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Change in serum cardiac and inflammatory biomarkers level following chemotherapy treatment among female breast cancer patients attending at Tikur Anbessa Specialized Hospital

By

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

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Certificate

This is to certify that this MSc thesis prepared by Muluabay Getie, entitled as: *Change in serum cardiac and inflammatory biomarkers level following chemotherapy treatment among female breast cancer patients attending at Tikur Anbessa Specialized Hospital: A retrospective study at the oncology department of Tikur Anbessa Specialized Hospital, Ethiopia,2019* and Submitted in partial fulfillment of the requirements for the degree "Master of Science in Medical Biochemistry" in the department of medical Biochemistry complies with regulations of the university and meets the accepted standards with respect to originality and quality.

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Declaration

I declare that this research paper entitled: ***Change in serum cardiac and inflammatory biomarkers level following chemotherapy treatment among female breast cancer patients attending at Tikur Anbessa Specialized Hospital: A retrospective study at the oncology department of Tikur Anbessa Specialized Hospital, Ethiopia, 2019*** is my original work and has not been presented for any degree in any other university, and that all sources of materials used for the research have duly been acknowledged.

Muluabay Getie

Signature: _____

Date: _____

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ABREVIATIONS AND ACRONYMS

5 - FU	5-Fluoro Uracil
ACS	Acute Coronary Syndrome
ANT	Anthracyclines
BNP	Brain Natriuretic Peptide
CHD	Coronary Heart Disease
CK	Creatine Kinase
CK-MB	Creatine Kinase Myocardial Band
CRP	C-reactive protein
CVD	Cardio Vascular Disease
CY	Cyclophosphamide
DOX	Doxorubicin
ECG	Electrocardiographic
FAC	Fluorouracil Adriamycin Cyclophosphamide
Hs-CRP	High sensitive C - reactive protein
IL-6	Interliukin-6
LVD	Left Ventricular Dysfunction
TNF-α	Tumor necrosis factor alpha
TnI	Troponin I
TnT	Troponin T

ABSTRACT

Chemotherapy is long been recognized that a well- established therapeutic approach for several malignancies including breast cancer. But the clinical efficacy of this drug is often limited by its related cardio toxicity. The assessment of multiple cardiac biomarkers can be used in identifying patients at increased risk and adverse outcomes from chemotherapy. This approach has significant importance to predict which patients will have adverse cardiac effects from chemotherapy as well as treat damage at an earlier stage, preserve patient health, and improve quality of life.

Objective: The aim of the present study was to evaluate changes in cardiac and inflammatory biomarkers level following chemotherapy treatment among female breast cancer patients attending at Tikur Anbessa specialized hospital.

Materials and methods: Hospital based retrospective study was conducted in 40 female breast cancer patients at TASH. Elecsys 2010 Troponin-T immunoassay analyzer (to measure serum cTnT level) , Roch/hitachi cobas c 502 analyzer (to measure serum CKMB and CRP levels) and R and D systemic luminex performance assay(to measure serum IL-6 and TNF- α) were used in 40 paired breast cancer patient samples retrieved from a repository specimen of an ongoing thematic research project. Data were analyzed using SPSS software version 23 package and a p value < 0.05 was considered statistically significant.

Results: In the female breast cancer patients receiving chemotherapy treatment, the mean serum cTnT, CKMB, CRP and TNF-α levels were significantly increased than their respective baseline values. No significance difference was found in the level of IL-6 between the base line and during chemotherapy treatment. 20%, 15%, 14% of the breast cancer patients had elevated cTnT, CK-MB and CRP levels after the three cycles of chemotherapy treatment respectively and 15% of patients had a baseline elevated levels of CRP compared with their respective cutoff value.

Conclusion: Cardiac biomarkers are significantly increased up on chemotherapy treatment

Key words: chemotherapy, cardio toxicity, cardiac biomarkers, inflammatory markers

1. INTRODUCTION

1.1. Background

1.1.1. Overview **of cancer**

Cancer can be defined as a disease in which a group of abnormal cells grow uncontrollably by disregarding the normal rule of cell division. Normal cells are constantly subjects to signals that dictate whether the cell should divide, differentiate into another cell or die. But, cancer cells develop a degree of autonomy from these signals, resulting in uncontrolled growth and proliferation (Hejmadi, 2009). If this proliferation is allowed to continue and spread it can be lead to death (Torre *et al*., 2016).

The disease affects worldwide countries of all income (Mathers, 2008). According to the recent GLOBOCAN 2018 estimation of cancer incidence worldwide, there were an estimated 18.1 million new cancer cases and 9.6 million cancer deaths. One in 5 men and one in 6 women worldwide develop cancer during their lifetime, and one in 8 men and one in 11 women die from the disease. Worldwide the 5-year survival prevalence is estimated to be 43.8 million (Bray *et al*., 2018).

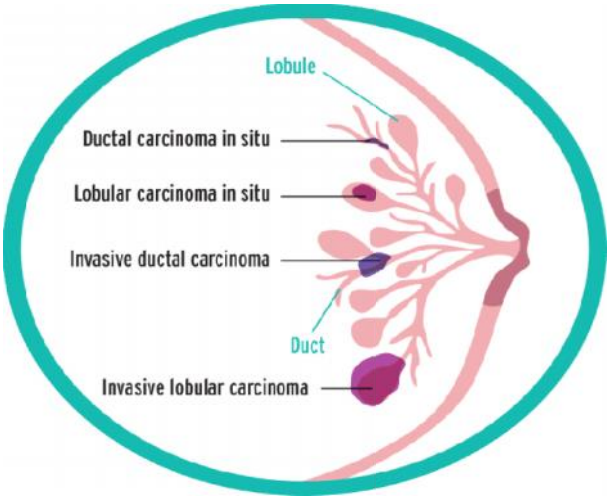
In both sexes combined, lung cancer is the most commonly diagnosed cancer (11.6% of the total cases) and the leading cause of cancer death (18.4% of the total cancer deaths), closely followed by female breast cancer (11.6%), prostate cancer (7.1%), and colorectal cancer (6.1%) for incidence and colorectal cancer (9.2%), stomach cancer (8.2%), and liver cancer (8.2%) for mortality (Bray *et al*., 2018).

In Africa, cancer is also an emerging public health problem with about 715,000 new cancer cases and 542,000 cancer deaths occurred in 2008 on the continent. These numbers are expected to double in the next 20 years. The increment of its burden in economically developing countries is mostly due to population aging and growth as well as, increasingly, an adoption of cancer-associated lifestyle choices including smoking, physical inactivity, and ‘‘westernized" diets (Jemal *et al*., 2011). The estimated number of new cases each year is expected to rise from 10 million in 2002 to 15 million by 2025, with 60% of those cases occurring in developing countries. Breast cancer in women and prostate cancer in men have now become the most commonly diagnosed cancers in many Sub-Saharan African countries, replacing cervical and liver cancers(Jemal *et al*., 2012)

According to the report obtained from an estimate of cancer incidence in Ethiopia in 2015 using population-based registry data conducted at Tikur Anbessa Specialized Hospital, 21,563 males and 42,722 female's incident cancer cases were diagnosed (Memirie *et al*., 2018).

1.1.2. Epidemiology

Breast cancer is an ancient disease and was described by the Egyptians 3000 years before Christ. Subsequently, various articles about breast cancer and its treatment were written by Greek and Roman physicians (Rayter and Mansi, 2008). It is a multi-factorial disease and characterized by the uncontrolled growth of abnormal cells in the milk-producing glands of the breast or in the passages (ducts) that deliver milk to the nipples (Asegaonkar *et al*., 2017).



The disease begins in breast tissue, which is made up of glands for milk production, called lobules, and the ducts that connect lobules to the nipple (Lauby-Secretan *et al*., 2015).

On the basis of origin, breast cancers are of two types namely, ductal carcinoma (constitute 80-90% breast cancer cases) and lobular carcinoma (constitutes10-20% breast cancer cases) *(*Sarkar and Mandal, 2011).

Fig. 1: Breast cancer origins on female breast, adapted from (https://www.bebrcaware.com)

Although the etiology of breast cancer is unknown, numerous risk factors may influence for the development of the disease. Established risk factors by epidemiologic studies include race, ethnicity, family history of cancer, and genetic traits, as well as modifiable exposures such as increased alcohol consumption, physical inactivity, exogenous hormones, and certain female reproductive factors (Weir *et al*., 2007).

Breast cancer is the most frequently diagnosed cancer(folowed by colorectal and lung cancer) and the leading cause of cancer death(followed by lung and colorectal cancer) in women worldwide. The disease also affects men, but it is rare among men and there is a pronounced female-to-male disparity in breast cancer incidence (Coughlin and Cypel, 2013). Each year more than one million women are diagnosed with breast cancer worldwide and over half of whom will

die from the disease. It accounts for 23% of the total cancer cases and 14% of the cancer deaths (Jemal *et al.*, 2011). According to the report by GLOBOCAN, approximately 2.1 million new breast cancer diagnoses were estimated in 2018, contributing about 11.6% of the total cancer incidence burden (Bray *et al*., 2018).

In Africa, the disease is the most commonly diagnosed cancer and the second leading cause of cancer death among women in 2008 which accounts 92,600 cases, and 50,000 deaths (Jemal *et al*., 2012). In the region, breast cancer survival is tends to be poorer, most likely because of a combination of a late stage at diagnosis and limited access to timely and standard treatment (Jemal *et al*., 2011).

Based on the 2012 GLOBOCAN report in Ethiopia, there was an estimated age-standardized incidence rate of 19.5 per 100,000 and an estimated age-standardized death rate of 11.8 per 100,000 females. The disease accounts for about 5.8% of the country's total national mortality (Ferlay *et al*., 2013).

According to the report revealed by Memirie et al. breast cancer is the commonest cancer in Ethiopia too, which constituting 33% of the cancers in women and 23% of all cancers identified from the Addis Ababa cancer registry (Memirie *et al*., 2018).

1.1.3. Breast cancer treatment

Despite an increasing incidence of breast cancer, the disease-specific mortality has been declining in the majority of developed countries. This is due to a significant advancement in early screening /diagnosis and novel treatments (Bloom *et al*., 2016). Most importantly the use of systemic therapy in early breast cancer is undoubtedly a major reason for that. Therefore nowadays the cancer treatments are more and more effective and could reduce the recurrence rate and cure, or at least transform cancer into a chronic disease with long-term survival. Breast cancer prognosis and treatment options are generally based on tumor-node-metastasis (TNM) staging. Lymphovascular spread, histological grade, hormone receptor status, ERBB2 (formerly HER2) over expression, co morbidities, and patient menopausal status and age are also important (Maughan *et al*., 2010).

Currently, treatment for breast cancer patients includes surgery, radiotherapy, chemotherapy, targeted therapy, or endocrine therapy, as a treatment strategy with a view to eradicating distant micro metastatic deposits. Chemotherapy is the most effective and widely used treatment in most types of malignancies (Aslam *et al*., 2014).

1.1.4. Chemotherapy treatment

Chemotherapy is the use of medicines or drugs to treat a disease, such as cancer. Chemotherapy treatment was developed and used since World War I from the chemical weapon program of the United State of America (USA). Since that era, it becomes one of the most important treatments for cancer disease (DeVita and Chu, 2008). Unlike surgery and radiation therapy which remove, kill, or damage cancer cells in a certain area, chemotherapy can work throughout the whole body. Hence this treatment option can kill cancer cells that have metastasized or spread to parts of the body far away from the primary (original) tumor. The choice of appropriate adjuvant therapy is based on the stage of the disease, the functional status and co-morbid condition of the patient, and the clinico-pathological and molecular characteristics of the tumor (Valachis and Nilsson, 2015).

The main mechanism for its working is by attaching and destroying the cancer cells which are characterized by their high multiplication and growth speed (Abdul and Hassan, 2012). It is estimated that, in 2012, there were at least 13.5 million cancer survivors treated with chemotherapy in the United States (CĂINAP *et al*., 2016).

