidermal growth factor receptor 2

HTGL…….….hepatic triglyceride lipase

LDL…….Low density lipoprotein

LDL-C ……..... Low density lipoprotein cholesterol

LPL…….Lipoprotein lipase

PAX2 ……....Paired box gene 2 /pared box protein 2

PEGME……..…Polyethylene-glycol-methyl ester

POD…….. Peroxidase

PR……..….Progesterone receptor

PVS……..…polyvinyl sulfonic acid

SERM……. Selective estrogen receptor modulator

SERMs……..…..... Selective estrogen receptor modulators

TASH…….….Tikur anbesa specialized hospital

TC……...Total cholesterol

TG…….....Triglyceride

TC…….…..Total cholesterol

VLDL…….…....Very low density lipoprotein

VLDL-C ……...…..... Very low density lipoprotein-cholesterol

WHO….....World health organization

Operational definitions

Case: Female breast cancer patient who is confirmed by hematologist or histologist.

Dyslipidemia: synonymous with hyperlipidemia or lipid abnormalities is abnormally elevated levels of any or all lipids and or lipoproteins in the blood. By the same token it is a defect in lipoprotein metabolism; example, increased cholesterol, increased triglyceride, increased low density lipoprotein, and decreased high density cholesterol.

Neoadjuvant chemotherapy is the use of chemotherapy alone prior to definitive surgery or radiation therapy. It's given before primary therapy.

Adjuvant therapy: Is the additional cancer treatment after primary treatment to lower the risk of reemergence.

1. INTRODUCTION

1.1. Overview of breast cancer

Cancer is the second largest killer disease. Cancer accounts for high morbidity and high mortality rate throughout the world **(**Sharma and Ray, 2000**).** Cancer starts when a cell begins to divide and grow in an uncontrolled and abnormal way. Overtime these cells cluster to form a tumor. Cancer that is detected early can potentially be cured when the tumor is small enough to be completely removed surgically (Mishra *et al*., 2004).

Breast cancer is a malignant tumor originating from the breast tissue, most commonly from the inner lining of milk ducts or the lobules that supply the ducts with milk. Breast cancer primarily affects women. Breast cancer is the most common cancer in women worldwide with nearly 1.7 million new cases diagnosed and 521,900 deaths in 2012 (second most common cancer overall). This represents about 12% of all new cancer cases (14.1 million) and breast cancer alone accounts for 25% of all cancer cases and 15% of all cancer deaths among females (Gomez *et al*., 2010).

However, it occasionally affects men; accounting for 0.2% of all cases in men. The female to male ratio of breast cancer prevalence is 100:1 (Gomez *et al*., 2010 Giordano et al., 2002). Breast cancer is similar in men and women; however, breast cancer in men is more frequently hormone receptor positive and may be more sensitive to hormonal therapy (Giordano et al., 2002). The risk appears to be higher with inherited breast cancer A2 (BRCA2) rather than breast cancer A1 (BRCA1) gene mutations (Gomez *et al*., 2010). Men tend to be diagnosed at an older age than women and at a later disease stage. Tumors of the male breast are more likely to express the estrogen and progesterone receptors (PRs) and less likely to overexpress HER-2 than breast cancers in women (Gomez *et al*., 2010).

Breast cancer is the leading cause of cancer death in less developed countries (Release, 2013). In the developing world, although definitive data is not available, it is widely accepted that the incidence of breast cancer is increasing due to increase in life expectancy, urbanization, adoption of western lifestyles and others. In 2008, the number of new cases of breast cancer in women from Africa was estimated to be 92,600 cases with alarming 54% (50,000) deaths from breast cancer (Mandal *et al*., 2014).

In Ethiopia, cancer is one of the non-communicable diseases among the major causes of illnesses and death. Breast cancer is one of the top two cancer types having a lion's share for the high women deaths in the country (Ababa *et al*., 2012). Over a period of sixteen years, 1997-2012, more than 50 cancer types, a total of 16,622 new cases were registered in TASH. Out of this, 3460 (prevalence=20.8%) were new cases of breast cancer representing approximately 216 cases per annum (Abate *et al*., 2016).

1.2. Literature review

1.2.1. Serum lipid profiles with respect to breast cancer and CVD

Cardiovascular diseases (CVD) and cancer are the leading causes of death worldwide (Rodrigues et al., 2014). Dyslipidemia (abnormal levels of lipids and lipoproteins in the blood) is already shown to play a major role in the etiopathogenesis of CVD. Dyslipidemia, which is a strong predictor of CVD, may also be important to predict the incidence of breast cancer (Smith, 2007). Lipoprotein fractions can induce cancer cells proliferation and migration and 27-hydroxycholesterol, a primary metabolite of cholesterol was shown to promote estrogen receptor positive (ER+) breast cancer growth (Nelson et al 2013; Mcdonnell *et al*., 2014).

Biochemically, complexes of variable proteins and lipid compositions called lipoproteins are responsible for transport of lipids throughout the body. The plasma lipoproteins are spherical macromolecules made up of lipids and specific proteins called Apo lipoproteins or Apo proteins. The lipoprotein complexes include high-density lipoproteins (HDL), low-density Lipoproteins (LDL), very-low-density lipoproteins (VLDL), and chylomicrons (CM). The complexes play key roles in maintaining their component lipids soluble while they transport them in the blood and providing an efficient mechanism for transporting their lipid contents to (and from) the body tissues (Marks *et al*, 2005).

In humans, the transport system is less efficient than in other animals and, as a consequence, humans are vulnerable to gradual deposition of lipid substances (dyslipidemia) —especially cholesterol—in body tissues. This is a potentially life-threatening occurrence when the lipid deposition contributes to plaque formation, causing the narrowing of blood vessels (atherosclerosis) (Harvey and Ferrier, 2011).

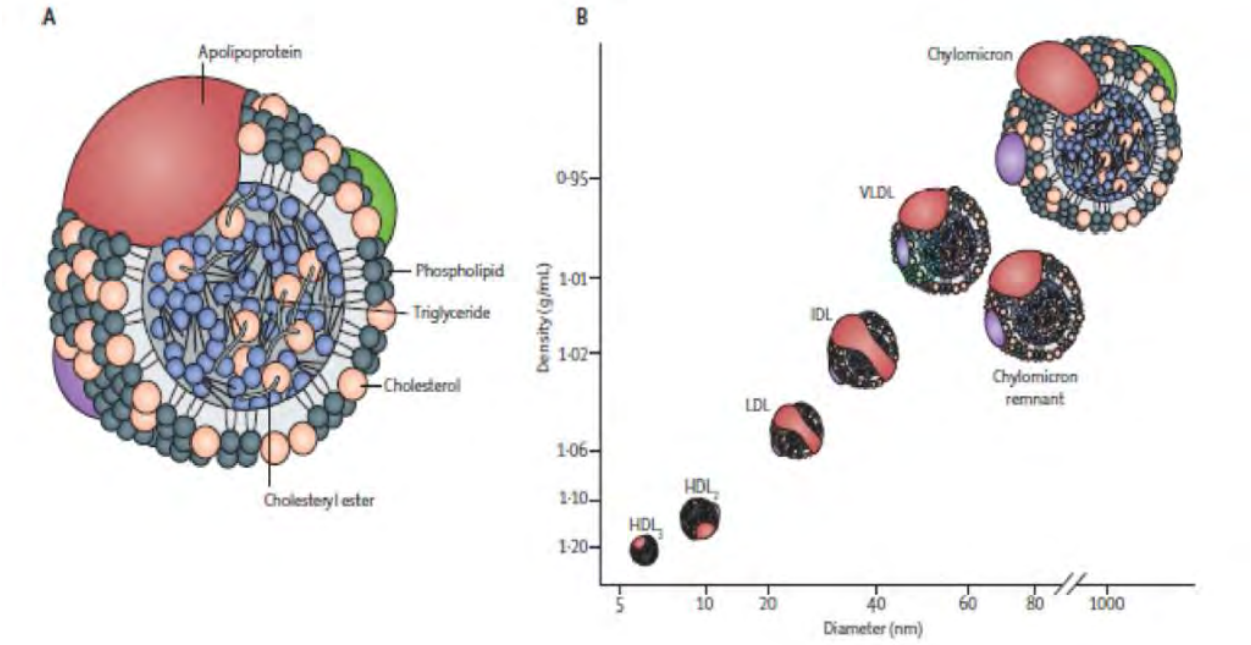

Figure 1. Structure, types and densities of lipoproteins in the blood from Braun Wald's Heart Disease (Mann *et al*., 2015)

LDL particles, associated with the Apo lipoprotein molecule Apo lipoprotein B, contain much less triacylglycerol than their VLDL predecessors, and have a high concentration of cholesterol and cholesteryl esters. The primary function of LDL particles is to provide cholesterol to the peripheral tissues where they are internalized through the LDL receptor by receptor-mediated endocytosis. Genetic defects that result in loss of function of LDL receptor cause inherited hyperlipidemias (Harvey and Ferrier, 2011).

Increased concentrations of serum LDL cholesterol (LDL-C) are associated with an increased risk of myocardial infarction and stroke, and reaction of LDL-C with reactive oxygen species is an early step in atherosclerotic plaque formation. Individuals who inherit one copy of a defective LDL receptor-related gene (heterozygous Familial Hypercholesterolemia), left untreated, often have myocardial infarctions in their 30s and 40s. If a person is homozygous for these mutations (homozygous Familial Hypercholesterolemia), they have extremely high serum LDL-C levels and can have myocardial infarctions in their late teens and early 20s (Mooradian, 2009).

High density lipoprotein (HDL) contains Apo lipoprotein A-1 (ApoA-I). Formation of HDL occurs in the liver and intestine, which both synthesize and secrete ApoA-I. Shortly after secretion as a

lipid poor protein, ApoA-I interacts with the cholesterol–phospholipid transporter ABCA1 (ATP Binding Cassette A1) expressed by hepatocytes and enterocytes to acquire lipids, thereby generating a nascent HDL particle (Grummer and Carroll, 1988).

Plasma lipid profiles are measured for cardiovascular risk prediction and have now become almost a routine test. The test includes four basic parameters: total cholesterol (TC), triglycerides (TG), HDL cholesterol (HDL-C) and LDL-C. Worldwide, there is broad variation in serum lipid profile patterns among different population groups. Increased serum levels of TC, TG, LDL-C, and decreased serum HDL-C level are known to be associated with major risk factors for CVD. There is a strong relationship between LDL-C concentrations and CVD risk (Choudhury *et al*., 2014). In addition, most frequently, in breast cancer patients serum TC, LDL-C and TG levels are constantly raised, while HDL-C level is decreased (Yadav *et al*., 2012; Ray and Husain, 2001). Therefore, dyslipidemia, which is a characteristics of metabolic syndrome, in breast cancer patients makes them more vulnerable to CVD.

Although dyslipidemia is one of widely recognized risk factors for CVD as well as breast cancer, the pathophysiology of the association between them is still not completely understood. Several mechanisms have been postulated to explain this association. Atherosclerosis – caused in great part by dyslipidemias – leading to structural changes that result in the decreased elasticity of large arteries, is generally viewed as the principal pathophysiologic alteration contributing to the development of arterial hypertension, which is another risk factor for CVD (Oparil *et al.,* 2003).

Plethora of studies have prospectively examined if increased lipid levels are correlated with the afterward development of breast cancer. In most studies, HDL-C levels show an independent and inverse relation with the development of breast cancer (Furberg *et al*., 2004; Michalaki *et al*., 2005 and Kim et al., 2009). Increased TG, higher TC, and higher LDL-C levels have been found to be associated with an increased risk of breast cancer in some studies, but not in all (Ray and Husain, 2001 and Yadav *et al*., 2012).

1.2.2. Breast cancer symptoms, risk factors and strategies to reduce these risks

The most common symptoms of breast cancer are lump in the breast, change in breast shape, dimpling of the skin, and fluid coming from the nipple, red scaly patch on the skin, unexplained weight loss and abnormal bleeding (American Cancer Society, 2007).