1.1.6. Toxicity of chemotherapy

From the total 132 cancer chemotherapy drugs that are approved by the US Food and Drug Administration, 56 of which drugs have been reported to cause oxidative stress (Aslam *et al*., 2014). Anticancer drugs mainly anthracyclines, 5-fluorouracil and cyclophosphamide exert prominent cardio toxicity (Iqubal *et al*., 2018). It is proved that chemotherapy drugs can damage both the cancer cells and the normal cells. This occurs largely by the means of genotoxicity partially caused by the production of reactive oxygen species, which does not specifically damages the cancer cells but also the normal cell (Conklin, 2004, Lee and Longo, 2011).

A proportion of the cancer patients suffer from only mild side effects whereas others may suffer from serious side effects. The occurrence of specific side effects will vary according to the chemotherapy or medications used. The most common side effects experienced are nausea and vomiting, anemia, hair loss, bleeding (thrombocytopenia), hyperuricemia bone marrow depression, alopecia, mucositis, etc. and even death may also occur in severe case (Abdul and Hassan, 2012).

1.1.7. Cardiac toxicity due to chemotherapy

As chemotherapeutic agents are becoming more effective and the population of cancer survivors increases, the morbidities that emerge from chemotherapy treatment have become more clinically relevant. Chemotherapy-induced cardiovascular morbidities encompass several of these negative side effects including systemic hypertension, thromboembolic events and heart failure (Bloom *et al*., 2016). Several adjuvant therapies against breast cancer can potentially cause a wide range of acute and late cardiac complications (Valachis and Nilsson, 2015). Therapy-related risk factors for chemotherapy-induced cardio toxic events include drug type, total dose administered during a day or a cycle, cumulative dose, administration schedule, route of administration, association with other cardio toxic drugs or concomitant radiotherapy (Csapo and Lazar, 2014).

According to the National Cancer Institute, cardio toxicity defines as "toxicity that affects the heart "which can be acute, which occurs during or soon after treatment and is transient, or chronic and can be categorized into type I (early onset) and type II (late onset) (Said *et al*., 2017).

Type I (early onset) related cardio toxicity occurs immediately after administration of chemotherapy and induces cell death (especially cardiomyocytes such as that seen during doxorubicin treatment) after exceeding a threshold level of cellular damage (Bloom *et al*., 2016). This type of cardio toxicity is detected through a reduction in left ventricle ejection fraction (LVEF) and augments one's vulnerability to future cardiovascular damage. It is characterized by damage to the microstructure of cardiac myocytes and results in cell death via necrosis or apoptosis. And this damage is generally considered irreversible (Christenson *et al*., 2015).

Type 2 cardio toxicity is spurred by cardiomyocytes dysfunction. Therefore, in contrast to type I cardio toxicity, type II cardio toxicity results in cardiac myocyte dysfunction with the notable absence of micro structural disruption. This type of impairment resolves with the completion of therapy and sometimes even during its continuation. This occurs like in those patients with trastuzumab administration who experience asymptomatic decreases in LVEF (Bloom *et al.,* 2016, Henri *et al.*, 2016).

The side effects of adjuvant breast cancer therapy, including cardio toxicity, remain clinically important (Bird and Swain, 2008) since 1979 after Von Hoff *et al* initially reported cardio toxicity in adult cancer patients as clinical congestive heart failure (CHF), characterized by

pulmonary edema, fluid overload, and effort intolerance. The authors reported a 2.2% overall with a cumulative doxorubicin dose-dependent incidence of CHF of 3%, 7%, and 18% at 400, 550, and 700 mg/m^2, respectively (McGowan *et al*., 2017).

The cardiac side effects of chemotherapy drugs have been shown to affect the quality of life and overall survival, regardless of the prognosis related to cancer (Henri *et al*., 2016). Cardiovascular disease (CVD) is now an important competing risk of long-term morbidity and mortality among breast cancer survivors (Curigliano *et al*., 2016). Conventional chemotherapy is associated with an increased risk of cardiac damage, including left ventricular dysfunction (LVD) and heart failure (HF), treatment-induced hypertension, vasospastic and thromboembolic ischemia, as well as rhythm disturbances, including conduction system damage and potentially QTc prolongation, that may be rarely life-threatening (Perez *et al*., 2011, Bowles *et al.*, 2012).

Besides characterizing chemotherapy-induced cardiac toxicity, methods for detecting, treating and preventing these clinical complications are very important. There are many techniques that have been applied for detecting progression of underlying cardiac disease from direct cardio toxic effects of chemotherapeutic agents. Biomarkers play a crucial role in identifying patients undergoing treatment who are at high risk for cardio toxicity and may assist in the identification of a low-risk cohort that does not necessitate continued intensive screening (Christenson *et al*., 2015).

1.2. Literature review

1.2.1. Role of cardiac biomarkers in the diagnosis of cardiac injury

The cardiovascular damage due to a variety of different chemotherapeutic agent is tremendous and, there is great interest in early detection of these side effects. Even if there is no effective means of accurately detecting and predicting the myocardial damage occurring due to chemotherapy treatment, biomarkers have the potential to fill this void (Christenson *et al*., 2015). These are a measurable substance that are objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (Boswood, 2013). The ideal biomarker has either high sensitivity or specificity and it also should be measurable and reproducible with a cost-effective assay.

Ideally, the use of biomarkers would be able to improve clinical outcomes for patients at risk for, or with known cardio toxicity (Shah *et al*., 2017). There are a great many circulating markers which can be altered by the presence of cardiovascular diseases. A strategy based on the use of

biochemical markers, in particular, cardiac troponins has developed in the last 15 years for early real-time identification, assessment, and monitoring of antitumor drug-induced cardio toxicity (Curigliano *et al*., 2016). Even if cardiac troponin is the first and well-studied blood biomarkers identified to detect cardiac damage, there are also other potential markers of cardio toxicity that have been investigated in small studies. These include like the markers of oxidative stress and inflammation (glutathione peroxidase, high-sensitivity C-reactive protein, interleukins), serum creatine kinase (CK) and serum creatine kinas myocardial band (CK-MB) (Curigliano *et al*., 2016).

1.2.2. Cardiac Troponin as a Marker of Cardio toxicity

Troponin is a protein complex located on the thin filament of striated muscles consisting of the three subunits namely Troponin T (TnT), Troponin I (TnI) and Troponin C (TnC) each having different structure and function (Nigam, 2007). The three units of troponin complex TnI (the inhibitory troponin), TnT (the tropomyosin binding troponin) and TnC (the calcium mediated troponin), along with tropomyosin is located on the actin filament and is essential for the calcium-mediated regulation of skeletal and cardiac muscle contraction (Babuin and Jaffe, 2005). Of the three troponins, TnT and TnI are being used as the biochemical markers for the diagnosis of myocardial injury. The troponins found in cardiac tissue (cTn) have a different amino acid sequence than that present in troponin of skeletal muscles. This makes cTn more specific for the diagnosis of myocardial injury (Nigam, 2007). Although they are normally undetectable, troponins may increase within 2 or 3 hours after cardiac damage occurs. This release kinetics is related to the distribution of these proteins within the myocardial cell. About 94-97% of these troponins are bound to myofibril and only 3% of cTnI and 6% of cTnT is free in the cytoplasm. When the myocardial damage occurs, the cytosolic troponins reach the blood stream quickly resulting in a rapid peak of serum troponin observed during the first few hours. This is followed by the release of structurally bound troponin resulting in a second peak lasting for several days. These detectable serum levels of cTn are an indicator of heart muscle damage (Nigam, 2007). Measurement and interpretation of troponin is part of the diagnosis of acute myocardial infarction (AMI) and workup of possible cardiac chest pain (Curigliano *et al*., 2016, Shah *et al*., 2017).

According to the guideline on the diagnostic criteria set by ESC,ACC,AHA,WHF expert consensus document, the term myocardial injury should be used when there is evidence of

elevated cardiac troponin values (cTn) with at least one value above the 99th percentile upper reference limits (URL). The guidelines also recommend the use of the 99th percentile of a healthy population as a cut-off for acute myocardial infarction using an assay with acceptable precision. An acceptable precision has been defined by consensus as a coefficient of variation <10%. For cardiac troponin T, the 99th percentile value of a healthy population is 0.01 ng /ml) (Thygesen *et al*., 2018).

Studies have shown that troponins may detect cardio toxicity at a preclinical phase, long before any reduction in LVEF has occurred, in patients treated with antitumor drugs (Curigliano *et al*., 2016). Its release in the blood stream is predictive of prognosis in myocardial infarction and other forms of heart disease (Doyle *et al*., 2005). Although it is known that cardiac troponin elevation is frequently associated with high anthracycline (AC) dosages, there have been studies demonstrating injury at low-to-moderate AC doses (<300 mg/m^2 (Moazeni *et al*., 2017). This blood marker is central to the universal definition of the acute coronary syndrome (ACS), and its release is predictive of prognosis in myocardial infarction and other forms of heart disease (Doyle *et al*., 2005).

Multiple studies have established the validity of cTn in detecting cardiovascular disease in patients receiving oncologic therapy, specifically addressing surveillance during therapy. Its measurement and determination is able to predict, at least 3 months in advance, the occurrence of a clinically significant dysfunction of the left ventricle. The early increase of the troponin concentration also predicts the degree and severity of future left ventricular dysfunction (Reagan, 2010). A study conducted at the University of Minnesota, USA, on eighteen consecutive pathologically diagnosed breast cancer patients taking anthracycline (doxorubicin with a median dose of 240 mg/m^2) chemotherapy drugs reported an increased levels of hs-cTnT to 19 pg/ml (P = 0.001) (Blaes *et al*., 2015). A related study done in Turkey on stage 2 and stage 3 breast cancer patients treated with epirubicin - containing adjuvant chemotherapy, elevated amount of cTnT (>0.01ng/mL) was also observed in 26 (63%) of their study participants after one cycle of chemotherapy treatment. The authors also recorded a decrease in mean left ventricular ejection fraction at baseline and one year after as 61±8% and 56±7% (p < 0.0001) (Erdim *et al*., 2009).

But a prospective study done in Germany on 99 cancer patients who were under treatment with ANT, taxanes, and/or trastuzumab was conducted to measure the change in troponin for early detection of cardio toxicity at baseline and at 3 and 12 months was contradicted with the above

two studies that authors conclude the occurrence of cardio toxicity could not be detected either by troponin (Jungandreas *et al*., 2014).

1.2.3. Ceratinine kinase myocardial band (CK-MB) as a marker of cardio toxicity

Creatine kinase (CK) is an enzyme and it was introduced in 1965 as a biochemical marker for myocardial damage and it is one of the oldest markers in detecting cardiac injury (Al-Hadi and Fox, 2009). CK is composed of two subunits, M and/or B. The three different pairs of these units combine to give rise to three different isoenzymes namely, CK-BB, CK-MB and CK-MM. It is understandable that many organs contain CK, but the distribution of isoenzymes is different in each one. Skeletal muscle is very rich in the MM isoenzyme, while brain, stomach, intestine, bladder and lung contain primarily the BB isoenzyme. The MB isoenzyme has been found in appreciable amounts (15 - 20 %) only in myocardial tissue (Schumann *et al*., 2002). CK-MB is the heart specific isoenzyme that exists in large quantity in heart muscle. About 15-40% of the total CK activity of heart muscle is due to CK-MB, the rest is largely due to the CK-MM isoenzyme. As a result, CK-MB has been the gold standard method for the diagnosis of acute myocardial infarction (AMI) in many laboratories (Al-Hadi and Fox, 2009).

Increased level of CK-MB is frequently interpreted as objective evidence of myocardial injury clinically. The appearance of CK-MB in serum reflects its unique presence in myocardial tissue and supports in the diagnosis of suspected myocardial infarction (Christenson *et al*., 1999, Al-Hadi and Fox, 2009, Emokpae and Nwagbara, 2017).

CK-MM is predominantly seen with striated muscle and myocardium, CK-MB only constitutes 0 –3% of CK in skeletal muscle and is elevated in myocardial disease. CK-MB isoenzyme comprises around 20% of total CK in myocardial tissue damage versus that of normal individuals where by one would find it constituting a lower percentage (only around 1% in this tissue) (Jagannadharao *et al*., 2010).