The causes of breast cancer are not fully known. However, researchers have identified a number of factors that increase one's chances of getting breast cancer. These are called risk factors. Risk factors do not cause breast cancer, but can increase the chances of getting breast cancer. Some women have many risk factors, but never get breast cancer. And, some women have few or no risk factors, but do get the disease. Breast cancer risk factors includes family history of breast cancer; personal history of breast cancer; early menarche (< 12 years); late menopause (> 55 years); aging; alcohol; late age at first full-term pregnancy (> 30 years); never breastfed a child; recent oral contraceptive use; high fat diet; tobacco smoke; obesity (postmenopausal); recent and long-term use of hormone replacement therapy; high-dose radiation to chest; lack of physical activity (American Cancer Society, 2007 and Petracci *et al*., 2011). More recently, Cholesterol has emerged as an important risk factor for breast cancer although the mechanisms by which this occurs are not well understood (Mcdonnell *et al*., 2014).

Among the best-studied risk factors for breast cancer are loss of function mutations in the genes coding for the tumor-suppressor proteins BRCA1 and BRCA2 (breast cancer 1 and 2, early onset) but they account for less than 10% of cases *(*Shah *et al*., 2014*)*. Less well defined, although supported by a wealth of epidemiological data, are other risk factors, primarily associated with the development of ER+ (estrogen receptor positive) breast cancer (Petracci *et al*., 2011).

Obesity increases the risk of postmenopausal breast cancer (Bhat *et al*., 2013). Risk is about 1.5 times higher in overweight women and about 2 times higher in obese women than in lean women *(*La Vecchia *et al*., 2011). Breast cancer risk associated with excess weight is likely due, in part, to high estrogen levels because fat tissue is the largest source of estrogen in postmenopausal women. This association might also be explained by the higher levels of insulin among obese women. Obesity is a risk factor for type II diabetes, which has also been linked to increased risk for postmenopausal breast cancer *(*De Bruijn *et al*., 2013).

The possible strategies that may help reduce the risk of breast cancer include avoiding weight gain and obesity, engaging in regular physical activity, and minimizing alcohol intake. The increased risk of breast cancer associated with the use of combined menopausal hormone therapy should be considered when evaluating treatment options for menopausal symptoms. Women who choose to breastfeed for an extended period of time may also lower their breast cancer risk (Kushi *et al*., 2012). Furthermore, applying appropriate mode of treatment can also reduce the risk of breast cancer among patients at high risk.

1.2.3. Breast Cancer Treatment

Treatment of breast cancer is complex and involves surgery, radiotherapy, chemotherapy, targeted therapy and hormonal therapy can be given depending on the estrogen receptor status. Systemic therapy (includes chemotherapy, targeted therapy and hormonal therapy) is treatment that travels through the bloodstream and can affect and treat all parts of the body, not just one area. Systemic therapy work through different mechanisms. Chemotherapy drugs generally work by attacking cells that grow quickly, such as cancer cells. Targeted drugs are newer and work by attacking specific molecules in or on cells that may be more common or active in cancer cells. Hormonal therapy works by either blocking the body's natural hormones or lowering the levels of those hormones, which act to promote cancer growth (Wolff and Davidson, 2001).

1.2.4. Tamoxifen, its mechanism of action and serum lipid profiles in breast cancer patients

Estrogen, like all other steroid hormones is able to cross cell membranes and bind in a specific manner to their receptors to form a specific hormone-receptor complexes. These complexes bind to specific DNA sites in estrogen dependent tissues called hormone responsive elements and cause increased transcription of various genes. The end result is increased cell growth, proliferation and protein synthesis and enzyme synthesis (Kumar *et al*., 1987), with concurrent carcinogenesis.

Patients with ER+/PR+ breast cancer can be given hormonal therapy (also called endocrine therapy) to lower estrogen levels or to block the effects of estrogen on the growth of breast cancer cells. In spite of the numerous choices in endocrine therapies, tamoxifen, which was discovered in 1967 (Jordan, 2006), has remained the "gold standard" of first-line hormonal therapy in patients who have tumors expressing hormone receptors and it has been the main stay of hormonal

treatment of all phases of breast cancer and represents a major therapeutic advance for clinical practice (Lake & Hudis, 2002).

Tamoxifen, which is one selective estrogen receptor modulators (SERMs), is a class of drug that act on the ER. A characteristic that distinguishes these substances from pure ER agonists and antagonists (that is, full agonists and silent antagonists) is that their action is different in various tissues, thereby granting the possibility to selectively inhibit or stimulate estrogen-like action in various tissues. Tamoxfen is one of SERM and it acts differently in breast and other tissues like bone and uterus (Bertelli *et al*., 1988). Tamoxifen is a treatment that blocks the effects of estrogen and is routinely used to treat both premenopausal and postmenopausal ER+ breast cancer patients. It is a SERM that works both by decreasing factors that increase the growth of breast cells and by increasing factors that decrease the growth of breast cells (Sanchez *et al*., 2006).

Treatment of ER+ breast cancer with tamoxifen for at least 5 years has been shown to reduce the rate of recurrence by approximately 40%-50% throughout the first decade, and reduces breast cancer mortality by about one-third through out the first 15 years (Davies *et al*., 2011). More recently, studies have shown that extended use of tamoxifen (10 years versus 5 years) further reduces the risk of breast cancer recurrence and mortality, so clinical practice guidelines now recommend consideration of adjuvant tamoxifen therapy for 10 years. Serious side effects contain a small increased risk of stroke, uterine cancer, pulmonary embolism and vision problems. Common side effects include weight loss, irregular periods, and hot flashes. It might cause damage to the baby if taken during breastfeeding or pregnancy (Davies *et al*., 2012).

Tamoxifen is a prodrug, having relatively little affinity for ER. It is metabolized in the liver by the cytochrome P450 isoform CYP3A4 and CYP2D6 into active metabolites such as N-desmethyl-4-hydroxytamoxifen (endoxifen) and 4-hydroxytamoxifen (afimoxifene) (Desta et al., 2004) which have 30-100 times further affinity with the estrogen receptor than tamoxifen itself. These active metabolites compete with estrogen in the body for binding to the ER. In breast tissue, 4-hydroxytamoxifen acts as an ER antagonist so that transcription of estrogen-responsive genes is inhibited (Wang *et al*., 2004). Four (4)-hydroxytamoxifen binds to ERs, the ER/tamoxifen complex recruits other proteins known as co-repressors and then binds to DNA to modulate gene

expression. Some of these proteins include silencing mediator of retinoid and thyroid hormone receptor and nuclear receptor co-repressor (Shang *et al*., 2000).

Tamoxifen role can be controlled by a number of many variables including growth factors (Massarweh et al., 2008). Tamoxifen needs to block growth factor proteins such as erb-b2 receptor tyrosine kinase 2 (ERBB2) / human epidermal growth factor receptor 2 (HER2) because elevated levels of ERBB2 are shown to occur in tamoxifen resistant cancers. Tamoxifen need a protein PAX2, which is encoded by Paired box gene 2 (PAX2 gene)**,** for its full anticancer activity (Hurtado *et al*., 2008). In the existence of high PAX2 expression, the tamoxifen/estrogen receptor complex is capable to suppress the expression of the pro- proliferative ERBB2 protein. When the nuclear receptor coactivator 3, which is also known as amplified in breast 1 (AIB-1), expression is higher than PAX2, tamoxifen/ER complex upregulates the expression of ERBB2 causing in stimulation of breast cancer growth. Four (4)-hydroxytamoxifen binds to ERs competitively in tumor cells and other tissue targets, producing a nuclear complex that reduce DNA synthesis and inhibits estrogen effects. It is a nonsteroidal agent with potent anti-estrogenic properties, which compete with estrogen for binding sites in breast and other tissues. Tamoxifen causes cells to remain in the G0 and G1 phases of the cell cycle (Liu *et al*., 2013).

Hormonal treatment may alter serum lipid levels and, therefore, may contribute to CVD in patients with cancer. Estrogen therapy seems to have a mixed effect on serum lipid levels with a significant decrease in the levels of TC and LDL-C, an increase in HDL-C and triglyceride concentration (Bulusu *et al*., 1982 and Schaefer *et al*., 1983).

Recently, authors have reported that changes of lipid profiles in Japanese postmenopausal women treated with tamoxifen were relatively favorable, while exemestane and anastrozole had no clinically significant effect on the serum lipids (Hozumi *et al*., 2010). According to Hozumi et al. (2016), the activity of LPL (lipoprotein lipase) and HTGL (hepatic triglyceride lipase), the key enzymes of triglyceride metabolism, decreased significantly as a result of tamoxifen treatment. However, the mean mass of lipoprotein lipase significantly increased after tamoxifen treatment and the authors have concluded that tamoxifen might increase inactive lipoprotein lipase.

Another study has shown that serum lipid profiles were significantly improved in breast cancer patients who receive treatment with tamoxifen which can effect lipid metabolism by increasing

the level of HD-C and reduce the level of TC and LDL-C and the authors have suggested that tamoxifen has cardio-protective effects on lipid metabolism (Morad *et al*., 2016).

Gupta et al. (2006) suggested that in pre-menopausal and postmenopausal patient's TC and LDL-C levels were reduced significantly, whereas, TG, VLDL-C and HDL-C were not altered. Comparison of the effects of tamoxifen in pre-menopausal and postmenopausal patients on lipid profile revealed that fall in TC and LDL-C was significantly higher at both 3 and 6 months in postmenopausal patients. Based on their investigation, the authors conclude that tamoxifen favorably alter the markers of cardiovascular risk in both pre-menopausal and postmenopausal patients of breast cancer.

In prospective study conducted on 109 patients indicate that tamoxifen has an impact on the serum lipid profile of breast cancer patients, the mean serum total TC levels after tamoxifen treatment, as well as the serum LDL-C levels, were lower than the baseline levels, with statistically significant differences which means that treatment with tamoxifen lowered the serum TC and LDL-C levels. However, the change in the mean serum TG levels in these patients was not statistically significant (Lin *et al*., 2014).

1.2. Statement of the Problem

Breast cancer is the most common cancer in women worldwide and the second most common cancer overall. It is the second leading cause of cancer death in American women, exceeded only by lung cancer and the leading cause of cancer death in less developed countries including Ethiopia (Release, 2013). In Ethiopia, breast cancer is one of the top two cancer types having a lion's share for the high maternal deaths in the country (Ababa *et al*., 2012).

Breast cancer and lipid abnormalities are well known to frequently coexist. The coexistence of breast cancer and lipid abnormalities have many clinical implications. Epidemiologic studies reported that elevated LDL-C and TG, and reduced HDL-C levels are important risk factors for developing CVD (Gordon *et al*., 1977 and Iso et al., 2001). In women over the age of 60, CVD becomes the leading cause of mortality. Myocardial infarction is the main cause of death in women in this age group (Wiseman, 1994).

It is known that estrogen induces hyperlipidemia through its multiple effects on lipid metabolism, including increased synthesis of TG and VLDL-C and decreased activity of LPL and HTGL. Elevated levels of triglycerides are associated with a decreased level of sex-hormone-binding globulins, resulting in increased amounts of free estradiol and increased breast cancer risk (Kakaiya *et al*., 2013).

Tamoxifen, used for breast cancer treatment, is essentially anti-estrogenic, but it has some estrogenic activities in non-breast tissues. The effects of tamoxifen on lipid metabolism may be attributable to its complex combination of anti-estrogenic and estrogenic activities (Hozumi et al., 2016). Tamoxifen has an impact on lipid profiles and currently, however, it has been suggested that its estrogenic activity is mainly responsible for the changes in lipid parameter (Lin *et al*., 2014).

Because marked hyperlipidemia is a potent risk factor for life-threatening acute pancreatitis and arteriosclerosis, blood lipid levels should be tested periodically during tamoxifen treatment, even if the patients are normolipidemic during the pretreatment stage. Therefore, the effects of tamoxifen on lipid metabolism should not be ignored. However, detection of lipid profiles among breast cancer patients treated with tamoxifen based hormonal therapy has not been investigated yet in our country, Ethiopia. Therefore, this study is aimed at measuring the serum lipid profiles among breast cancer women treated with tamoxifen and those newly diagnosed for breast cancer and make a comparison between the two groups.

1.3. Significance of the study

Breast cancer is one of the commonest causes of cancer mortality in females. Lipid abnormalities in breast cancer become one of the risk factors for CVD, and this should cautiously be intervened. Treatment of breast cancer is complex and involves surgery, radiotherapy, chemotherapy and hormonal therapy depending on estrogen receptor status of the disease in the individual patient. Tamoxifen, has been the main stay of hormonal treatment of all phases of breast cancer and represents a major therapeutic advance for clinical practice (Wolff and Davidson, 2001).

Evaluation of lipid profiles is important as long term users of tamoxifen continue to increase. This is because life threatening adverse effects of dyslipidemia impacts negatively on the quality of life of the survivors, and therefore need to be controlled. Several studies conducted in different countries reported that tamoxifen as a hormonal therapy for breast cancer, has a favorable effect on lipid profile in postmenopausal women and it has a favorable cardio-protective effects (Gupta *et al*., 2006 and Morad *et al*., 2016).

Tamoxifen has been reported to have beneficial cardiovascular effects and the benefits of prophylactic tamoxifen therapy, including potential protection against CHD and osteoporosis, in addition to decreasing the risk of breast cancer should outweigh the risk of deleterious side-effects. Protection against CVD could thus be a health benefit of both disease therapy and preventative treatment with tamoxifen. But studies related to the effects of tamoxifen on serum lipid profiles in breast cancer patients have not yet investigated in Ethiopia and so this study is expected to add information regarding tamoxifen induced lipid profile changes in Ethiopian.

The findings of this study might provide information on the challenges faced during tamoxifen-based treatment and can be used as baseline for next studies since such a study was not previously done in the country. The information generated from this study will help clinicians to consider other safer options of treatment, better preventive protocol and make appropriate intervention as early as possible. The results obtained may also help patients to be protected from adverse effect of the drug and promote immediate recovery. It also helps in influencing the development of appropriate policies, plans and intervention programmers for the prevention and management of hormonal therapy related health problems. This in turn, might improve the quality of care for breast cancer patients treated throughout the country.

2. OBJECTIVES

2.1. General objective

To evaluate and compare serum lipid profiles between newly diagnosed and tamoxifen treated breast cancer females attending the oncology clinic at TASH.

2.2. Specific objectives

- To measure and compare fasting serum TC, TG, HDL-C and LDL-C levels in newly diagnosed and tamoxifen treated women with breast cancer
- To calculate body mass index (BMI) in newly diagnosed and tamoxifen treated women with breast cancer
- To compare lipid profile parameters between premenopausal and postmenopausal breast cancer women patients receiving tamoxifen

3. MATERIALS AND METHODS

3.1. Study Design

Comparative cross-sectional study design was used to determine serum lipid profile levels in newly diagnosed and tamoxifen treated women with breast cancer in the oncology clinic of TASH.

3.2. Study Area

The study was conducted at the oncology unit of Tikur Abesa Specialized Hospital (TASH). The hospital is a large referral teaching hospital, under the administration of Addis Ababa University, located in Addis Ababa, Ethiopia. The hospital has 800 beds and gives diagnostic and treatment service for about 370,000-400,000 patients per year (Stohr *et al*., 2007). The oncology unit of TASH is the only oncology unit for the country and has an outpatient department, which gives service to new and follow-up patients and an in-patients department, which has 19 beds. Professionally, the unit has 3 oncologists with palliative specialist, 1 general practitioner, 6 residents, and 12 nurses. TASH offers comprehensive cancer treatment within the country. Being a public hospital, TASH offers the lowest cost for these services compared to the private hospitals.

3.3. Source Population

All breast cancer patients referred to TASH during the study period were included in the study.

3.3.1. Study subjects and study duration

All tamoxifen treated and newly diagnosed women with breast cancer at TASH. This study was conducted from December 2016 to June, 2017.

3.4. Inclusion and Exclusion Criteria

3.4.1. Inclusion criteria

All breast cancer women treated with tamoxifen for at least three months at TASH were included in this study. All new medically diagnosed breast cancer women were used as positive controls.

3.4.2. Exclusion Criteria

Women breast cancer patients with medical contraindications to hormonal therapy, thromboembolic complications, endometrial cancer and history of cardiac illness were excluded in this study.

3.5. Sampling method and sample size determination

Convenience sampling method was applied and used to get the calculated number of study participants in the study period. By using semi-structured questionnaire, the patients were selected and all pieces of information of patients needed for the study were collected. The sample size was determined based on prevalence of breast cancer (20.8%) in Ethiopia as reported by Abate et al. (2015), using single population proportion formula with a confidence interval (CI) of 95%.

$$\mathbf{n = (Z\alpha/_2)^2\, p\, (1\text{-}p)/d^2}$$

Where **n** is minimum sample size required; **Z1-α/2** is the standard normal variable at (1-α) % confidence level and α (level of significance), usually 95% confidence level is used = 1.96; **P** is estimate of the prevalence rate of breast cancer female patients in the population; **d** is the margin of sampling error tolerated, assume to be 0.05. So, n after adjustment is approximately 253 patients, but due to budget constraint only 103 (51 newly diagnosed & 52 treated with tamoxifen) patients were enrolled.

3.6. Study variables

3.6.1. Dependent variables

- Serum TC level
- Serum HD-C level
- Serum TG level
- Serum LDL-C level

3.6.2. Independent variables

- Age
- Place of residence
- Marital status

- Family history
- Menopausal status
- BMI
- Clinical stage of breast cancer

3.7. Blood sample and data collection procedures

After the study participants had been asked for their consent to be interviewed and to give sample, blood was collected under aseptic conditions i.e., skin over the vein was cleaned by 70% alcohol and blood (5ml) was withdrawn from the study participants, who had fasted overnight, by qualified health care oncology professional nurses in the oncology clinic. Then, specimen was transferred into serum separator tube and it was allowed to stand for 30 minutes at room temperature to allow complete clotting and clot retraction; and centrifuged at 4000 rpm for 10 minutes to extract the serum.

The extracted serum was then transferred into a clean and sterile container and stored at – 80°C until it was used. After the data collection was completed, the extracted serum was used to determine the levels of TC, HDL-C and TG by using calibrated fully-automated Mind ray, clinical chemistry analyzer according to the reagent manufacturer's instruction in central laboratory of Zewuditu referral hospital. The reagents were commercially available in readymade kits. LDL-C was calculated using the Friedwald formula (Friedewald *et al*., 1972). Safety precautions were taken while handling blood and disposing it.

In addition, primary data and information about the patients like current medications, relevant previous medical and medication histories were recorded using data abstraction format through reviewing patients' medical chart and the questionnaire was filled by face to face interview and some anthropometric indicators were also assessed and measured side by side.

3.8. Test principles of the analytes

3.8.1. Serum total cholesterol concentration

Principle: The method for the measurement of serum TC involves the use of three enzymes: cholesterol esterase (CE), cholesterol oxidase (CHOD) and peroxidase (POD). Cholesterol esters are first hydrolyzed to release free cholesterol and triglycerides using cholesterol esterase. The free cholesterol is then oxidized by CHOD to generate H_2O_2. The hydrogen peroxide reacts with phenol and 4-aminoantipyrine in the presence of POD to generate a colored quinoid dye product, the absorbance of which is measured at 540 nm, and is proportional to the concentration of TC in the original sample. The reaction sequence is as follows:

Cholesteryl ester + H_2O $\xrightarrow{CE}$ cholesterol + fatty acid

Cholesterol + O_2 $\xrightarrow{CHOD}$ cholest- 4-en-3-one + H_2O_2

$2H_2O_2$ + 4-aminophenazone + phenol $\xrightarrow{POD}$ 4-(p-benzoquinone- monoimino)-phenazone + 4 H_2O

Reagent composition:

R1: 200 mmol/L PIPES pH 7.0, containing 1 mmol/L sodium cholate, > 250 U/L cholesterol esterase, >250 U/L cholesterol oxidase, > 1 KU/L peroxidase, 0.33 mmol/L 4-aminoantipyrine, 4 mmol/L phenol, 2 g/L non-ionic surfactant, and commercial biocides.

R2: 5.18 mmol/L cholesterol standard.

Procedure: One ml of the working reagent (R1) was mixed with 10 µl of serum sample. After 5 minutes of incubation at 37^0C, the absorbance was measured at 500 nm against the reagent blank. Desirable or normal cholesterol levels were considered to be those below 200 mg/dL.

3.8.2. Serum triglyceride concentration

The method is based on the enzymatic hydrolysis of serum or plasma triglyceride to glycerol and free fatty acids by LPL. The glycerol is phosphorylated by adinosine triphosphate (ATP) in the presence of glycerolkinase (GK) to form glycerol-3-phosphate (G-3-P) and adenosine diphosphate (ADP). G-3-P is then oxidized by glycerphosphate oxidase (GPO) to form dihyroxyacetone phosphate and hydrogen peroxide. A red coloured product is formed by the peroxidase (POD) catalyzed coupling of 4-aminoantipyrine (4-AA) and phenol with H_2O_2, the optical density at 540 nm of which is proportional to the concentration of triglyceride in the sample. The reaction sequence is as follows:

Triglycerides + 3H2O $\xrightarrow{\text{LPL}}$ glycerol + 3 free fatty acids

Glycerol + ATP $\xrightarrow{\text{GK}}$ G-3-P + ADP

Glycerol-3-phosphate + O2 $\xrightarrow{\text{GPO}}$ dihydroxyacetone phosphate + H_2O_2

H_2O_2+ 4-AA + 4-chlorophenol $\xrightarrow{\text{POD}}$ Quinoneimine + H_2O + HCl +4-chlorophenol

Reagent composition

R1 = monopipes buffer (pH 7.5) 50 mmol/L, LPL ≥ 1300U/L, GK ≥ 400U/L,POD ≥ 500 U/L, 4-AA 0.25mmol/L, phenol 5mmol/L, Mg^{2+} = 4.5 mmo/l, ATP= 2mmol/l , sodium azide (0.05%)

R2= Triglycerides standard: - Glycerol 2.25mmol/L, equivalent to 200mg/dl of glycerol trioleate,

Procedure: -Ten microliter of serum was mixed in a cuvette with 1ml of triglyceride mono reagent R1, and then incubated at room temperature for 5 minutes. Then the optical density was read at 600 nm, against reagent blank and compared with standard triglyceride concentrations used as samples.

Desirable or normal fasting triglyceride levels are considered to be those below 200 mg/dL, and are further categorized as Borderline, 200-400 mg/dl; High, 400-1,000 mg/dl; and Very High (>1000 mg/dl).

3.8.3. High density lipoprotein (HDL) cholesterol concentration

AMS-direct method for the quantitative determination of HDL-C in serum was used.

Principle:- LDL, VLDL and chylomicrons (CM) bind to polyvinyl sulfonic acid (PVS) and polyethylene-glycol-methyl ester (PEGME) present in the reagent and forming (LDL+VLDL+CM)PVS/PEGME complex. Only HDL-C reacts with the enzymes cholesterol oxidase (CHOD) and cholesterol esterase (CE). The reaction is as follows:

HDL+LDL+VLDL+CM $\xrightarrow{\text{PVS/PEGME}}$ HDL+ (LDL+VLDL+CM) PVS/PEGME

HDL $\xrightarrow{\text{CHOD+ CE}}$ Fatty acids +H_2O_2

$2H_2O_2$ + 4-aminoantipyrine (4-AA) + N, N-Bis (4-sulfobutyl) 3-methylaniline ($_{TODB}$) $\xrightarrow{\text{POD}}$ $5H_2O$ + Quinone dye

Reagent composition

Reagent I: MES buffer (PH 6.5) = 50mmol/l; TODB= 2mmol/l; PVS =2mmol/l; PEGME =0.05mmol/l; MgCl2= 1mmol/l; EDTA=1mmol/l

Reagent II: MES buffer (PH 6.5) = 50mmol/l; CE = 50 KU/l; CHOD=30 KU/l; POD = 30 KU/l; 4-AA = 4mmol/l; detergent = 0.003%

HDL-C standard, 40mg/dl

Procedure:

I, Precipitation

Serum (0.2ml) was mixed with 0.4 ml of Precipitating Reagent I in a test tube and allowed to stand for 10 min at room temperature. Then, the sample was centrifuged for 10 min. at 10,000 rpm and the supernatant containing HDL removed and further processed for cholesterol determination.