Despite the data that the recent ESC/ACC recommendation for redefinition of myocardial infarction established cardiac troponin (cTn) testing as the "gold standard" for diagnosis (Ottani *et al.*, 2000), the use of CK-MB testing in clinical practice is also remains common place and often cTn and CK-MB levels are both tested (Newby *et al*., 2006).

According to many consensus values, the reference range of CK-MB level for a healthy people (37^{o}c) is < 25 u/L and for suspecting of myocardial infarction diagnosis using the CK–MB

activity based on long term experience is a value of CK-MB > 24 u/L (Stein, 1985, Klein *et al*., 2001, Thomas *et al*., 2005).

Studies showed that CK-MB levels increased in cancer patients up on chemotherapy treatment. A study done at the University of Minnesota on breast cancer patients revealed a significant increment in the level of CK-MB after their breast cancer patients treated with doxorubicin (*P*= 0.02) (Blaes *et al*., 2015).

1.2.4. C-reactive protein (CRP) as a marker of cardio toxicity

C-reactive protein is a classical acute phase reactant protein from pentraxin family (consists of five identical polypeptide chains that form a five-member ring having a molecular weight of 105000 Daltons (Danesh *et al.*, 2004). It was discovered by Tillett and Francis in 1930 and named as CRP because of its high binding affinity to C-polysaccharide of *Streptococcus pneumonia* (Wilson *et al*., 2006*).* Despite these early findings, it was not until the 1990s that cardiovascular interest in CRP was revitalized. In the mid 1990s, immunoassays for CRP (hs-CRP), with greater sensitivity than those previously routine uses, revealed that increased CRP values strongly predict future coronary events (Shrivastava *et al*., 2015). It is synthesized by hepatocytes in response to inflammation, trauma, and tissue damage. Its production is regulated by interleukin 6 (IL- 6). Both genetic and environmental factors influence an individual's basal CRP concentration, and thus circulating CRP levels in apparently healthy people can vary from 0.1 to 10 mg/L. Increased CRP concentrations have been reported in many diseases, including cardiovascular diseases, type 2 diabetes, arthritis and many types of cancers (Heikkilä *et al*., 2007).

An expert panel assembled by the Centers for Disease Control and Prevention (CDC) and the AHA to improve cardiovascular risk stratification in primary prevention populations, termed CRP as an independent marker of cardiovascular risk . The panel also recommends the use of CRP as part of global risk prediction in asymptomatic individuals, particularly those deemed at intermediate risk for CVD by traditional risk factors (Shrivastava *et al*., 2015).

CRP has emerged as one of the most important novels inflammatory biomarkers in CHD patients in identifying patient groups who might benefit from particular treatment strategies. Many large-scale prospective studies demonstrate that CRP strongly and independently predicts adverse cardiovascular events, including myocardial infarction, ischemic stroke, and sudden cardiac death in individuals both with and without overt CHD. It is also believed to be both a marker and

a mediator of atherosclerosis and CHD (Shrivastava *et al*., 2015). The elevation of this protein is also a predictive of decreased LVEF and diastolic dysfunction in the context of stable coronary artery disease, myocardial infarction and congestive heart failure (Wilson *et al*., 2006). A study reported that a moderate rise in CRP levels is also seen in chronic inflammatory states (Asegaonkar *et al*., 2015). In addition to assessing future CHD risk in asymptotic individuals, growing bodies of studies suggest that elevation of hs-CRP levels predicts a poor cardiovascular prognosis. In a meta-analysis of many prospective studies, elevated hs-CRP was shown to predict future risk of ischemic stroke, peripheral arterial disease and CHD (Wilson *et al.*, 2006).

A study conducted in patients with breast cancer treated with dose-dense chemotherapy incorporating trastuzumab and lapatinip, detectable CRP level was reported in 74 of 92 study participants. That is 78 % of their study subjects show detectable increment in the levels of CRP (Morris *et al*., 2011a). Another study was conducted in India with a total of 30 cases on the levels of CRP among cancer patients before and after chemotherapy treatment. Their finding revealed that CRP levels of cancer patients were significantly higher than those of the healthy subjects and the CRP levels increased after chemotherapy treatment. They also reported that there is more increase in serum CRP level in every patient after chemotherapy (Arpita *et al*., 2016).

1.2.5. Inflammatory cytokines (Tumor necrosis factor alpha (TNF-~) and interleukin-6 (IL-6) as a marker of cardiac injury

Inflammatory markers have been identified as potential indicators of heart failure and future adverse events (Iqbal *et al*., 2012). In addition to detecting subclinical heart failure, cytokine levels may offer valuable information in clinical decision-making processes, such as selection of an appropriate therapeutic intervention and monitoring response to therapy. Elevated levels of circulating proinflammatory cytokines are associated with disease progression and adverse outcomes in chronic heart failure patients which can be also observed after chemotherapy treatment .Large scale studies in animal and human models have established evidence of changes in inflammatory cytokine levels after administration of chemotherapy drugs (Vistnes *et al*., 2010). A study showed that tissue and serum levels of TNF-α, interleukin 1 beta (IL-1β), and IL-6 in rats were elevated after 5-FU administration before histological evidence of tissue damage (M Logan *et al*., 2008). Several studies have shown raised levels of inflammatory cytokines such as TNF α, IL-1and IL-6 in heart failure patients in plasma and circulating leukocytes, as well as

in the failing myocardium itself (Gullestad *et al*., 2012). Inflammatory response and cytokine elaboration are integral components of the host response to tissue injury and plays a particularly active role after myocardial infarction. Cytokines are released by the host myocardium to modulate tissue repair and adaptation after injury. Cytokines such as TNF-α or IL-6 are elaborated soon after myocardial ischemic injury and can acutely regulate myocyte survival or apoptosis and trigger additional cellular inflammatory response (Gullestad *et al*., 2012). Inflammatory cytokines are expressed by all nucleated cell types residing in the myocardium, including the cardiac myocyte. For instance, TNF-α is produced and secreted by many cell types including: immune cells, endothelial cells, epithelial cells, smooth muscle cells, and cardiac myocytes (Tangpong *et al*., 2008). It is a classic biomarker of inflammatory processes which has displayed clinical utility as a marker in cases of heart failure. A study found that–TNF-α level was associated with abnormal left atrial function and advanced left systolic and diastolic dysfunction-in those patients diagnosed with heart failure (Iqbal *et al*., 2012).

Like TNF-α and other several inflammatory biomarkers, IL-6 has been also associated with and predicted the risk of future cardiovascular events (Held *et al*., 2017). It is associated with an increased incidence of myocardial infarction and mortality among patients with acute coronary syndrome (Wainstein *et al*., 2017).

1.1.8. FAC based drug regimens and their possible mechanisms in causing cardio toxicity

FAC is an abbreviation for a chemotherapy combination drug regimens used alone or together with other therapies to treat breast cancer. It includes the chemotherapy medicines 5-fluorouracil, doxorubicin hydrochloride (Adriamycin) and cyclophosphamide which is administered intravenously for cancer patients in every 3 weeks for six cycles (Shenkier *et al.*, 2004). The most studied chemotherapeutic agents associated with adverse cardiac events are anthracyclines (Doxorubicin). The drug is used in the treatment of many adult malignancies like breast cancer, sarcoma, lymphoma, or gynecological cancer. Anthracyclines are currently used in more than 50% of regimens contributing to the overall survival rates in excess of 75% (Csapo and Lazar, 2014). Nowadays, 32% of breast cancer patients, 50 to 60% of childhood cancer survivors, 57 to 70% of elderly lymphoma patients are treating with anthracycline containing chemotherapy drug regimens (McGowan *et al*., 2017). Though the broad use of this drug has dramatically improved cancer survival statistics, life-altering cardiac sequelae from anthracyclines remain a problem, with a range of 5% to 23% of patients developing late-onset heart failure secondary to

anthracycline induced cardio toxicity (Geisberg and Sawyer, 2010). The cardio toxic effect induced by this drug was first reported in 1967 with a clinical presentation of heart failure in children treated with doxorubicin (DOX) for leukemia and it can be acute, early onset chronic (within days or weeks) and chronic progressive cardio toxicity (weeks to months after drug administration (Alkuraishy *et al*., 2017).

Despite there are many postulated mechanisms by which DOX can cause cardiac injury, the most commonly accepted theory implicated inhibition of TOP2β and the formation of free radicals and superoxide due to doxorubicin metabolism in cardiomyocytes (Mobaraki *et al*., 2017).

In the free radical theory, the reaction was initiated by loss of an electron from DOX that triggered the formation of DOX semiquinone radical by a reduced flavoenzyme such as NADPH-cytochrome P450 reductase. Under the normoxic condition, this semiquinone radical can readily react with oxygen-generating superoxide anion (O_2^-), which could be neutralized in to relatively stable and low-toxic hydrogen peroxide (H_2O_2) by superoxide dismutase, or further changed to other ROS or reactive nitrogen species (RNS) in a sequence of reactions known as the redox cycling (Cappetta *et al*., 2017).

Dangerously, H_2O_2 and O_2^- may also generate highly reactive and toxic hydroxyl radicals (OH^-) during the iron-catalyzed Haber- Weiss reaction. The semiquinone radical forms a complex with iron leading to the formation of an anthracycline-iron (DOX) (Fe^{2+}) free radical complex (Schimmel *et al*., 2004). The DOX (Fe^{2+}) complex then reduces oxygen to produce superoxide and to regenerate DOX. The superoxide is dismutated into hydrogen peroxide and oxygen. This causes an increase in superoxide and a decrease in nitric oxide formation. The consequential formation of peroxynitrite could also play a role in the cardio toxicity thus lipid per oxidation may be initiated from the combination and arrangement of superoxide, hydrogen peroxide and free iron (Alkuraishy *et al*., 2017). The strict connection between anthracyclines and mitochondria lays in the high affinity for cardiolipin, a phospholipid located in the inner mitochondrial membrane, where anthracyclines being retained at higher concentration, disrupt electron-transport chain and thus inducing more ROS production.

The specific vulnerability of cardiac cells to the oxidative stress would be due to relatively low levels of antioxidant enzymes in the heart. A study conducted using a rat hearts and concluded that doxorubicin is able to cause an additional decrease in the a priori low intensity of the antioxidant enzymes in rat hearts (Inoue and Tani, 2014).

Different studies reviled that the myocardial damage due to DOX undergoes apoptosis (programmed cell death) which is initiated by the formation of oxidative free radicals. Arola and his colleague in their study on "Acute doxorubicin cardio toxicity involve cardiomyocyte apoptosis" found that a 34 apoptotic cell death in rat cardiomyocytes and bovine aortic endothelial cells upon exposure to doxorubicin (Arola *et al.*, 2000).

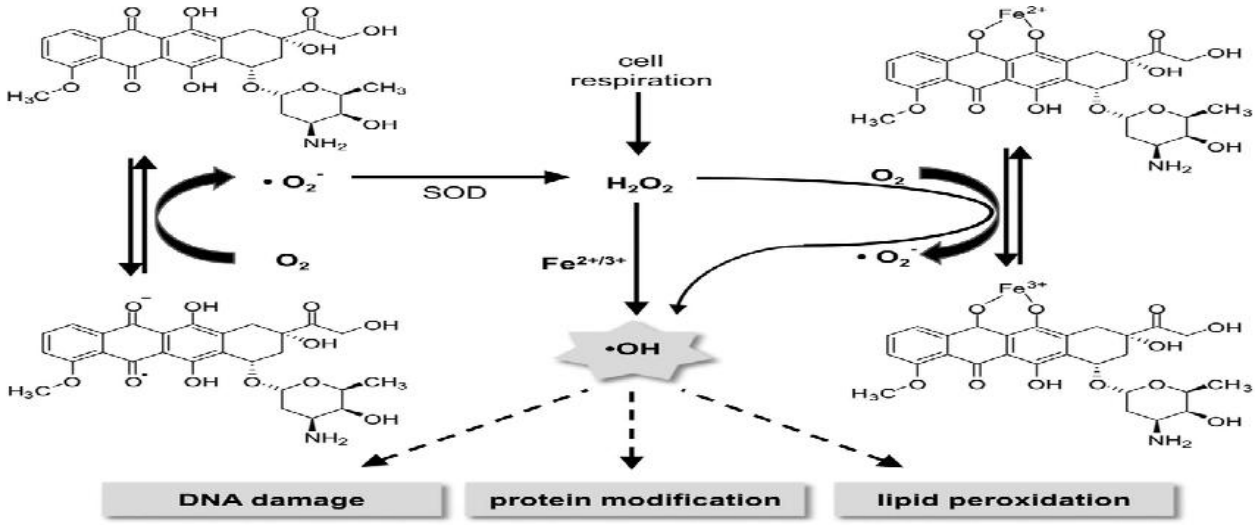


Fig. 2: Model of doxorubicin-mediated generation of reactive oxygen species (ROS) by redox cycling and Fenton's reaction (Henninger and Fritz, 2017). Doxorubicin produces ROS by redox cycling at its semiquinone structure. In the Fenton's reaction, iron catalyses the generation of OH radicals (•OH) from H2O2. SOD: superoxide dismutase.