II. Colorimetry

Supernatant (50 microliter µl) containing HDL was mixed in a cuvette with 1ml of cholesterol reagent II, and then incubated at room temperature for 10 minutes. Then the optical density was read at 600 nm, against reagent blank, as described under the method for TC determination. A low HDL-C concentration is considered to be a value below 40 mg/dl. HDL-C values were also used in the calculation of LDL-C (as shown below)

3.8.4. Low density lipoprotein (LDL) cholesterol concentration

Most of the circulating cholesterol is found in three major lipoprotein fractions: VLDL, LDL and HDL. TC = VLDL-C + LDL-C + HDL-C. LDL-C is calculated from measured values of TC, TG and HDL-C according to the relationship: LDL-C = TC - HDL-C – (TG/5) where TG/5 is an estimate of VLDL-C and all values are expressed in mg/dl. Desirable levels of LDL-C are those below 100 mg/dl in adults.

3.9. Body Mass Index (BMI) measurement procedures

The weight of the breast cancer women patients was measured using a standard balance, and the height was measured by using a height measuring device attached to the balance. BMI was then calculated from the body weight (kg) and height (meter) as follows: BMI = Weight (in kg) / (Height in m)^2 (Tambe *et al*., 2010). Using the WHO classification (WHO, 1997), four categories of BMI can be identified as follows: underweight, <18.5 kg/m^2; normal, >18.5–24.9 kg/ m^2; overweight, >25.0–29.9 kg/ m^2; and obese, >30 kg/ m^2.

3.10. Data quality control and management

- In order to assure quality, data was collected by four oncology nurses who have basic knowledge on cancer therapy care services.
- The data collection questionnaire was well prepared and all variables were filled on the data extraction format daily.
- Most laboratory procedures were handled by professional laboratory technologists but some of them were handled by the investigator.
- All the tests were standardized and fully-automated.

3.11. Data processing and analysis

After checking for completeness and cleaning, the data obtained from laboratory analyses of the blood samples and questionnaires were performed by coded and entered into SPSS version 23 package and the different variables were tested and analyzed. Simple descriptive statistics was used to present the socio-demographic and clinical characteristics of the study subjects; and continuous variables were presented as mean ± standard deviation and were compared using inferential statistics (student t-test and One-way ANOVA) to test the significance of mean differences between and among groups respectively. Other associations were performed with Pearson's correlation coefficient. When P-value is less than 0.05, the difference was considered statistically significant, and the difference was considered highly significant when P-value is less than 0.001.

3.12. Ethical consideration

Before starting data collection and preliminary study, ethical clearance letter with reference number SOM/DRERC/BCHM061/2009 was obtained from the Departmental Research and Ethics Review Committee, Department of Biochemistry, College of Health Sciences, Addis Ababa University. Then a letter informing the department of hormonal therapy about the study was written from ethical Committee of department of Medical biochemistry and permission was obtained from department of hormonal therapy unit to access data from study population. The objective of the study was briefly clarified and explained for each participant, before enrolling any of the eligible study participants. Samples and data were collected after informed consent had been obtained from the study participants. Confidentiality, anonymity, neutrality, accountability and academic honesty was maintained throughout the study, for example, by using codes. The findings of the study will be disseminated for health care professionals and other concerned bodies for better care of breast cancer patients.

4. RESULTS

4.1. Socio-demographic and clinical characteristics of study participants

The present study enrolled a total of 103 breast cancer female patients, of which 52 (50.5%) were receiving tamoxifen as hormonal therapy for 3 months and above duration, and 51 (49.5%) were new medically diagnosed breast cancer female patients who did not start any cancer therapy. The average age of tamoxifen treated and new medically diagnosed breast cancer women was 42.48 and 46.53 years respectively with the standard deviations (SD) of 10.82 and 12.43 years ranging from 26 to 78 and 28 to 76 years respectively. The majority of the breast cancer female patients were found within the age group of 31-50 years (**Figure 2**).

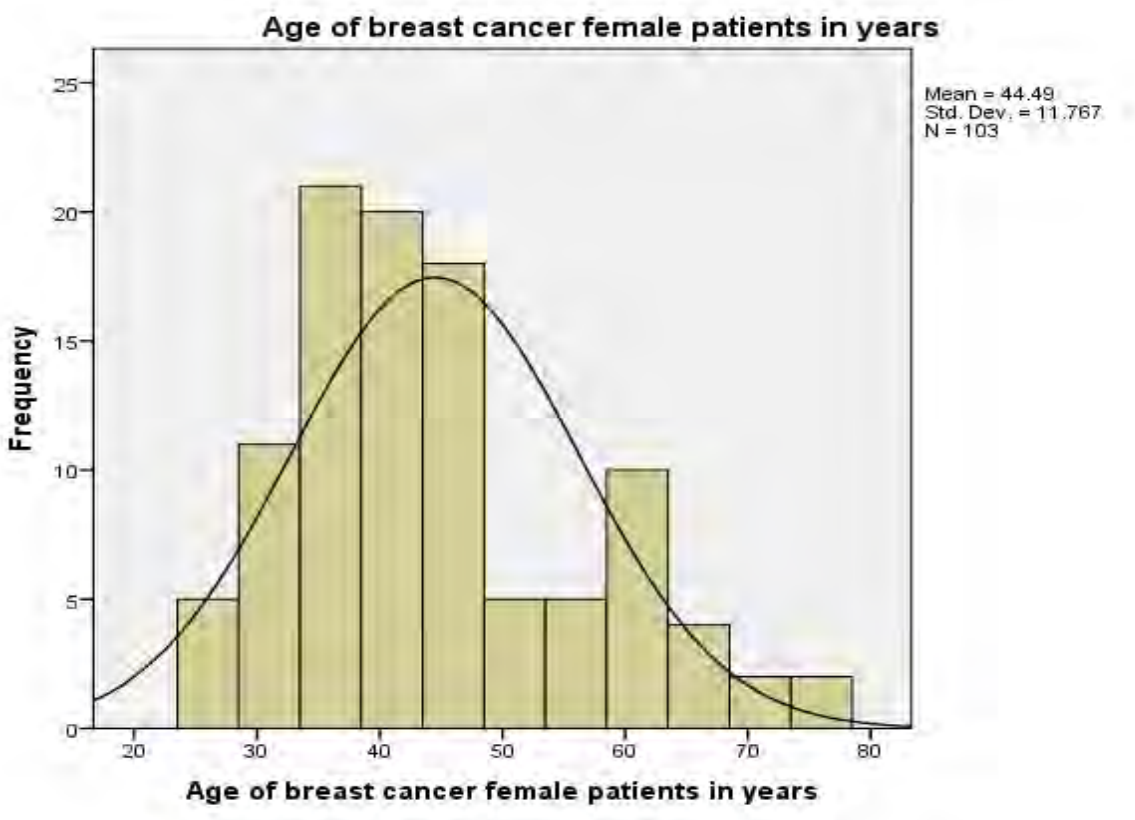

Figure 2: Age distribution of the breast cancer female patients in years following at TASH, Ethiopia

The study also showed that the majority of the study participants were found in Addis Ababa and Oromia region, 45.6% and 34%, respectively. Most of the breast cancer female patients in the

study were married (77.7%). While all the study participants had no history of alcohol drinking, and cigarette smoking behavior (**Table 1**).

About 16.5% of study participants were found to have family history of breast cancer. Of the study participants, 60.2% were premenopausal. Among breast cancer women receiving tamoxifen, 67.3% were premenopausal, while 47.1% were postmenopausal among those newly diagnosed group. About 54.8% of tamoxifen treated breast cancer women patients were in clinical stage I and II and the rest (45.2%) were in stage III and IV. In newly diagnosed group, 45.2% were in clinical stage I and II but the rest 54.8% were in stage III and IV (**Table 1**).

Table 1. Socio-demographic and clinical characteristics of newly diagnosed and tamoxifen treated breast cancer female patients at TASH, Ethiopia

Characteristics	Groups	Treated cases (n=52)	Untreated cases (n=51)	N=103
Age [a]		42.48±10.82	46.53±12.43	44.49±11.77
Region [b]	Addis Ababa	42.3	49.0	45.6
	Oromia	32.7	35.3	34.0
	Amhara	9.6	5.9	7.8
	Tigray	3.8	0	1.9
	SNNP	11.5	9.8	10.5
Marital status [b]	Married	88.5	66.7	77.7
	Single	5.8	5.9	5.8
	Divorced	3.8	9.8	6.8
	Widowed	1.9	17.6	9.7
Alcohol consumption [b]	Yes	0	0	0
	No	100	100	100
Cigarette smoking [b]	Yes	0	0	0
	No	100	100	100
Family history of breast cancer [b]	Yes	21.2	11.8	16.5
	No	78.8	88.2	83.5

Menopausal status [b]	premenopausal		67.3	52.9	60.2
	Postmenopausal		32.7	47.1	39.8
Clinical stage [b]	Stage I and II		54.8	45.2	50.7
	Stage III and IV		45.2	54.8	49.3
Height (m) [a]			1.59±.07	1.60±.06	1.60 ± .06
Weight (Kg) [a]			57.33±8.90	61.13±12.25	59.21±10.81
BMI (in kg/m^2) [a]			22.67±3.57	23.85±4.71	23.26±4.20
BMI- categories [b]	Underweight		5.8	3.9	4.9
	Normal		67.3	66.7	67.0
	Overweight		23.1	15.7	19.4
	Obese		3.8	13.7	8.7
Surgery [b]	Yes	mastectomy	55.8		
		conservative	11.5		
	No		32.7		
Radiation [b]	Yes		23.1		
	No		76.9		
Chemotherapy [b]	Yes	adjuvant	23.1		
		Neoadjuvant	11.5		
		palliative	17.3		
	No		48.1		
Duration of tamoxifen treatment in months [b]	3-6		36.5		
	7-12		30.8		
	13-24		17.3		
	25-48		15.4		

[a]Age, continuous variable, is expressed as mean ± standard deviation; [b]for the rest of the variables, qualitative, the numbers are in percent out of the total (52,51 and 103) patients.

The study revealed that the average BMI is normal (23.26 kg/m^2) in the study participants. However, about 23.1% and 15.7% of the cases with and without tamoxifen treatment respectively were overweight. And also, among the tamoxifen treated patients, 3.8% were obese and 13.7% of the cases without treatment were obese (**see Table 1**).

In addition, about 29 (55.5%) and 6 (11.5) cases obtained mastectomy and conservative surgical treatment respectively before the beginning of tamoxifen therapy. About 12 (23.1%), 12(23.1%) and 6(11.5%) of the cases were received radiotherapy, adjuvant and neo-adjuvant chemotherapy respectively prior to tamoxifen treatment. Furthermore, most of the tamoxifen treated breast cancer female patients, 36.5 %, and 30.8 %, were found to have duration of treatment for 3-6 and 7-12 months respectively, and some of them (17.3 % and 15.4%) were found to have treatment for 13-24 and 25-48 months respectively as shown in the **Table 1**.

4.2. Levels of lipid panels in the breast cancer female patients

The mean levels of lipid profiles in new newly diagnosed and tamoxifen treated breast cancer female patients are shown in **Table 2**. The result of this study showed that the average serum TC levels of newly diagnosed untreated and tamoxifen treated breast cancer female patients were 234.75±63.67 mg/dl and 200.65±50.19 mg/dl, and the levels of LDL-C were found to be 157.67± 57.28 mg/dl and 120.93±40.83 mg/dl respectively. In addition, our result showed that in newly diagnosed untreated and tamoxifen treated breast cancer female patients, the average TG levels were 157.35 ±73.52 mg/dl and 146.60±77.43 mg/dl, the levels of HDL-C were 45.61 ± 16.41 mg/dl and 50.40±20.15 mg/dl respectively (**see Table 2**).

Independent samples t-test showed that the TC and LDL-C levels were significantly higher ($p<0.05$) in breast cancer female patients who received no therapy than breast cancer female patients who received tamoxifen treatment. However, serum levels of TG and HDL-C were higher and lower respectively but not significant ($p<0.05$) in newly diagnosed breast cancer female patients than breast cancer female patients treated with tamoxifen (**see Table 2**).