Other cytostatics chemotherapy drugs more frequently correlated with cardio toxic side effects are antimetabolite agents (5-Fluorouracil), alkylating agents (Cyclophosphamide Carboplatin, Cisplatin) , taxanes (paclitaxel, docetaxel),) small molecule tyrosine kinase inhibitors (lapatinib, imatinib, sorafenib, sunitinib) and trastuzumab, a monoclonal antibody directed against the human epidermal growth factor receptor-2 (HER2), used in the treatment of metastatic breast neoplasm (Csapo and Lazar, 2014).

In 5-fluorouracil treatment, cardiac symptoms generally occur during the initial hours following the start of therapy (Schlitt *et al.*, 2014). Its cardio toxic effect is the second most common cause of chemotherapy induced cardio toxicity, followed by anthracycline (Broder *et al.*, 2008). Cardio toxicity induced by 5 - FU often presents as myocardial ischemia, but to a lesser extent cardiac

arrhythmias, hyper and hypotension, left ventricular dysfunction, cardiac arrest and sudden death. According to many scientific reports, the incidence of cardio toxicity related to 5-FU ranges from 1.2% to 18% with mortality ranging between 2.2% and 13%. This wide variation is believed to be related to dose dependency as well as the frequency of drug administration. With shorter bolus regimens, the incidence of cardio toxicity typically lies between 1.6% to 3% of cases but with more prolonged regimens, these percentages increase to 7.6% to 18% (Sorrentino *et al*., 2012).

The adverse effect due to cyclophosphamide (CY) treatment is relatively well tolerated at lower doses but when the cumulative dose exceeded 600 mg/m^2, it may cause acute cardio toxicity. The incidence of fatal cardiomyopathy associated with CY varies from 2.0% to 17.0%, depending on the different regimens and patient populations (Gado *et al*., 2013). Based on reports by multiple studies, the incidence of heart failure after high doses of CY is varied widely from author to author (less than 5% and 10-29%) (Auner *et al*., 2002, Zver *et al*., 2008).

CY, which is a prodrug, can be activated by the hepatic cytochrome P-450 (CYP) enzyme system to produce 4-hydroxy cyclophosphamide (HCY), which forms in equilibrium with aldocyclophosphamide (AldoCY) (Kurauchi *et al*., 2017). Depending on cell type, AldoCY may, through the chemical process of β-elimination, decompose to form cytotoxic phosphoramide mustard (PM) and by product acrolein, or may be mediated by aldehyde dehydrogenase (ALDH),to inactive metabolite *o*-carboxyethyl-phosphoramide mustard (CEPM) (Nishikawa *et al*., 2015)

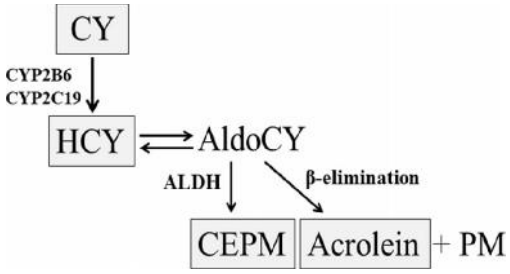


Fig.3: Cyclophosphamide metabolic pathway(Kurauchi *et al.*, 2017)

CY: cyclophosphamide, CYP2B6: cytochrome p2b6, CYP2C19: cytochrome p2c19, HCY: hydroxyl-cyclophosphamide, AldoCY: aldocyclophosphamide, ALDH: aldehyde dehydrogena-se, CEPM: o-carboxyethyl phosphoramide mustard, PM: phosphoramide mustard

1.3. Statement of the problem

Despite an increasing incidence of breast cancer, advances in cancer therapy like earlier detection and highly effective multimodal treatments have resulted in significant improvement in long-term survival for many types of cancer. As a result, breast cancer has become a largely curable disease and a chronic illness. Chemotherapy plays a vital role in the overall health

improvement of different types of cancer survivors including breast cancer. Hence, the disease specific mortality rate has been declining and the survival of patients has been increased in different parts of world (Bloom *et al*., 2016). But the clinical efficacy of chemotherapy is often limited by its related cardio toxicity. The development of cardio toxic events, even when they are asymptomatic, not only has a negative impact on the patient's cardiac prognosis but it also considerably restricts the therapeutic opportunities. Cardiac dysfunction and heart failure are among the most serious cardiovascular consequences of systemic cancer treatment. Conventional chemotherapeutics, such as anthracyclines, antimetabolite, and cyclophosphamide, can induce permanent myocardial cell injury, leading to acute or chronic LVD (Curigliano *et al.*, 2016). In a recent comprehensive review of breast cancer survivors in the United States, women were noted to be at significantly increased risk of death caused by CVD, exceeding their risk of death from the initial cancer itself (Bodai and Tuso, 2015).CVD is the predominant cause of mortality in breast cancer patients over 50 years of age and is a more common contributor than cancer to mortality among older cancer survivors (Siegel *et al*., 2015).

A study on the childhood cancer survivors also showed that, 15 to 25 years after diagnosis, survivors of childhood cancer have an 8.2-fold higher rate of cardiac death compared with the age-matched and sex-matched national average. Compared with controls, long-term childhood cancer survivors had 15-fold increased rates of congestive HF, 10-fold higher rates of CVD, and 9-fold higher rates of stroke life-threatening, and fatal events in the Childhood Cancer Survivor Study (Armstrong *et al*., 2014).

The clinical manifestations of cardio toxicity cover a broad spectrum of disorders, ranging from mild transient arrhythmias to potentially lethal conditions such as myocardial ischemia or infarction and cardiomyopathy (Csapo and Lazar, 2014).

Nowadays securing cardiac function is an ongoing challenge for the pharmaceutical industry and the physicians who have to deal currently with these adverse reactions (Magnano *et al.*, 2014). In the developed countries, it is a primary goal for cardiologists and oncologists for identifying cancer patients taking chemotherapy treatment who are at risk of cardiac damage.

The appropriate management should include better detection of those patients at risk, the development of preventive strategies and the early treatment of cardio toxicity when it does appear (Csapo and Lazar, 2014).

To detect subclinical myocardial damage, time and expensive monitoring of cardiac functions are recommended, during and after chemotherapy (Dolci *et al*., 2008). Hence, the side effect of chemotherapy-induced cardiac damage become worse when it comes to resource-limited countries where there is limited access of treatment sites and strategies and no enough laboratory set up for early screening, identification and stratification of patients at risk of cardiac injury before, during and after chemotherapy treatment.

The common adopted diagnostic approach to detect and identifying patients at risk of cardiac chemotherapy-induced cardiac damage is mostly relied on the estimation of left ventricular ejection fraction by echocardiography and angiography with a radionuclide (Ky *et al*., 2014). This method is more traditional, inadequate and shows a low diagnostic sensitivity (with overall diagnostic sensitivity for AMI of 70-81% and as many as 30-50% of patients may initially present with normal or non-diagnostic ECG) (Al-Hadi and Fox, 2009). It has also a low predictive power toward early prediction of subsequent decline in function with treatment and detection of myocardial injury (Dolci *et al*., 2008).

But the assessment of multiple cardiac biomarkers has been shown to be of incremental utility in identifying patients at increased cardiac risk and adverse outcomes from chemotherapy as well as treat damage at an earlier stage, preserve patient health, and improve quality of life (Ky *et al*., 2014, Henri *et al*., 2016).

The 2007 ACC/AHA guidelines for the management of MI also emphasize troponins and CK-MB as potentially important markers of cardiac injury (Curigliano *et al*., 2016). Therefore the use of easily detectable cardiac biomarkers in blood, such as cTn, cardiac natriuretic peptides (CNPs), hs-CRP, CK-MB and other potential cardiac biomarkers including inflammatory cytokines are very recommendable in the screening of high-risk patients for the detection of early subclinical cardio toxicity (Horacek *et al*., 2007). Early detection of cardio toxicity using these cardiac biomarkers is also crucial for applying preventive and supportive therapeutic strategies (Horacek, 2011).

1.4. Significance of the study

At present, cardiac biomarkers have been evaluated inadequately in assessing chemotherapy induced cardiac injury. Only a few studies have been published worldwide and noon in our country in addressing the role of biochemical markers of functional and structural myocardial injury in patients treated for cancer. Therefore our study, evaluation of serum cardiac and

inflammatory biomarkers to assess cardiac toxicity (injury) induced by chemotherapy agents will add some important findings to the existing scientific data and help as a stepping stone for other interested researchers in our country in this area of study.

This study will help to set up a risk stratification method of the country cancer patients receiving chemotherapy treatment and treating them accordingly with considerable cardiac care. In addition, our work of evaluating serum levels of cardiac and inflammatory biomarkers in breast cancer patients receiving chemotherapy at TASH is significantly an important study to improve the overall society health problem with drug-induced cardiac damage.

Furthermore, this study will help to add some new information to the existing related data that are done before elsewhere and may give the insight to develop better chemotherapy treatment outcome. It also gives a starting point for other broad and nationwide research for those interested researchers on cardiac injury and related topics.

1.5. Hypothesis of the study

Null hypothesis (H_0) - there is no significant difference in the mean value of serum levels of cardiac biomarkers (cTroponin, CK-MB, CRP) and inflammatory cytokines (TNF-α and IL-6) in breast cancer patients at baseline and after the three cycles of chemotherapy treatment.

Alternative hypothesis (H_1) - there is significant difference in the mean value of serum levels of cardiac biomarkers (cTroponin, CK-MB and CRP) and inflammatory cytokines (TNF-α and IL-6) in breast cancer patients at baseline and after the three cycles of chemotherapy treatment.

2. Objectives

2.1. General objective

To evaluate changes in serum cardiac (Troponin, CK-MB and CRP) and inflammatory (IL-6 and TNF- α) biomarkers level following chemotherapy treatment among female breast cancer patients attending at Tikur Anbessa specialized hospital

2.2. Specific objectives

- To evaluate serum levels of cTnT, CK-MB, CRP, TNF-α and IL-6 of female breast cancer patients at baseline and after the three cycles of chemotherapy treatment
- To compare serum cardiac levels of cTnT, CK-MB ,CRP TNF-α and IL-6 of female breast cancer patients before and after the three cycles of chemotherapy
- To examine the associations of demographic and clinical features of female breast cancer patients with their serum cardiac and inflammatory biomarkers

3. Materials and methods

3.1. Study area and duration

This study was conducted from July 2017 to June 2019 at the oncology department of Tikur Anbessa Specialized teaching Hospital, Addis Ababa, the capital of Ethiopia. Addis Ababa is situated in central Ethiopia at an elevation of about 2440 meters (8000 ft.) above sea level. The population of the city is estimated to be about 3,384,569 according to 2007 census Population and Housing Census Report of 2007. Tikur Anbessa specialized teaching hospital is the largest general public referral hospital and the only chemotherapeutic service center in Ethiopia.