Table 2. Levels of lipid profiles in newly diagnosed and tamoxifen treated breast cancer female patients attending oncology clinic at TASH, Ethiopia

Variables	Untreated cases (n=51)	Treated cases (n=52)	P-value
TC (mg/dl)	234.75±63.67	200.65±50.19^{+}	.003
TG (mg/dl)	157.35 ±73.52	146.60±77.43	.471
LDL-C (mg/dl)	157.67± 57.28	120.93±40.83^{++}	.000
HDL-C (mg/dl)	45.61 ± 16.41	50.40±20.15	.189

Values are expressed as mean ± standard deviation

Among the 103 breast cancer female patients, only 21 (20.4 %) had desirable level of LDL-C which is below 100 mg /dl (the cut-off value for the metabolite). Nonetheless, the rest of the patients 82(79.6 %) had undesired level of LDL-C i.e., greater than 100 mg /dl. On the other hand, while only 38 (36.9 %) of the breast cancer female patients showed undesired level of HDL-C, in most of the patients (63.1 %) levels of serum HDL-C were found to be with in normal range which is above 40 mg/dl **(see Figure 3).**

In the whole group of breast cancer female patients, 42(40.8 %) had normal serum TC level which is below 200 mg /dl, (the cut-off level for the metabolite). But the remaining patients 61(59.2%) had abnormal level of TC (greater than 200 mg /dl). Whereas 81 (78.6 %) of the breast cancer female patients had desirable level of serum TG, 22 (21.4%) patients had abnormally high serum TG level which is above 200mg/dl. In general, the tamoxifen treated female breast cancer patients had a better lipid profile than the non-treated breast cancer patients (**see Figure 3 below**).

Table 3. Normal and abnormal levels of lipid profiles expressed in percent (%) in a total of 103 breast cancer female patients attending oncology clinic at TASH, Ethiopia

Variables	Cut points	Untreated cases (n=51)	Treated cases (n=52)	N=103
TC (mg/dl)	<200	17.5	23.3	40.8
	≥200	32.0	27.2	59.2
TG (mg/dl)	<200	37.9	40.8	78.6
	≥200	11.7	9.7	21.4
HDL-C (mg/dl)	≥40	30.1	33.0	63.1
	<40	19.4	17.5	36.9
LDL-C (mg/dl)	<100	5.8	14.6	20.4
	≥100	43.7	35.9	79.6

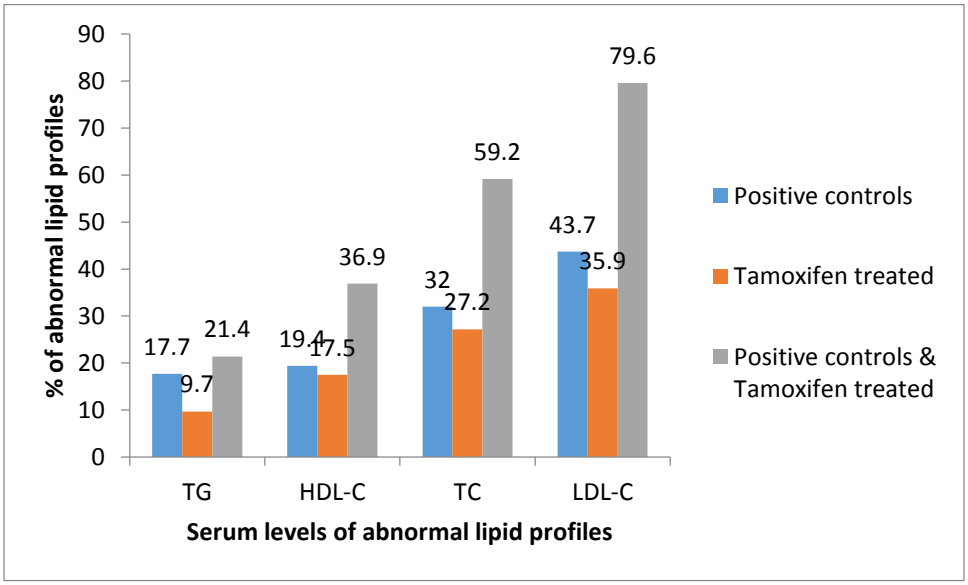

Figure 3. Newly diagnosed and tamoxifen treated breast cancer female patients having abnormal levels of lipid profiles expressed in percent out of total number of patients (n=103) at TASH, Ethiopia

The comparison of lipid profiles between pre-and post-menopausal tamoxifen treated breast cancer patients are shown in the **Table 4**. The levels of TC, TG, HDL-C and LDL-C (mean ± SD) in premenopausal cases with tamoxifen treatment were 205.31±46.22, 146.63±75.65, 48.91±18.97 and 127.07±37.75 while in the postmenopausal group their levels were 191.06±57.83, 146.52±83.34, 53.47±22.69 and 108.28±45.09 respectively (**see Table 4**). The independent samples t-test showed that there is no mean significant differences in serum lipid profiles including BMI between premenopausal and post-menopausal breast cancer patients with tamoxifen therapy ($p>0.05$).

Table 4. Comparison of BMI and lipid values between pre- and post-menopausal tamoxifen treated breast cancer women patients

Parameters	Cases on tamoxifen treatment (n=52)		
	Premenopausal (n=17)	Postmenopausal (n=35)	p-value
BMI (Kg/m2)	22.97±3.79	22.06±3.08	.396
TC (mg/dl)	205.31±46.22	191.06±57.83	.342
TG (mg/dl)	146.63±75.65	146.52±83.34	.997
HDL-C (mg/dl)	48.91±18.97	53.47±22.69	.450
LDL-C (mg/dl)	127.07±37.75	108.28±45.09	.121

Values are expressed as mean ± standard deviation

4.3. BMI, duration of treatment, clinical features, and the dependent variables

In both cases of with and without treatment, one-way ANOVA with Tukey post hoc test showed that there was no a statistical significant variation in lipid profiles ($p>0.05$) among the different clinical stages of breast cancer. In female breast cancer patients receiving tamoxifen, correlation analyses showed that age was not correlated with serum lipid profiles. However, bivariate, Pearson correlation, analyses showed that age was positively correlated with serum, LDL-C ($r = 0.377$, $p < 0.05$); TC ($r = 0.355$, $p < 0.05$), TG ($r = 0.258$, $p >0.05$, insignificant) and negatively correlated with serum HDL-C ($r = -0.169$, $p > 0.05$) in newly diagnosed breast cancer women.

In the tamoxifen treated women, there was no association between BMI and the lipid profiles. On the other hand, in newly diagnosed breast cancer women, BMI had statistically significant positive correlation with the serum levels of TC ($r = 0.361$, $p < 0.01$) and LDL-C ($r = 0.333$, $p < 0.05$). In addition, there was insignificant positive correlation between BMI and TG ($r = 0.226$, $p >0.05$) levels but no association was observed between BMI and HDL-C levels.

Similar results were observed using One-way ANOVA with Tukey post hoc test which showed that there was significantly higher serum TC and LDL-C levels in the obese newly diagnosed groups as compared to those who were underweight and normal ($p<0.05$) (**see Figure 4& 5**). The mean serum levels of the former and the later were 294.14 mg/dl and 207.20 mg/dl respectively. In addition, 236.88 mg/dl and 155.13 mg/dl were the average serum TC and LDL-C levels respectively among overweight newly diagnosed breast cancer female patients.

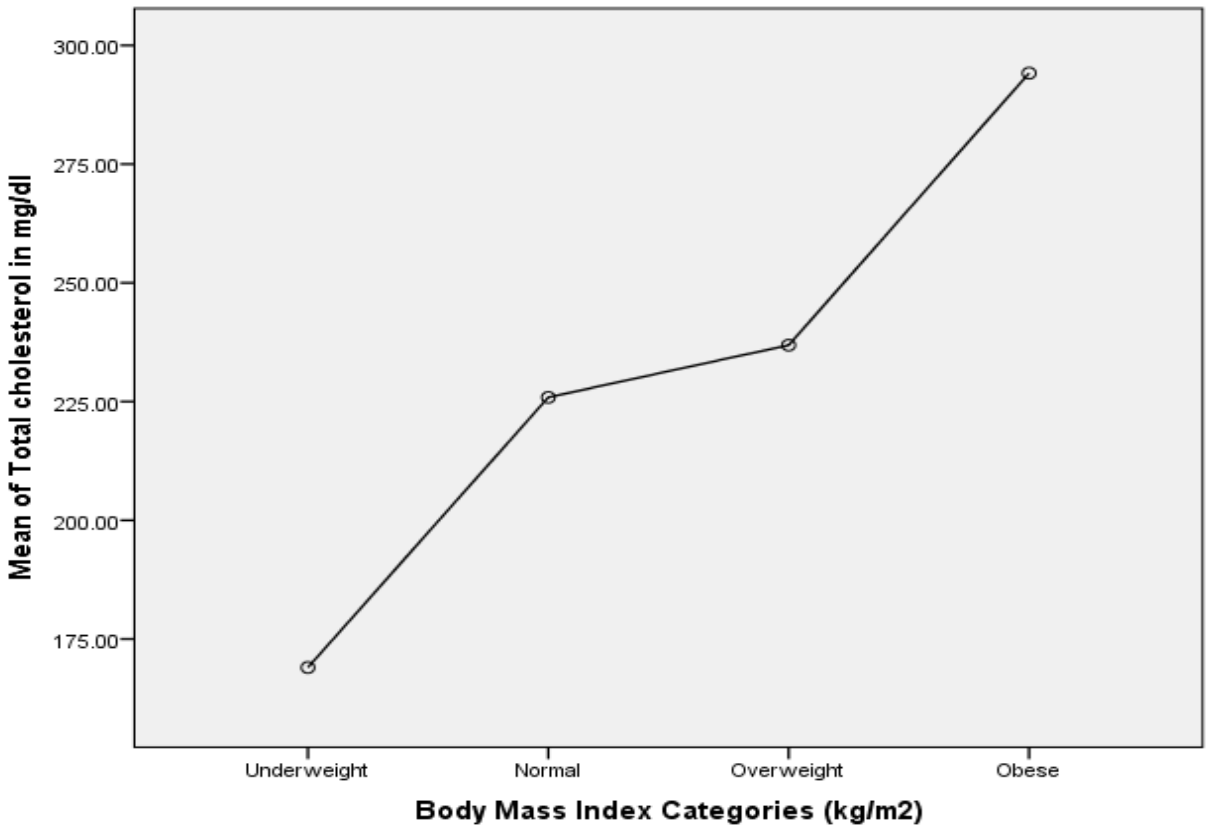

Figure 4. The mean plot of TC (mg/dl) in new medically diagnosed breast cancer women with respect to their BMI (kg/m^2) categories