3.2. Study design

Hospital-based retrospective study was conducted to evaluate the serum levels of cardiac biomarkers (Troponin, CK-MB, and CRP) and inflammatory cytokines (TNF- α and IL-6) among female breast cancer patients on chemotherapy treatment at Tikur Anbessa Specialized Hospital, Addis Ababa. For this study we have used frozen serums specimens from a repository of our earlier study thematic project (To investigate predisposing genetic deriving factor for aggressive nature of Ethiopian breast cancer and establish application of breast cancer data base).

3.3. Population

3.3.1. Source of population

The source of population for this study was all repository specimens of female breast cancer patients attended chemotherapy treatment at the oncology department of TASH with in the study period.

3.3.2 Study population

The study population for this study was all repository specimens of women with breast cancer received consecutive systemic chemotherapy treatments for at least three cycles at the oncology department TASH during the time interval of the study.

3.4. Eligibility criteria

3.4.1. Inclusion criteria

Fully documented and matched samples of patients that were received at least three consecutive cycles of chemotherapy treatment without interruption were included from the total repository specimens of a thematic research project (To investigate predisposing genetic deriving factor for aggressive nature of Ethiopian breast cancer and establish application of breast cancer data base).

3.4.2. Exclusion criteria

- Incomplete and inadequate samples
- Known ischemic and valvular heart disease
- Recorded advanced organ failure
- Non paired samples
- Other malignancies

3.5. Sampling method and sample size determination

A total of 40 paired samples (40 samples before chemotherapy and 40 matched samples after three cycles of chemotherapy) were used in this study using a convenient sampling method. When calculating the sample size requirements for this study, a number of factors are taken into consideration including sample availability, cooperation and attrition, practical constraints such as time and finance, adequate left over samples for our measurement and analysis, (Miles, 2003). Therefore by examining the sample size of other comparable studies carried out internationally assessing cardiac biomarker levels in female breast cancer patients, sample size varies from study to study. However, power analysis and sample size calculation were not reported in any of these studies. Therefore based on logistical feasibility and uncertainties in sample size considerations, 40 paired samples were included in the study. This sample size was adequate to observe the change in cardiac and inflammatory markers to observe cardiac toxicity induced by chemotherapy treatment as described by others (Erdim *et al*., 2009, Frères *et al*., 2018).

3.6. Variables

3.6.1. Dependent variables

- Serum Troponin level
- Serum CK- MB level
- Serum CRP level
- Serum TNF-α level
- Serum IL-6 level

3.6.2. Independent variables

Linked socio-demographic and clinical variables such as:

- BMI
- Age
- Tumor site
- Drug protocol type
- Tumor stage
- Radiation exposure to chest
- Smoking status
- Alcohol use habit
- Chat chewing habit and menopausal status were also retrieved and considered as independent variables for this study.

3.7. Test principles of the laboratory analytes

3.7.1. Determination of serum concentrations of Cardiac troponin level

Serum level of cardiac hs-TnT concentrations was measured by using a commercially available kit, Elecsys Troponin-T immunoassay (Roche Diagnostics, Indianapolis, IN, USA) both at baseline and after the breast cancer patients received three cycles of chemotherapy treatment. The kit has a measuring range of 3-10,000 pg/mL, a lower detection limit of 3 pg/mL) and the 99^{th} percentile cut-off point of 10 pg/mL). According to the diagnostic criterion for an AMI, a hs-cTnT level > the 99^{th} percentile (0.01ng/mL) was considered as elevated (Sherief *et al.*, 2012, Xu *et al*., 2013).

The test principle is a sandwich principle in which cTnT is sandwiched by biotinylated monoclonal anti-TnT antibody and monoclonal anti-TnT antibody labeled with ruthenium complex. A biotinylated monoclonal troponin T-specific antibody and a monoclonal TnT-specific antibody labeled with a ruthenium complex[a] then react to form a sandwich complex. The reaction mixture is aspirated into the measuring cell where the micro particles are magnetically captured onto the surface of the electrode. Then the sandwich complex is bound to the solid phase via interaction of biotin and streptavidin present on streptavidin coated micro particles that are magnetically captured to the electrode surface. After removal of the unbound substances, application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

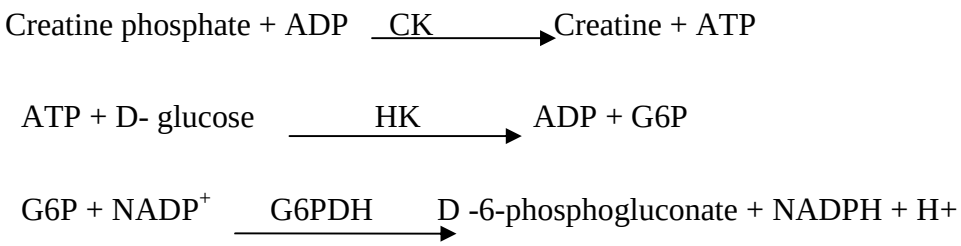
3.7.2. Determination of serum concentrations of hs-CRP level

Serum level of CRP was measured immunoturbidometrically (latex high-sensitive assay) using Roch/hitachi cobas c 502 analyzer (Roche Diagnostics, Indianapolis, IN, USA) with a lower detection limit of 0.15 mg/L) and a measuring range of 0.15- 20.0 mg/L (Ridker, 2003). Based on the instruction of the analyzer and the guideline set by international federation of clinical chemistry and laboratory medicine / Community Bureau of Reference of the Commission of the European Communities (BCR IFCC/CRM 470, the reference value of CRP for adult population is < 5 mg/L. In our study values above this reference point was taken as elevated.

The test is based on the principle of the latex agglutination. When latex particles complexed human anti-CRP is mixed with a patient's serum containing CRP, a visible agglutination reaction will take place within 2 minutes. The CRP reacts with the specific antibody producing insoluble immune complexes. Human CRP agglutinates with latex particles coated with monoclonal anti - CRP antibodies. The precipitate is determined turbidometrically. The turbidity caused by these immune complexes is proportional to the CRP concentration in the sample and can be measured spectrophotometrically. In healthy adults, the concentrations of CRP in serum have been reported to be at 1 mg/L (Järvisalo *et al*., 2002).

3.7.3. Determination of serum concentrations of CK-MB level

Serum level of CK-MB was measured by immunological UV assay using Roche/ Hitachi Cobass c 502 analyzer (Roche Diagnostics, Indianapolis, IN, USA) USA) which has a lower detection limit of 3 u/L and a measuring range of 3-500 u/L (Schumann *et al.*, 2002). For suspecting of myocardial infarction diagnosis using the CK-MB activity based on long term experience is a value of CK - MB > 24 u/L (Stein, 1985, Thomas *et al*., 2005). In measuring CK-MB level from the serum the immunological UV assay follows the following test principles. The CK-M subunits are inhibited by specific antibodies. Since CK-BB occurs rarely in serum it is assumed that the CK-B activity is derived from CK-MB present in the specimen. The activity of the CK-B subunits is determined and multiplied by two to provide an estimated activity of the CK-MB. The CK is activated by N-acetylcysteine (NAC). In a primary reaction, the activated CK catalyzes the dephosphorylation of creatine phosphate to form creatine and ATP. In a coupled reaction catalyzed by hexokinase (HK), glucose is phosphorylated by ATP to form -glucose-6-phosphate (G6P). Finally, glucose-6 phosphate dehydrogenase (G6PDH) catalyzes the oxidation of G6P by NADP+ to form 6-phosphogluconate and NADPH.



The rate of the NADPH formation is directly proportional to the catalytic CK- MB activity and it is determined by measuring the increase in absorbance photo metrically.

3.7.4. Determination of serum IL-6 and TNF- ~ level

The concentration of TNF α and IL-6 in the serum of patients at baseline and after the three cycles of chemotherapy treatment was determined by the use of R and D systemic luminex assay. The mechanism of determination employed the quantitative sandwich enzyme immunoassay technique.

The kit for human IL-6 Luminex Performance Assay (Minneapolis, Minnesota United States) with a catalog number of, LUH206 has a measuring range of 3.00 - 2400 pg/ml and sensitivity of 1.11pg/mL.

The human TNF-α High Sensitivity Luminex Performance Assay kit (Minneapolis, Minnesota United States) with catalog number LHSC210 has also a detection range of 0.82 - 3350 pg/mL with a sensitivity of 0.54pg/mL.

A Luminex Performance Assays afford the benefit of multi analyte analysis of cytokines in a complex sample.

Principle and procedure: analyte-specific antibodies are pre-coated on to color-coded micro particles. Micro particles, standards and samples are pipetted in to wells and the immobilized antibodies bind the analytes of interest. After washing away any unbound substances, a biotinylated antibody cocktail specific to the analytes of interest is added to each well. Following a wash to remove any unbound biotinylated antibody, streptavidin-phycoerythrin conjugate (Streptavidin-PE), which binds to the biotinylated antibody, is added to each well. A final wash removes unbound Streptavidin-PE and the micro particles are re-suspended in buffer and read using a Luminex or Bio-Plex analyzer. One laser is micro particle-specific and determines which analyte is being detected. The other laser determines the magnitude of the phycoerythrin-derived signal, which is in direct proportion to the amount of analyte bound.

3.8. Anthropometrical measurement procedure

The weight of the breast cancer patients was measured using a standard balance, and the height was measured by using a height measuring device attached to the balance. Body Mass Index (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/m2

Normal, >18.5–24.9 kg/m2

Overweight, >25.0–29.9 kg/m2 and

Obesity >30 kg/m2; the participants' ages were also recorded.

3.9. Data quality assurance and measurement

The data collection questionnaire was well prepared and all variables were filled on the data extraction format.

- All the laboratory procedures were handled by professional laboratory technologists.
- All the tests were standardized and automated.
- Clinical chemistry analyzer, as well as the reagents were also calibrated and kept to adequate temperature and appropriate wavelength according to the kit instruction to conduct the standard procedure.

3.10. Data processing and analysis

After checking for completeness and cleaning, processing and analysis of the data obtained from laboratory analyses of the blood samples and questionnaires was performed by coding and entering the data in to SPSS software version 23 package and the different variables were tested and analyzed. The results of the descriptive statistics were expressed as frequency and percentage. Simple descriptive statistics were used to present the socio-demographic and clinical characteristics of the study subjects. Continuous variables were presented as mean ± standard deviation and were compared using paired t-tests for groups. Other associations were also performed with Pearson's correlation coefficient. A p-value of < 0.05 at 95% confidence level was considered to be statistically significant in all the analyses.

3.11. Ethical consideration

Before starting data collection and preliminary study, ethical clearance letter with protocol number M.Sc.15c/17 was obtained from the Departmental Research and Ethics Review Committee, Department of Biochemistry, College of Health Sciences, Addis Ababa University.

3.12. Dissemination and utilization of the result

After completion of this research, the results of the study will be submitted and presented to Biochemistry Department; Addis Ababa University. The findings of the study will be also disseminated through publications and presentation in scientific conferences and workshops for health care professionals and other concerned bodies for better care of cancer survivors than ever.

4. RESULTS

4.1. General characteristics of the study participants

This study enrolled 40 female breast cancer patients on chemotherapy treatment with a mean age of 44.50±9.05 years. The majority (62%) of them were found within the age group of below 50 years; and as far as menopausal status concerned, 22 (55%) of the participants were in pre menopausal status. Regarding their residency, most of the breast cancer patients in this study were Addis Ababa residents (40%) followed by Oromo (27.5%), Amhara (22.5%) and others (10%). While 8 (20%) of the study participants had a history of alcohol drinking behavior, only 2 (5 %) of them did smoke cigarette; and 1 (2.5%) had a habit of chat chewing (**Table 1)**.

The present study also revealed that the average BMI of our study subjects falls in the normal range (22.59 Kg/m^2). About 6 (15%) and 9 (22.5%) of the breast cancer patients were under weight and overweight respectively; and only 1 (2.5%) of them were obese. Among the breast cancer patients in this study, 11(27.5%) were found to have family history of breast cancer and in 21(52.5%) of the breast cancer patients, the tumor were located on the left side of the breast.