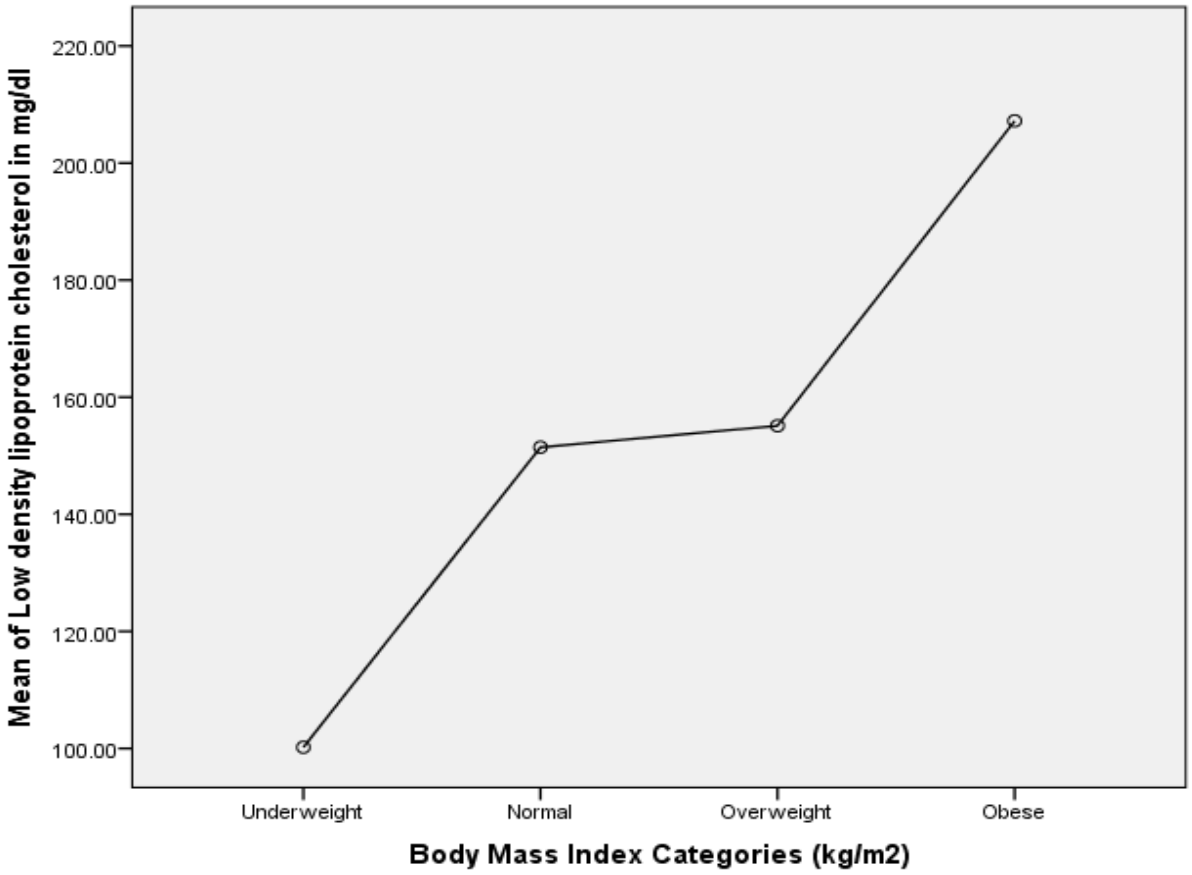

Figure 5. The mean plot of LDL-C (mg/dl) in new medically diagnosed breast cancer women with respect to their BMI (kg/m^2) categories

One-way ANOVA with Tukey post hoc test also showed that in breast cancer females patients receiving tamoxifen for 3-6 months, there was significant reduction in total cholesterol and LDL-C levels as compared to those who had followed the care for 7-12 and 13-24 months ($p < 0.05$) (**Figure. 6&7**). The peak reduction in both TC and LDL-C values occurred from 3-6 months. But duration of tamoxifen treatment had no significant effect on serum TG and HDL-C levels.

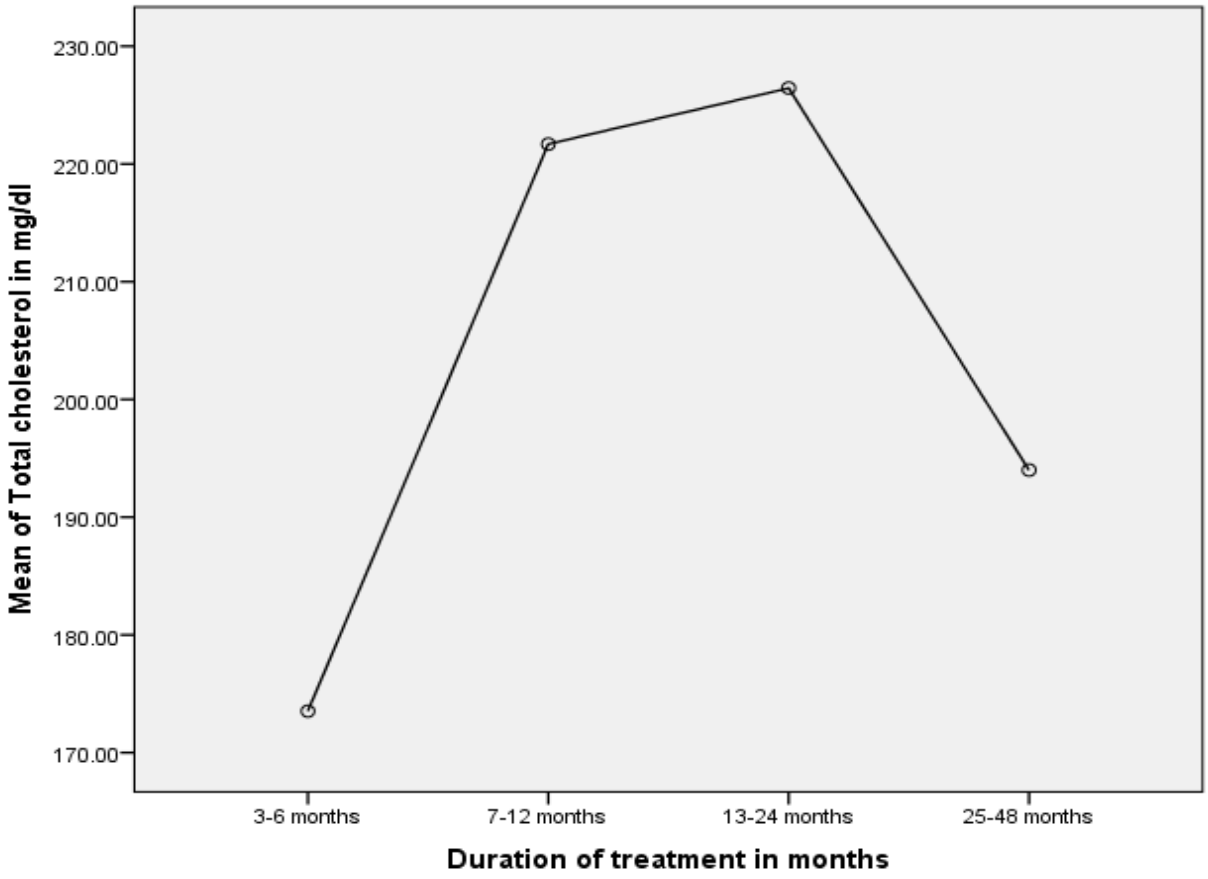

Figure 6. The mean plot of TC (mg/dl) in breast cancer female patients with tamoxifen therapy with respect to their duration of treatment in months

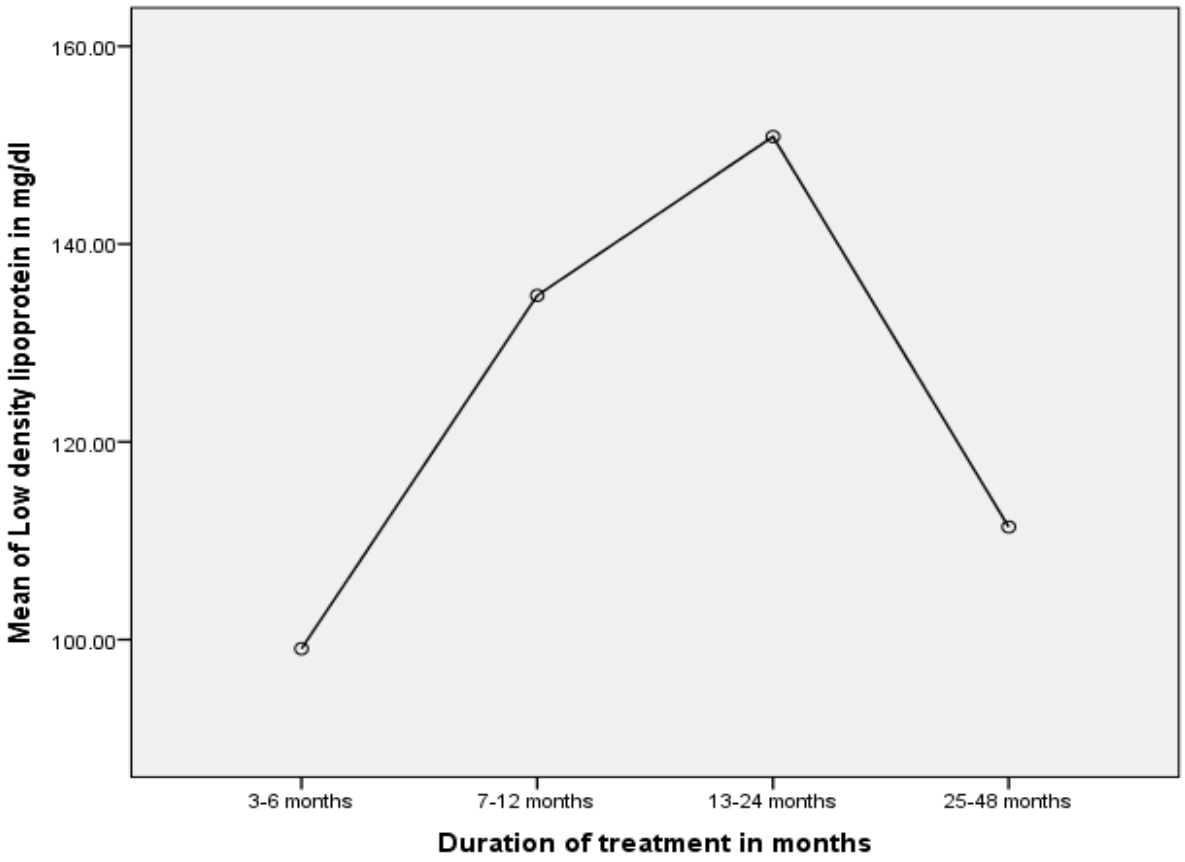

Figure 7. The mean plot of LDL- C (mg/dl) in breast cancer female patients with tamoxifen therapy with respect to their duration of treatment in months

5. DISCUSSION

A total of 103 breast cancer female patients were involved in this study at Tikur Anbesa Specialized hospital, Ethiopia. The study focused on evaluation and examination of the serum lipid parameters (LDL-C, HDL-C, TC and TG) in 51 new medically diagnosed and 52 tamoxifen treated breast cancer female patients. The mean age of the breast cancer female patients was 44.49 years and the majority of them were found within the age group of 31-50 years (64%). As opposed to the west where breast cancer among women is usually common after menopause, but here in Ethiopia most of the breast cancer patients appear to have the disease in their premenopausal ages. This requires a further investigation and a protracted definitive study to find out the reason and cause.

The result of this study revealed, that in newly diagnosed women with breast cancer the average levels of serum TC and LDL-C were significantly higher whereas the mean TG and HDL-cholesterol levels were found within their respective normal ranges compared to tamoxifen treated women.. This indicates that new medically diagnosed untreated breast cancer women had a deranged TC, LDL-C and TG levels as compared to those women receiving tamoxifen. This implies that tamoxifen, as a treatment strategy, tends to control lipid profile among female breast cancer patients.

Cancer as a disease seems to disturb lipid metabolism and alters the normal lipid profiles. This could be because cancer cells mainly depend on glycolysis to fulfill their energy demand. The carbon skeletons of amino acids obtained from degradation of proteins is used for gluconeogenesis that constantly supplies the glucose for energy production in cancer cells. Moreover, pentose shunt is also undergone at faster rate in cancer cells which produces NADPH for reductive biosynthetic processes of cholesterol, fatty acid, TG and nucleotide biosynthesis. This could be the main reason why the newly diagnosed breast cancer patients have higher TC, LDL-C and TG than the tamoxifen treated patients. Cancer cells abnormally proliferate and hence undergo uncontrolled cell division, which in turn requires reductive biosynthesis at the different phases of the cell division cycle. The obese breast cancer females had a higher of TC, LDL-C and TG levels than the patients with normal BMI and moreover the nontreated newly diagnosed breast cancer patients had also a higher TC and TG level than the tamoxifen treated breast cancer patients. This clearly shows that obesity, which is a result of abnormal deposition of fats is again a result of abnormal

synthesis and deposition of fats during cancer. This condition of dyslipidemia aggravates the disease and could lead to complications of the disease. Tamoxifen acts as estrogen antagonist in breast tissue and blocks the effects of estrogen like lipogenesis.