About 9 (22.5%) of our chemotherapy treated female breast cancer patients were in clinical stage II, 26(65%) were in stage III and the reset 5(12.5%)) were in stage IV. Regarding treatment protocols, the majority 31(77.5%) were treated with FAC combination anti cancer drugs followed by AC 7 (17.5 %) and FAC + taxane 2(5%). In addition all of the breast cancer patients in this study underwent surgery (**Table 1)**.

Table 1: Sociodemographic, clinical, anthropometric and histopathological features of the breast cancer patients at the oncology clinic of TASH, Addis Ababa, Ethiopia, 2019

Variables		Frequency(n=40)	percentage
Age [a] (Mean ±SD)		44.50±9.05(26-60)	
Residency [b]	Addis Ababa	16	40
	Oromo	11	27.5
	Amhara	9	22.5
	Others	4	10
Menopausal status	Pre	22	55
	Post	18	45

Smoking habit	Yes	2	5
	No	38	95
Alcohol consumption	Yes	8	20
	No	32	80
Chat chewing	Yes	1	2.5
	No	39	97.5
Body mass index MI(kg/m^2)categories	<18	6	15
	18-24	24	60
	25-30	9	22.5
	>30	1	2.5
Family History of breast cancer	Yes	11	27.5
	No	29	72.5
Drug protocol	FAC	31	77.5
	AC	7	17.5
	FAC + Taxane	2	5
Tumor site	Right breast	19	47.5
	Left breast	21	52.5
Clinical tumor stage	I	0	0
	II	9	22.5
	III	26	65
	IV	5	12.5
Neo adjuvant therapy	Yes	7	17.5
	No	33	82.5
Surgery status	Yes	100	100
	No	0	0

Key: Age[a], 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 40 patients, FAC = 5-fluorouracil, Adriamycin, cyclophosphamide, AC = Adriamycin, cyclophosphamide

4.2. Serum cardiac biomarkers of the study participants

Serum cardiac and inflammatory marker tests (cTnT, CK-MB, CRP, TNF-α and IL-6) were performed at baseline and after the three cycles of chemotherapy treatment for 40 breast cancer participants. Accordingly, in the sample of breast cancer patients at the base line, all patients had normal level of cTnT and CK-MB levels below the cutoff point (< 0.01 ng/mL and < 24 u/L) respectively. But regarding the CRP level 6(15%) of the study participants showed base line elevation above the cutoff value (≥ 5mg/L) **(Table 2**).

After the breast cancer patients took three cycles of chemotherapy treatment, elevated amount in the levels of cardiac troponin (≥ 0.01pg/mL) , CK-MB (>20 u/L) and CRP (≥ 5 mg/L) was detected in 8 (20%),6(15%) and 14 (35%) of them respectively (**Table 2**).

Table: 2 Percentage (absolute number) of the study participants having abnormal levels of serum cardiac biomarker at baseline and after the three cycles of chemotherapy treatment at oncology clinic of TASH, Addis Ababa, Ethiopia, 2019

Variables **Cardiac Markers**	**Categories (cutoff points)**	**Base line value (N)**	**After 3 cycles of chemo. (N)**
cTnT	< 0.01 ng/mL	40(100 %)	32(80%)
	≥ 0.01 ng/mL	0(0%)	8(20%)
CK-MB	≤ 24 u/L	40(100%)	34(85%)
	> 24 u/L	0(0 %)	6(15%)
CRP	< 5 mg/L	34(85%)	26(65%)
	≥ 5 mg/L	6(15%)	14(35%)

Key: N = Number, % = percentage, cTnT = cardiac troponinT, CK-MB = Creatine kinase myocardial band, CRP = C- reactive protein

The mean levels of serum cardiac biomarkers in breast cancer patients at base line and after the three cycles of chemotherapy treatment are shown in (**Figure 4)**.

Before initiation of chemotherapy treatment the average levels of cTnT and CK-MB were found to be 4.25±1.90 pg/mL and 13.87±5.49 u/L respectively. In addition, our study shows that the mean values of CRP before the start of any chemotherapy treatment were 2.17±2.15 mg/L **(Figure 4).**

We had also evaluated the mean values of these cardiac biomarkers after patients had taken three cycle of chemotherapy treatment, and we found deviations from the pretreatment statuses. Accordingly, the average serum level of cTnT was recorded as 7.26 ± 4.11 pg/mL, which shows significant increment than the baseline value (p = 0.000001). Similarly a significant higher mean serum levels of CK-MB and CRP were found with a value of 16.17 ± 8.34 u/L (p = 0.029) and 3.87 ± 4.70 mg/L (p = 0.006) respectively (**Figure 4).**

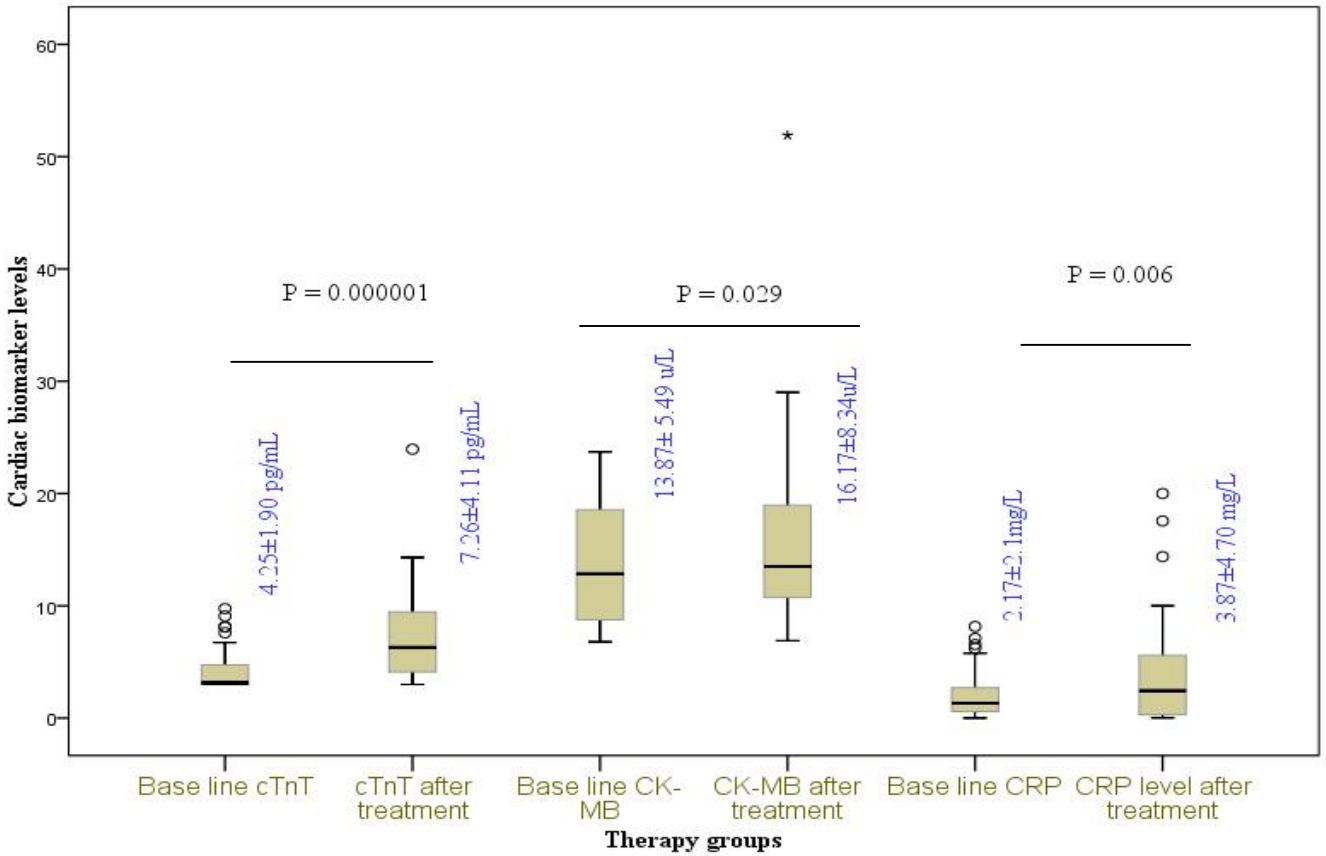


Figure 4: Comparisons of mean value of cardiac biomarkers level between the base line and after the three cycles of chemotherapy treatment of breast cancer patients at oncology Clinic of TASH, Addis Ababa, Ethiopia, 2019

Key: cTnT = cardiac troponinT, CKMB = creatine kinase myocardial band, CRP = C – reactive protein. Data are expressed as Mean ± SD; P value ≤ 0.05 was statistically significant.

4.3. Levels of inflammatory cytokines (IL-6 and TNF - α) in breast cancer patients

In the study samples, the levels of inflammatory cytokines (IL-6 and TNF-α) were also measured at base line and after three cycles of chemotherapy treatment.

The study revealed that base line mean serum levels of TNF-α and IL-6 levels were found to be 4.21±2.31 pg/mL and 6.41±11.41 pg/mL respectively **(figure 5).** In this study, deviations in the mean value of inflammatory cytokines were seen after the breast cancer patients had completed three cycles of chemotherapy treatment and recorded as 4.70 ±1.84 pg/ml for TNF- α and 2.90 ± 1.84 pg/mL for IL-6 **(figure 5).**

To see if there is a significant change in inflammatory markers (IL-6 and TNF-α) between the base line and after the three cycles of chemotherapy, paired samples t - test was done. The test revealed that levels of TNF-α increased significantly after chemotherapy with (p = 0.001, < 0.05). But the mean value of IL-6 between the baseline and after chemotherapy showed no statistically significance difference (p = 0.275, > 0.05) (**figure 5**).

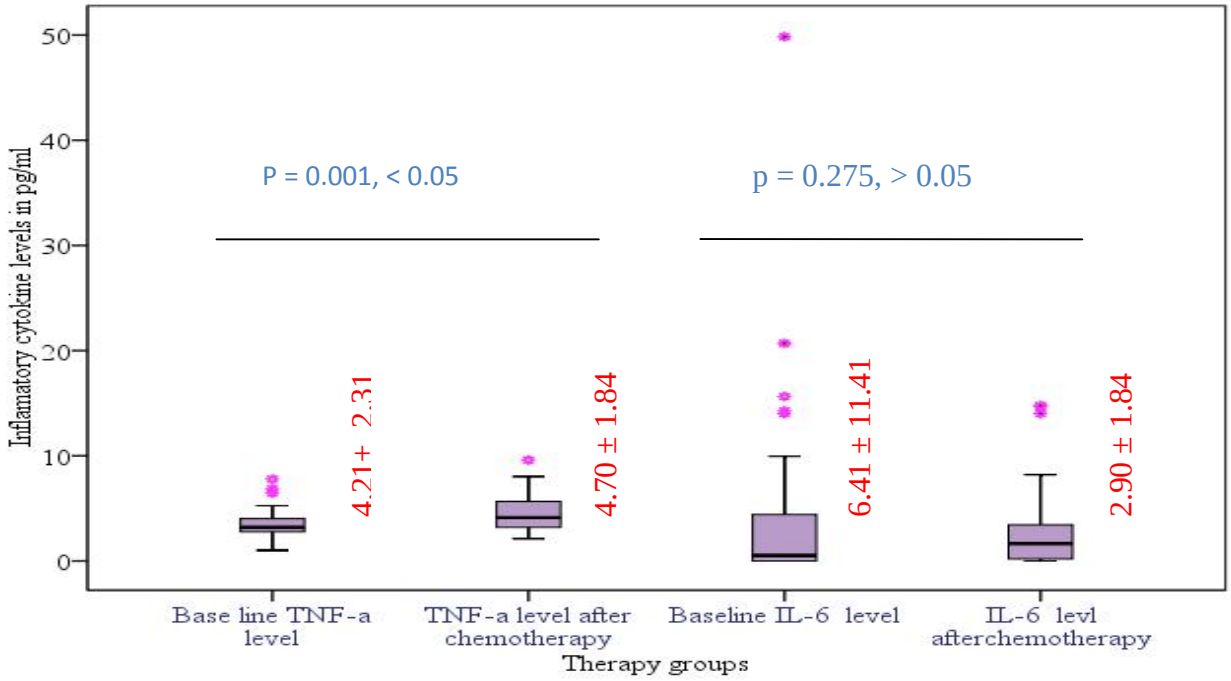


Figure 5: Comparisons of mean value of inflammatory (TNF-α and IL - 6) level between the base line and after the three cycles of chemotherapy treatment of breast cancer patients at oncology Clinic of TASH, Addis Ababa, Ethiopia, 2019

Key: IL-6 = interleukin 6, TNF-a = tumor necrosis factor alpha

4.3. Correlation analysis between the dependent variables before chemotherapy treatment

The correlation analysis showed no significance association between the dependent variables before chemotherapy treatment **(Table 3)**.