Epidemiologic studies have reported that elevated LDL-C and TG levels, and reduced HDL-C levels are important risk factors for developing CVD (Gordon *et al*., 1977 and Iso *et al*., 2001). Overall, a 1 mg/dL increase in HDL-C decreases the risk of CHD by 2-3% (Gordon et al., 1989). A 10 mg/dL increase in LDL-C increases the risk of CVD by 12% (Howard *et al*., 2000).

The present cross-sectional study showed that the effect of tamoxifen as a hormonal therapy in females with breast cancer produced a significant decrease in serum LDL-C (an important risk factor for CHD) compared with newly diagnosed breast cancer female patients who were not receiving any cancer therapy. The results demonstrated that serum LDL-C levels were reduced by tamoxifen, in agreement with the results of previous studies conducted in different parts of the world (Rossner and Wallgren, 1984; Liu and Yang, 2003; Gupta *et al*., 2006; Lin *et al*., 2014 and Morad *et at*., 2016). This may be due to its partial estrogenic activity, since estrogen is known to reduce serum LDL-C levels by increasing LDL particle clearance through LDL receptor up regulation (Windler *et al*., 1980). The results of this study were in contrast with previous studies (Caleffi et al., 1988 and Sharma et al., 2001)), which reported that the serum LDL-C levels remained unchanged after tamoxifen treatment.

Our finding showed that LDL-C levels were significantly increased in newly diagnosed breast cancer women compared with those who were receiving tamoxifen. This elevated serum LDL-C, which is more susceptible to oxidation, may result in high lipid peroxidation in breast cancer patients. This may cause oxidative stress leading to cellular and molecular damage thereby resulting in cell proliferation and malignant conversion, which is in agreement with the work of (Rhaman, 2007).

In addition, this study showed that there was a statistically significant reduction in serum TC (another known risk factor for CVD) levels in breast cancer women receiving tamoxifen compared with newly diagnosed breast cancer women with no therapy. The reduction in TC levels may be due to anti-lipidemic effects of tamoxifen treatment and this is in line with other previous studies

(Gupta *et al*., 2006 and Morad et *at*., 2016). However, in another study, it was reported that the reason for reduction in serum TC levels was shown to be mainly a result of decreased levels of LDL-C (Rossner and Wallgren, 1984). The results of this study are in contrast with that of previous studies (Caleffi et al., 1988; Hozumi *et al*., 1999 and Sharma et al., 2001), which reported that the serum TC levels remained unchanged after tamoxifen treatment

The TC levels were significantly higher in newly diagnosed breast cancer women compared with those who were receiving tamoxifen. Some authors suggested that an increased serum TC level may play significant role in carcinogenesis (Ray and Husain, 2001 and Yadav *et al*., 2012). Cholesterol itself is not carcinogenic, rather a metabolite of cholesterol called hydroxycholesterol (27HC) (Mcdonnell *et al*., 2014) that mimics the hormone estrogen and can independently drive the growth of breast cancer may have such effect.

In previous studies on newly diagnosed breast cancer patients, several reports have shown that tumor progression from localized to metastatic disease is associated with declining HDL-C levels (Knapp *et al*., 1991 and Kokoglu *et al*., 1994). The decreased levels of HDL-C have been reported to be associated with increased levels of cytokines (Navab *et al*., 2004 and Peng *et al*., 2010), which have been shown to be related to both obesity and breast cancer. In this study, insignificant moderate increase in serum HDL-C levels were observed in breast cancer women with tamoxifen treatment as compared to those who were not receiving cancer therapy. This might be due to tamoxifen's estrogenic activity on serum HDL-C levels. Similar findings have been reported by numerous authors (Decensi et al., 1999; Gupta *et al*., 2006; Tominaga *et al*., 2010; and Morad *et al*., 2016). These observations suggest that HDL-C might play a key role for the prevention of CVD in breast cancer patients. HDL-C is known to play a central role in reverse cholesterol transport and to have antioxidant, anti-inflammatory and anti-thrombotic effects. These roles of HDL-C have been studied both epidemiologically and clinically, and it has been shown that there is an inverse correlation between the HDL-C level and the risk of coronary artery disease (Frick *et al*, 1984). The slight increase in HDL-C in the tamoxifen treated group in our study indicates that the drug has a protective effect of excess cholesterol accumulation in the vasculature and plays anti-atherogenic role.

Elevated levels of triglycerides are associated with a decreased level of sex-hormone-binding globulins, resulting in increased amounts of free estradiol and increased breast cancer risk

(Kakaiya *et al*., 2013). The previous study findings were inconsistent in serum TG levels upon tamoxifen treatment in breast cancer patients. Thus, in the study conducted in Iraq by Morad et al. (2016) reported that serum TG levels were significantly decreased in breast cancer women receiving tamoxifen. In our study finding, breast cancer female patients receiving tamoxifen as a hormonal therapy had an insignificantly decreased TG level, in line with (Gupta al., 2016). This suggests that tamoxifen treatment may decrease serum TG levels in breast cancer patients due to its antiestrogen effect, perhaps via inhibition of transcription of genes that are activated by the action of estrogen by attaching to estrogen receptors. Lipolysis is activated during many cancer cases mainly due to increases biosynthesis and release of cytokines by the adipose tissue.

In contrast, in other related previous studies, tamoxifen significantly increased serum TG levels (Sharma et al., 2001; Liu and Yang, 2003 and Tominaga *et al*., 2010). This may be due to its partial estrogenic activity, as it is well established that estrogen induces hypertriglyceridemia through its multiple effects on lipid metabolism, including increased synthesis of TG and decreased HTGL activity. HTGL participates in the catabolism of TG rich lipoprotein and the lowering of enzyme activity is accompanied by an increase in serum TG levels (Sawada *et al*., 2009). In this way, tamoxifen is considered to exhibit a strong estrogen agonistic effect on lipoprotein metabolism.

Tamoxifen therapy has convincingly demonstrated benefit for patients with ER+ breast cancer, regardless of age, lymph node status, or menopausal status (Bryant *et al*., 2001). In the present study, the comparative effects of tamoxifen on serum lipid profile were evaluated and compared between pre and post-menopausal women with breast cancer. However, among breast cancer female patients treated with tamoxifen there was no a statistically significant difference in mean serum levels of lipid profiles between premenopausal and postmenopausal patients, which implies that it has equivalent medical uses regardless of menopausal status. In addition, menopausal status can be directly related with age. This could be explained in such a way that among breast cancer women on tamoxifen treatment, there was no association between age and lipid profiles suggesting that it can be used for both premenopausal and postmenopausal women with breast cancer with similar effects on lipid profiles.

However, the levels of LDL-C has shown to be more lowered (despite the reduction is not significant) in postmenopausal women than in premenopausal women. This is in line with the results of related studies (Gupta *et al*., 2006 and Powles *et al*, 1990)). The latter reported that

among healthy women with higher risk for breast cancer treated with tamoxifen as primary prophylaxis, the reduction of LDL-C appears to be larger in postmenopausal women.

On the other hand, unlike breast cancer female patients receiving tamoxifen, Bivariate, Pearson correlation, analyses showed that age positively correlated with serum, LDL-C ($r = 0.377$, $p < 0.05$); TC ($r = 0.355$, $p < 0.05$), TG ($r = 0.258$, $p > 0.05$) and negatively correlated with serum HDL-C ($r = -0.169$, $p > 0.05$) in newly diagnosed breast cancer women with no therapy. This finding is in corroboration with previous studies (Bhat *et al*., 2012 and Laisupasin *et al*., 2013).

The duration of tamoxifen treatment had no a statistically significant effect on serum TG and HDL-C levels but in breast cancer females patients receiving tamoxifen for 3-6 months, there was significant reduction in TC and LDL-C levels as compared to those who had followed the care for 7-12 and 13-24 months ($p < 0.05$) .The peak reduction in both TC and LDL-C values occurred from 3-6 months, in compatible with a previous study conducted by Gupta et al. (2006) who reported that a peak decrease was achieved at 6 months. On the contrary, a peak reduction of these parameters occurred at 18 months in another study (Tominaga *et al*., 2010). Therefore, tamoxifen shows its effect on lipid profile in breast cancer patients after a prolonged administration of the drug. No wonder that the drug is given as prophylaxis for about 5 – 10 years in individuals who have a family history of breast cancer.

5.1. BMI, clinical features and the dependent variables

Anthropometric indicators are related with different pathological conditions. Generally, BMI is widely used as indicator to predict obesity. It has been hypothesized that the adult weight gain or increased BMI is a strong predictor of breast cancer risk. Particularly, obesity increases the risk of post-menopausal ER+ breast cancer by more than 50% according to Vrieling et al. (2010). This study tried to investigate the associations of BMI and lipid abnormalities in tamoxifen treated breast cancer women and those women patients with no therapy.

Concordant to the previous studies (Morad *et al*., 2016), the result of this study showed that there is no correlation between BMI and lipid profile in breast cancer women receiving tamoxifen as a hormonal therapy. In breast cancer patients with no therapy, BMI had significant positive correlation with serum TC and LDL-C levels and insignificant positive correlation with TG levels. This implies that there is an association between TC, LDL-C, BMI and breast cancer risk. This is in line with the findings of previous studies (McDonnell *et al*., 2014 and Mishra, 2015). But there was no association between BMI and HDL-C levels in both groups of patients.

Obese newly diagnosed breast cancer women patients had significantly higher serum TC and LDL-C levels (with the mean 294.14 mg/dl and 207.20 mg/dl respectively) as compared to those who had normal BMI. In addition, 236.88 mg/dl and 155.13 mg/dl were the average serum TC and LDL-C levels respectively in overweight newly diagnosed breast cancer women patients. This observations in relation to breast cancer risk could be explained as follows: Obese/overweight individuals are characterized by elevated levels of proinflammatory cytokines, insulin, bioavailable insulin-like growth factor-1 (IGF-1) and leptin which stimulate breast tumor cell proliferation through several signaling pathways.

6. CONCLUSIONS

The findings in this study confirm that serum lipid profile was significantly improved in breast cancer female patients who received treatment with tamoxufen when compared with new medically diagnosed breast cancer female patients before therapy. This improvement was in terms of significant reduction in TC, LDL-C and TG (despite the reduction was not significant) accompanied by the elevation of HDL-C though it was insignificant. Serum TC and LDL-C levels were significantly higher in overweight and obese breast cancer women with no therapy. Obesity is reflected with increased lipid biosynthesis and increased serum lipid profile and hence it predisposes women to breast cancer. However, Tamoxifen through its complex effects on lipid metabolism protects lipid abnormalities and also has antioxidant role, which avoids oxidative stress that may lead to cancer complications.

7. STRENGTHS AND LIMITATIONS OF THE STUDY

The study can express its strength that it includes some demographic, clinical and BMI claimed to be associated with the variables under study. In addition, the anthropometric indicators, height and weight, are measured directly than by self-report. Despite the aforementioned strengths, this study has several weaknesses. First, because the sample size was small, it may be difficult to represent the whole breast cancer female patients in the population. Lack of previous study findings in Ethiopia for comparison is also another limitation. Life style, nutritional factors and dietary habits, waist circumference as well as waist to hip ratio were not taken into consideration in this study. Finally, the study is cross-sectional, but the ideal would be prospective, allowing patients to be followed over years. This means that cause-and-effect relationships cannot be definitively ascertained, but associations can be evaluated.

8. RECOMMENDATIONS

The following recommendations are suggested to further investigate and evaluate lipid profiles in breast cancer patients:

- Further studies could be conducted with larger sample size using prospective study design to see the effect of tamoxifen on lipid profiles along with estradiol hormone in breast cancer patients.
- Life style, nutritional factors and dietary habits should be assessed in further studies.
- There should be timely evaluation of lipid profile of the breast cancer patients and prescription of drugs whenever indicated so as to prevent CVD complications.
- Although BMI is widely used indicator to reflect obesity generally, it fails to account the proportion of weight related to muscle mass or regional distribution of excess fat in the body. Individuals having same BMI may significantly vary in their abdominal fat distribution or mass. For these reason, a measure of waist circumference and waist to hip ratio should be included in future studies.

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ANNEXES

Annex 1: Information sheet (English Version)

Research Project: Evaluation and comparison of serum lipid profiles between newly diagnosed and tamoxifen treated breast cancer women attending the oncology clinic at TASH, Addis Ababa, Ethiopia

Sponsoring organization: Department of Biochemistry, School of graduate studies,
College of Health Sciences, Addis Ababa University

Principal Investigator: Bihonegn Birhan (BSc in Biology, MSc in Medical Biochemistry candidate)

Advisors: Solomon Genetu (PhD),

Introduction

Dear the participants you are kindly requested to take part in this research project as a study participant voluntarily. Read the information provided in this sheet carefully and then respond freely and voluntarily to what the investigator interviews you.

Objective of the research project

This information sheet is prepared by the investigator and the advisors at AAU for a project with the objective of evaluation and comparison of serum lipid profiles between new medically diagnosed and tamoxifen treated breast cancer participants.

Procedure

If you agree to take part in the study, the investigator or a health worker will give you verbal and/or written information about the study and you will be given the consent form to sign, the physician or health professional will ask you some questions about your general health and perform a complete medical examination and assess whether you qualify to participate in the study. If you are fit for the study about 5 ml of blood samples will also be collected for only the laboratory examination of HDL-C, LDL-C, TC, TG and face to face interview for additional questions.

Discomforts and risks and benefits from participation

The degree of discomfort you may encounter in giving the sample is no more than when one does in his/her routine examination. But, there could be cases in which minor pain and change in color of your skin following the blood drawing occur transiently. The blood will be withdrawn by licensed health care professionals (nurses) in the hospital and appropriate care will also be taken.

You will not be provided with any direct incentives for your participation in the research. But the cost for general medical examination will be covered by the project. In addition, based on the results obtained from the research you will be cared accordingly or the results may serve you as a baseline data. In addition, the result of the study will be beneficial for the better prevention and care of breast cancer patients than before. Hence, you are indirectly benefiting other patients and the society in this aspect.

Confidentiality

All pieces of information about the patients will be kept confidential. Log books used in the laboratory will have no names but codes. The information sheet that links the coded number to patient name will be locked inside a box and it will not be revealed to anyone except your physician and the principal investigator. You have full right to withdraw from participating in this study at any time before and after consent even without explaining the reason. Your decision will not affect your right to get health service you are supposed to get otherwise.

Contact information: If you have any questions contact: Bihonegn Birhan: 0939906102

Annex 2: Informed consent (English version)

Department of Biochemistry, School of graduate studies, College of Health Sciences,
Addis Ababa University, Consent form for the participation of the study participants in the research project

Name of the study participant ……

Code number…..……

I have clearly been informed about the research project that it aims to evaluate and correlate serum lipid panels among new medically diagnosed and tamoxifen treated breast cancer patients. The objectives of the research project have clearly been explained to me and I have been told that the results obtained from me will help me as well as the community for better management of the disease. I had been also informed about the confidentiality of this research project. Moreover, I have also been well informed of my right to keep hold of information, decline to cooperate and make myself withdraw from the study. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation result/s.

I_____ hereby give my consent for providing the requested information and blood sample as the doctors find best for me.

Signature: _____ Date_____

Annex 3: Questionnaire (English version)

Dear respondents, you are kindly requested to give correct information accordingly. Thank you for your time and participation.

I. Personal socio-demographic, anthropometric and clinical information

Card no._____

1. Age (in years) _____
2. Marital status: Single
   Married
   Divorced
   Widowed
3. Residential area: Addis Ababa
   Oromia
   Amhara
   Tigray
   SNNP
   Others
4. Height (m) _____
5. Weight (in Kg) _____
6. Body Mass Index (kg/ m2) _____
7. Alcohol consumption: Yes No

If yes then,

Types of alcohol	Quantity		
	Daily	Weekly	Monthly
Bottles of beer			
Glasses of wine			
Pints of hard liquor			

8. Smoking history
   a. Currently a smoker, specific pack/year _____
   b. If stop smoking, the number of packs smoked per year before quitting_____
   C. Never smoked
9. Family history of breast cancer Yes No
10. Menopausal status: premenopausal
    Postmenopausal
11. Radiation therapy: (No or Yes)
12. Surgery: (No or Yes). If yes a. Breast conserving b. Mastectomy
13. Chemotherapy: (Yes or no). If 'yes' a. Neo-adjuvant b. Adjuvant c. palliative
14. Duration of of tamoxifen treatment: 3-6 months
    7-12 months
    13-24 months
    >24 months

Annex 4: Information sheet (Amharic version)

የተሳታፊዎች የፈቃደኝነትና መተማመኛ መረጃ መስጫ ቅፅ በአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የሕክምና ባዮኬሚስትሪ ትምሕርት ክፍል፡ ጥናቱን ስፖንሰር ያደረገዉ ተቋም አዲስ አበባ ዩኒቨርሲቲ ጤና ሣይንስ ኮሌጅ ነው፡፡

መረጃ መስጫ ቅፅ

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ባዮኬሚስትሪ ት/ክፍል ሁለተኛ ዲግሪ ተማሪ የመመረቂያ ጥናት ጽሁፍ ላይ እዲሳተፉ ተጋብዘዋል፡፡ እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊትከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነልዎትን ማንኛዉንም ሃሳብ ይጠይቁ፡፡

Evaluation and comparison of serum lipid profiles between newly diagnosed and tamoxifen treated breast cancer women attending the oncology clinic at Tikur Anbesa Specialized Hospital የጥናቱ ርእስ ሲሆን አላማውም የጡት ካንሰር ያለባቸው ታካሚዎች በደማቸው ውስጥ ያለውን የቅባት መጠን እንዲሁም ሌሎች ከካንሰር ጋር ግንኙነት ያላቸውን ነገሮች መጠንና ሁኔታ መለካት ነው፡፡ የጥናቱ ውጤት ለታካሚው ብሎም ለሌላው ማህበረሰብ የሚጠቅምና የተሻለ የጤና እንክብካቤ እንዲኖር የሚያደርግ ነው፡፡እናም እርስዎ በዚህ ጥናት ለመሳተፍ ጠቃሚና ምቹ ሆነው ተመርጠዋል፡፡ የእርስዎ በዚህ ጥናት ላይ የሚያደርጉት ተሳትፎ ሙሉ በሙሉ በበጎ ፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡

በጥናቱ ከተሳተፉ ለናሙና ይሆን ዘንድ 5ሚሊ ሊትር ያህል ደም በሆስፒታሉ ጤና ባለሙያዎች የሚሰጡ ሲሆን የደም ናሙናውን በሚሰጡበትም ሰአት ሁልጊዜ ለምርመራ ከሚሰጡበት የተለየ ህመምና አለመመቸት የለውም ለምናልባት ቢኖር ተገቢውን የጤና እንክብካቤ የሚያገኙ ይሆናል፡፡ በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኃላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋን በዚህ ሆስፒታል የሚሰጠዎ ማንኛዉም አገልግሎት ላይ ተጽዕኖ የለውም፡፡ በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅበዎታል፡

ግልጽ ያልሆነልዎ ጥያቄ ካለ

ሞባል፡ 0939906102 ቢሆነኝ ብርሃን

Annex 5: Informed consent (Amharic version)

የተሳታፊዎች ስምምነት ማረጋገጫ ቅጽ

የሚስጥር ቁጥር ----------

የተሳታፊዉ ስም ----------

እኔ ስሜ ከላይ የተገለጸዉ ግለሰብ የተፈለኩት በዚህ ጥናት እንድሳተፍ ሲሆን የጡት ካንሰር ያለባቸው ታካሚዎች በደማቸው ውስጥ ያለውን የቅባት መጠንና እንዲሁም ከጡት ካንሰር ሌሎች ጋር ግንኙነት ያላቸውን ነገሮች መጠንና ሁኔታ መለካት የሚለው ጥናት አላማና ጥቅም ተገልፆልኛል፡፡ ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የምሰጠዉ በአጠቃላይ የጥናቱን አላማና ጥቅም በመረዳትና በፍጹም ፈቃደኝነት ነው፡፡ በመጠይቁ ላይ የምሰጠዉ የእኔ መረጃ እንደማይባኩን እንደሚያዝም ተነግሮኛል፡፡

በተጨማሪም ጥናቱ ዉስጥ ላለመሳተፍ ከፈለኩኝ መብቴ የተጠበቀ እንደሆነና በማንኛውም ጊዜ ከጥናቱ በራሴ ዉሳኔ መዉጣት ጭምር መብቴ መሆኑንና ከጥናቱ በመዉጣቴ ምንም አይነት ችግር እንደማይደርስብኝ በሚገባ ተገልጾልኛል፡፡ ስለሆነም ሁኔታዉን በሚገባ በማጤን በፈቃደኝነት በምርምሩ ላይ ለመሳተፍ ፈቃደኝነቴን ሰጥቻለሁ፡፡

በተጨማሪም የምሰጠዉ የደም ናሙና ለCholesterol, Triglycerides, HDL-C እና LDL-C ምርመራዎች ብቻ እንደሚዉል ተነግሮኝ ተስማምቻለሁ፡፡ ማንኛዉንም ያልገባኝን ነገር የመጠየቅ እድል ተሰጥቶኝ በሚገባኝ ቋንቋ መልስ አግኝቻለሁ፡፡

በተጨማሪም የሁሉም የላብራቶሪ ምርመራ ዉጤቶች በጊዜዉ ለህኪሜ እንደሚሰጥልኝ እና ዉጤቱን ማወቅ ከፈለኩ ማግኘት እንደምችል ተነግሮኛል፡፡ በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤአለሁ፡፡ ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ፡፡

እኔ_____የተባልኩት ግለሰብ ይህን ሁሉ በማገናዘብ በምርምሩ ላይ ስለኔ መረጃ እና የደም ናሙና ለመስጠት ተስማምቻለሁ፡፡

ፊርማ ቀን

ተሳታፊ _____ _____

Annex 6: Questionnaire (Amharic version)

መጠይቅ

ውድ ተሳታፊ ቀጥሎ ያለውን መጠይቅ ለመሙላት ስለተባበሩን እናመሰግናለን፡፡

ካርድ ቁጥር_____

1. እድሜ (በአመት) _____
2. የትዳር ሁኔታ: ያላገባ
   ያገባ
   የፈታ
   የሞተበት
3. መኖሪያ ቦታ፡ አዲስ አበባ
   ኦሮሚያ ክልል
   አማራ ክልል
   ትግራይ ክልል
   ደቡብ ብሄር ብሄረሰቦች ህዝቦች ክል
   ሌሎች
4. ቁመት (በሜትር)_____
5. ክብደት (በኪ.ግ.) _____
6. የሰዉነት ክብደት ልኬት (ኪ.ግ. / ሜ^2) _____
7. 13. አልኮል ይጠጣሉ? አዎ አልጠጣም

አዎ ካሉ፡

የአልኮሉ አይነት	የሚጠጡት የአልኮል ብዛት መጠን		
	በቀን	በሳምንት	በወር
ቢራ በጠርሙስ			
ወይን በብርጭቆ			
አስካሪ መጠጥ በመለኪያ			

8. ሲጋራ ያጨሳሉ: አዎ አላጨስም
9. የጡት ካንሰር ያለበት ዘመድ አለዎት፡ አዎ የለኝም
10. የወር አበባ ያያሉ? አዎ አላይም
11. የጨረር ህክምና ታክመዋል? አዎ አልታከምኩም
12. የጡት ቀዶ ጥገና ተደርጎልዎታል? አዎ አልታከምኩም
    መልስዎ አዎ ከሆነ ጡትወ ሙሉ በሙሉ ነዉ የተወገደ ወይስ በከፊል
13. የኬሞቴራፒ ህክምና ታክመዋል? አዎ አልታከምኩም
    አዎ ከሆነ መልስወ ፡
    ሀ. ከቀዶህክምና በፊት ነዉ
    ለ. ከቀዶህክምና በኃላ ነዉ
    ሐ. ወይስ መልክቱ ብቻ እንደታየ ነዉ
14. ታሞክሲፊን መዉሰድ ከጀመሩ ስንት ጊዜ ሆነዎት: ከ3-6 ወር
    ከ7-12 ወር
    ከ13-24 ወር
    ከ24 ወር በላይ