Table 3: Correlation analysis between the dependent variables before chemotherapy treatment

Variables	cTnT		CKMB		CRP		TNF- α		IL-6	
	r	p	R	P	r	P	r	p	R	p
cTnT			0.152	0.349	0.180	0.266	-0.017	0.936	-0.068	0.740
CK-MB					0.291	0.068	0.113	0.581	0.310	0.123
CRP							0.211	0.300	0.046	0.822
TNF-α									0.309	0.124
IL-6										

Key: cTnT = cardiac troponinT, CK-MB = creatine kinase myocardial band, CRP = C – reactive protein, IL-6 = interleukin 6, TNF-α = tumor necrosis factor alpha

After our study participants had taken three cycles of chemotherapy treatment, the Pearson correlation analysis showed a significant positive correlation between some of the dependent variables. Accordingly, cTnT level was positively and significantly correlated with levels of CK-MB (r = 0.484, p = 0.002) and CRP (r = 0.325, p = 0.041). In addition to this, CK-MB and CRP were also positively correlated(r = 0.324, p = 0042).

Table 4: Correlation analysis between the dependent variables after chemotherapy treatment

Variables	cTnT		CK-MB		CRP		TNF-a		IL-6	
	r	p	R	p	R	p	R	P	R	p
cTnT			0.484**	**0.002**	0.325*	**0.041**	-0.158	0.442	-0.082	0.689
CKMB					0.324*	**0.042**	-0.108	0.600	0.158	0.440
CRP							-0.209	0.305	0.311	0.122
TNF-a									0.026	0.899
IL-6										

Key: cTnT = cardiac troponinT, CKMB = creatine kinase myocardial band, CRP = C – reactive protein, IL-6 = interleukin 6, TNF-a = tumor necrosis factor alpha

4.4. Correlation analysis of sociodemographic characteristics and the dependent variables before chemotherapy treatment

Bivariate, Pearson correlation test was carried out to determine any association (relationship) between serum levels of cardiac biomarkers and inflammatory cytokine with clinical and sociodemographic characteristics. In the breast cancer patients before the start of chemotherapy treatment, serum levels of CK-MB was positively and significantly associated with the patients age (r = 0.362, p = 0.022) **(Table 5**).

Table 5: Correlation analysis of sociodemographic characteristics and the dependent variables before chemotherapy treatment

Predictors	cTnT		CK-MB		CRP		TNF-α		IL-6	
	r	P	R	p	R	p	r	P	R	p
Age	0.094	0.564	0.362*	**0.022**	-0.109	0.505	-0.227	0.265	0.023	0.912
BMI	-0.277	0.083	-0.231	0.152	0.140	0.389	-0.172	0.402	-0.259	0.202
Menopausal status	0.022	0.892	-0.030	0.856	-0.034	0.835	-0.134	0.514	0.103	0.617
Tumor site	0.053	0.743	-0.071	0.662	-0.158	0.331	-0.152	0.459	-0.223	0.274
Smoking	-0.143	0.373	0.063	0.701	0.085	0.600	-0.246	0.225	-0.094	0.647
Alcohol	-0.301	0.059	0.072	0.660	0.194	0.231	0.057	0.781	0.176	0.391
Chat	0.123	0.448	0.142	0.384	-0.044	0.789	-0.016	0.937	0.085	0.681
Neo-adjuvant	0.096	0.555	-0.060	0.714	-0.092	0.571	-0.095	0.644	-0.069	0.736
Family history of BC	0.023	0.889	-0.203	0.209	-0.065	0.693	0.187	0.360	0.018	0.932
Tumor stage	0.017	0.933	0.010	0.951	0.220	0.173	0.165	0.421	-0.311	0.122

** .Correlation is significant at the 0.01 level (2-tailed), * Correlation is significant at the 0.05 level (2-tailed)

Key: r = Pearson correlation coefficient, p = p value for Pearson correlation, TnT = troponin T, CK-MB, creatine kinase myocardial band, CRP = C - reactive protein, TNF- α = tumor necrosis factor alpha, IL - 6 = interleukin six, BMI = body mass index

4.5. Correlation analysis of sociodemographic characteristics and the dependent variables after chemotherapy treatment.

Bivariate, Pearson correlation test was done to determine the association between serum levels of cardiac biomarkers and inflammatory cytokine with the independent variables (Table 6). After patients took the three cycles of chemotherapy treatment, cTnT was negatively associated with BMI (r = - 0.498, p = 0.008). In addition a significantly positive association also observed between serum IL-6 level and alcohol consumption habit of breast cancer patients (r = 0.642 , p = 0.0004) (Table 6).

Table 6: Correlation analysis of sociodemographic and clinical characteristics and the dependent variables after chemotherapy treatment

Predictors	cTnT		CKMB		CRP		TNF- α		IL-6	
	r	P	R	p	R	p	r	P	R	p
Age	0.302	0.058	0.146	0.367	0.144	0.374	-0.318	0.113	-0.117	0.569
BMI	-0.414**	**0.008**	-0.019	0.907	0.098	0.547	-0.217	0.288	0.371	0.062
Menopausal status	0.046	0.778	0.003	0.984	0.061	0.707	0.002	0.993	0.011	0.957
Tumor site	0.237	0.141	-0.168	0.301	0.002	0.989	-0.270	0.183	0.128	0.533
Smoking	-0.098	0.548	0.097	0.552	-0.032	0.845	0.262	0.196	0.058	0.778
Alcohol	-0.281	0.079	-0.055	0.707	-0.027	0.867	-0.120	0.559	0.642**	**0.0004**
Chat	0.154	0.344	0.021	0.064	-0.029	0.859	-0.050	0.807	0.042	0.837
Neo-adjuvant	-0.047	0.773	-0.100	0.540	-0.205	0.205	0.106	0.607	0.114	0.579
Type of chemo	0.054	0.743	0.128	0.432	0.160	0.323	-0.052	0.799	0.344	0.086
Family	-0.102	0.532	-0.180	0.268	-0.137	0.400	0.207	0.320	0.090	0.660

history										
Tumor stage	0.052	0.782	-0.024	0.884	0.140	0.390	-0.019	0.927	-0.213	0.297

**Correlation is significant at the 0.01 level (2- tailed), *Correlation is significant at the 0.05 level (2- tailed)

Key: r = Pearson correlation coefficient, p = p value for Pearson correlation, cTnT = troponinT, CK-MB, creatine kinase myocardial band, CRP = C - reactive protein, TNF- α = tumor necrosis factor alpha, IL-6 = interleukin six, BMI = body mass index

5. Discussion

With the scale up of using chemotherapy drugs and the increasing number of long term breast cancer survivors, patients often exposed for many different organ injury, most importantly the heart from toxic chemotherapy drugs (Bomzer, 2014). And the optimal treatment for chemotherapy-induced cardiomyopathy is prevention. Biomarkers allow individuals at risk for developing long-term cardiac complications to be identified, in many cases before any other clinical evidence is apparent (Blaes *et al*., 2015). So the need to monitor side effects of these drugs is crucial for the general health of the breast cancer patients and assessing of serum cardiac biomarkers (cTnT, CK-MB and CRP) and inflammatory cytokines (IL-6 and TNF-α) in breast cancer patients on chemotherapy treatment helps in early prediction of cardiac injury. With this, the present study evaluates these potential biomarkers from serum samples.

For evaluation, a total of 40 female breast cancer patients who were under chemotherapy treatment were involved in the study at TASH, Ethiopia. Data coming from this study ascertained that the mean age of female breast cancer patients was 44.50±9.05 yeas (range 26-60 years) and the majority (62%) of them was found within the age group of below 50 years old; and nearly 55% of our study participants were in pre-menopausal stage. This is in agreement with previous study done in Ethiopia and the authors reported that more than 70% of breast cancer cases were diagnosed below 50 years of age (Abate *et al*., 2016). But this result was contradicted with most of the report from developed countries in that more than two-thirds of breast cancer cases are diagnosed in women aged 50 years and older (Coughlin and Cypel, 2013). This could be due to differences in genetic and environmental issues like practice of physical activity, breast feeding patterns, differences in alcohol consumption and diet.

The results of the current study revealed that there was a high statistical significant difference ($p<0.05$) in the mean value of cardiac biomarkers and proinflammatory cytokine levels of female breast cancer patients between base line and after they receive three cycles of chemotherapy drugs. The significant higher mean levels of cTnT in our female breast cancer patient participants after the three cycles of chemotherapy is in agreement with the results of other different related studies done worldwide Agree with (Lipshultz *et al*., 2004, Kilickap *et al*., 2005, Katsurada *et al*., 2014, Blaes *et al*., 2015, Frères *et al*., 2018).

The likely explanation of this higher mean value might be due to the toxic effect of chemotherapy drugs on the cardiac which leads to the release of structurally bound troponin from the myofibril of necrotic cardiomyocytes in to the blood stream resulting significant increment of serum troponin (Nigam, 2007). According to Lipshultz and colleagues, serum cTnT increased during doxorubicin administration in doxorubicin-treated children, and it was decreased as a result of dexrazoxane therapy (Lipshultz *et al*., 2004). Another report also showed that increased serum cTnT level can be detected in the early stages of anthracycline therapy (Kilickap *et al*., 2005).

Troponins are normally undetectable, but their level may increase within 2 or 3 hours after cardiac damage occurs. This release kinetics is related to the distribution of these proteins within the myocardial cell. About 94-97% of these troponins are bound to myofibril and only 3% of cTnI and 6% of cTnT is free in the cytoplasm.

When the myocardial damage occurs, the cytosolic troponins reach the blood stream quickly resulting in a rapid peak of serum troponin observed during the first few hours. This is followed by the release of structurally bound troponin resulting in a second peak lasting for several days. These detectable serum levels of cTn are an indicator of heart muscle damage (Nigam, 2007). Therefore measurement and interpretation of troponin is important and it is part of the diagnosis of acute myocardial infarction (AMI) and workup of possible cardiac chest pain (Shah *et al*., 2017, Curigliano *et al*., 2016).

Regarding the serum levels of CK-MB, our study revealed a significant increased after the three cycles of chemotherapy treatment ($p = 0.029$), which was in agreement with Blaes et al in that they reported a significant higher mean level in their breast cancer patients after treated with doxorubicin ($p = 0.02$) (Blaes *et al*., 2017). Elevation in mean values of CK-MB level after chemotherapy treatment in this study can most likely be explained as of the combined effect of chemotherapy drugs to the heart, CK-MB released to the blood stream and its serum level become elevated to the detectable value. CK-MB is the heart specific isoenzyme that exists in large quantity in heart muscle than the rust of the body its appearance in serum reflects its unique presence in myocardial tissue and supports in the diagnosis of suspected myocardial infarction in clinical laboratories (Christenson *et al*., 1999, Al-Hadi and Fox, 2009, Emokpae and Nwagbara, 2017). However, our results contradicts with other reports (Horacek *et al*., 2007, Erdim *et al.*, 2009), which we fall short of justification.

In the present study, the prevalence of cardiac biomarkers abnormality levels have been summarized as per the criteria of the recent ESC, ACC, AHA and WHF reports to define myocardial injury and infarction. Accordingly, the term myocardial injury should be used when there is evidence of elevated cTn values with at least one value above the 99th percentile upper reference limit (URL). The guidelines also recommend the 99th percentile value of a healthy population (0.01ng/mL) as a cutoff point for acute myocardial infarction (Thygesen *et al*., 2018).
This study showed base line cTnT level remains below the cut off values in all patients (< 0.0l ng /mL) which is in lined with another comparable study done in Czech Republic (Horacek *et al.*, 2010). But after three cycles of chemotherapy treatment, an abnormal elevation was observed in 8 (20%) of our study participants. This finding was lower than the report done by different authors (Erdim *et al*., 2009, Lipshultz *et al*., 2012, Frères *et al*., 2018). Elevated amount of cTn in 26 (63%), 42% and 47% of the study participants were reported by Erdim et al, Frères et al and Lipshultz et al. respectively.
This variation could be due to the differences in sample size, age, drug protocol and duration of treatment cycle among studies.
A study done by Daniels et al. found that detectable TnT was associated with increased risk of death (hazard ratio 1.85, P = 0.001) in their study participant of 957 elderly adults (Daniels *et al*., 2008). Elevations in cTnT have been associated with structural and functional measures of heart disease; it is biologically plausible that individuals with elevated baseline cTnT levels and levels after treatment may both suggest these subgroups are more likely to have a decline in LVEF and at a higher risk of developing chemotherapy-induced cardiomyopathy (Blaes *et al*., 2015).
In the present study, CK-MB values > 24 u/L were considered elevated and suggesting cardiac injury associated with the treatment (Stein, 1985, Thomas *et al.*, 2005). Hence, in this study, baseline value of CK-MB Levels remained below the cutoff value (>24 u/L) in all of our study participants. But elevated amount was shown in 6 (15%) of the study subjects after they received three cycles of chemotherapy treatment. This finding is contradicted with a study done in Czech Republic and India by Horacek et al. and Datta et al. respectively that CK-MB level remained within the reference range in all patients of their study subjects (Horacek *et al*., 2007, Datta *et al*., 2015). The reason for this variation was not clear but it might be due to the non homogenization of the study subjects.

A huge number of available data suggested that conventional chemotherapy and targeted therapies are associated with an increased risk of cardiac damage including LVD and heart failure, treatment-induced hypertension, vasospastic and thromboembolic ischemia. It also associated with rhythm disturbances, including conduction system damage and potentially QTc prolongation that may be rarely life-threatening (Perez *et al*., 2011, Bowles *et al*., 2012).

Despite frequent attempts, the molecular mechanism of chemotherapy-induced cardio toxicity has not been fully identified yet. But there are many different postulated mechanisms for different chemotherapy drugs regarding the molecular mechanism of how they induced cardio toxicity (Christenson *et al*., 2015). For example, the mostly proposed mechanism of 5-FU induced cardio toxicity based on ultrasound and angiographic findings in different studies is through coronary vessel spasm (vasospasm) leading to ischemia to myocardium (Schimmel *et al*., 2004, Christenson *et al*., 2015). Cardio toxicity of cyclophosphamide is due to the effect of toxic metabolite on endothelial cells that causes severe myopericarditis and myocardial necrosis (Iqubal *et al*., 2018).

Topoisomerase II beta inhibition and formation of supper oxides and free radicals are well studied mechanisms among many speculated mechanisms by which anthracycline (DOX) induced tumor cytotoxic effect (Mobaraki *et al*., 2017). Anthracycline (DOX) intercalates with topoisomerase II β that reduces the activity of DNA synthesis and its topology (Moudgil and Yeh, 2016). As a result, it induces production of reactive oxygen species that disrupts the mitochondrial membrane integration. In addition, it reduces the oxygen binding capacity of hemoglobin since it form a complex with iron (Hanrahan *et al*., 2007, Mobaraki *et al*., 2017).

Our study also showed a significant increment in the mean value of hs-CRP level, a cardiac and an acute phase inflammatory marker, after the three courses of chemotherapy treatment in female breast cancer patients, which is in agreement with a report made by (Nuver *et al*., 2004). This high level of hs-CRP after chemotherapy is may be due to the non specific feature of chemotherapy drugs that kill the normal cells together with cancerous cells and brings more tissue damage (including cardiomyocytes) as a result of the dose and course of therapy. Another possible explanation may be since the smooth muscle cells of the human coronary arteries may also produce CRP as a local response to inflammation (Calabró *et al*., 2003).

The study participants having CRP level ≥ 5mg/l were higher (35%) after the third course of chemotherapy treatment than at base line (15%) which was lower than the prevalence reported by Morris and his colleague in that they found 47 % and 61% abnormal elevated levels of CRP at base line and after chemotherapy treatment respectively (Morris *et al*., 2011b). This variation is most probably due to difference in the length of follow-up in the study participant and difference in drug protocol; their participants were under dose dense chemotherapy treatment followed by trastuzumab and lapatinip for 18 months. But our study subjects were under conventional FAC combination drug treatment for 3 month follow up. The incidence of cancer treatment-induced CV injury varies widely, depending on the specific cancer therapy used, duration of therapy, and underlying patient co morbidities (Curigliano *et al*., 2016).

The current retrospective study showed that the average level of inflammatory cytokine TNF- α increased significantly after the breast cancer patients received three courses of chemotherapy treatment ($p = 0.001$). This is in line with a study conducted by Perik et al. in that they reported significant increased in median level of TNF- α after their breast cancer survivors study participants took 5 x FEC (five cycles of fluorouracil, epirubicin cyclophosphamide) ($p < 0.05$) (Perik *et al*., 2006). The increment in the levels of TNF-α during chemotherapy treatment could be due to the response of necrotic cells induced by the chemotherapy drugs. Chemotherapy drugs can induces the release of inflammatory cytokines from breast tumor cells, stromal cells, or peripheral leukocytes (Pusztai *et al*., 2004).

Damage-associated molecular patterns trigger leukocyte release of inflammatory cytokines, which drives systemic inflammation and is characterized by increased levels of tumor necrosis TNF-α, IL-6, and CRP (Thomas and Lip, 2017)**.**

In this study we found no significance change in the average level of IL- 6 after the female breast cancer patients received three cycles of chemotherapy treatment ($p = 0.27$). It was reported that in FAC treated study groups, IL-6 level showed no significant change (Pusztai *et al.*, 2004). Puszati et al. also reported a significant change of IL-6 in their paclitaxel treated groups which are contradicted with our finding. This could be probably because the expression levels of different inflammatory cytokines could be varies according to the chemotherapy drug type and their specificity as well as the protocol used.

In the present study, the correlation analysis revealed that there was no any significant correlation between the dependent variables in the sample before chemotherapy treatment. But, unlike the base line result, significant associations were observed between some of the dependents variable after the study subjects took three consecutive cycles of chemotherapy treatment. Accordingly, cTnT and CK-MB showed a significant positive correlation ($r = 0.484$, $p = 0.002$) which is in agreement with the previous study reported a significant correlation($r = 0.75$) between CK-MB and cTnT (Reddy *et al*., 2004).

In addition to this, the correlation analysis also shows that there was a positively significant correlation of CRP level with cTn and CK-MB ($r = 0.325$, $p = 0.041$ and $r = 0.324$, $p = 0.042$) respectively. This finding was in concordance with a study by Lim and his colleagues whom reported a significant correlations of CRP with TnT ($r = 0.35$, $P < 0.02$) and CK-MB ($r = 0.51$, $P < 0.01$) (Lim *et al*., 2014). Another study also found a strong positive correlation between CRP with CK-MB and cTnT ($p > 0.05$) (Gurunani *et al*., 2015). Similarly Aseri et al. reported that CRP correlated significantly with CK-MB ($r = 0.405$) (Aseri *et al*., 2014).

The current study tried to investigate the associations of some demographic and clinical features with cardiac and inflammatory biomarker abnormalities. Accordingly, patients BMI showed a negative association with serum cTnT levels among study participants both at base line (not significant) and after the three cycles of chemotherapy treatment (significant with $r = -0.414$, $p = 0.008$)

A comparable study which support our finding was done by Oreopoulos et al. and Stosovic et al. and found a mean value of TnT that was inversely correlated with body mass index (Oreopoulos *et al*., 2008, Stosovic *et al.*, 2014).

It is well-known that in the general population obesity is associated with risk of developing cardiovascular disease and increased mortality (Poirier *et al*., 2006). Obesity causes an increase in cardiac output and hence overall cardiac workload to meet the metabolic demands of the expanded adipose tissue. It has been linked to several cardiovascular changes; including hyper dynamic circulation, structural changes, sleep apnea, and overt heart failure. This combination of factors leads to a concentric left ventricular hypertrophy and cardiovascular effects, even without associated hypertension (Vasan, 2003).

However, several studies done worldwide have described an inverse correlation between BMI and mortality in patients with coronary artery disease. This could be probably due to the so called ‘‘obesity paradox’’.

Our study also revealed a significant positive correlation between the age and CK-MB levels of breast cancer patients ($r = 0.362$ $p = 0.022$). Studies showed that the risk for cardiovascular diseases increases with age (Blaes *et al*., 2017).

In this study we have found a significant positive correlation between the general alcohol consumption habit of breast cancer patients and serum IL-6 level after female breast cancer patients took the three cycles of chemotherapy treatment ($r = 0.642$, $P = 0.0004$).But this study did not included alcohol type and quantity as a result we failed to discuss the relation among different quantity and beverage type of alcohol that the study participants had taken.

This is supported by a study on the relationship of alcohol intake with inflammatory markers and plasminogen activator inhibitior-1 in well-functioning older adults by Volpato et al. and found a positive correlation between alcohol consumption habit and IL-6 level ($p < 0.001$). The probable justification of this could be because ethanol might modulate IL-6 production and clearance at several sites, including adipose tissue. In addition, ethanol might modulate the effect of IL-6 on the stimulation of acute phase protein production from hepatocytes (Volpato *et al*., 2004).

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6. Conclusion

The findings in this study showed an elevated serum cardiac biomarker values above their respected cutoff points in some of the breast cancer patients after they received three cycles chemotherapy treatment. It also revealed a significant increment in the levels of serum cardiac and inflammatory biomarkers in breast cancer female patients after they received three cycles of chemotherapy treatment when compared with the base line values. This significant increment of cardiac biomarkers up on chemotherapy treatment confirms that chemotherapy drugs have a potential cardio toxic effect. Therefore, it is highly recommended that study with serial measurement of cardiac biomarkers and echo-cardiography findings to assess functional abnormality are very crucial in cancer patients on chemotherapy for a better cardiac risk classification and treatment outcome.

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7. Strength and limitation of the study

As far as our knowledge concerned, this study is the first work attempted to investigate the pharmacokinetics of chemotherapy drugs on cancer survivor in Ethiopian breast cancer patients. In addition, this study is a retrospective study which helps us to see the different change in biomarker levels of each individual patient (cause and effect relationship).

Despite the aforementioned strengths, this study has several weaknesses. First because the sample size was small, it could not be easy to say the study represents the whole Ethiopian breast cancer patients under chemotherapy. Lack of previous study findings in Ethiopia limited the comparison of these study findings with other findings. The study did not include clinical findings like change in LVEF and compare with change in cardiac biomarker levels. Other variables capable of affecting cardiac function like blood pressure, HDL level, diabetes, family history of heart disease, drug dose and other psychosocial factors were not fully included under the study.

8. Recommendations

The following recommendations are suggested to further investigate the effect of chemotherapy drugs induced cardio toxicity in breast cancer patients:

- Life style and other psychosocial factors like economic status, educational level, dietary habits, and work type and habit should be assessed in further studies.
- Further studies could be conducted with larger sample size and full cycle follow up with incorporation of more variables using multiple cardiac blood markers including cardiac function assessment
- Early diagnosis and monitoring the cardiac status of each cancer patients at base line, in each subsequent cycle and post chemotherapy treatment is very important for a better care and treatment of the patients.

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10. ANNEXES

Annex 1: Information sheet (English Version)

Research Project: Change in serum cardiac and inflammatory biomarkers level following chemotherapy treatment among female breast cancer patients attending at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

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

Principal Investigator: Muluabay Getie (BSc in Biology, MSc in Medical Biochemistry candidate)

Advisors: Daniel seifu (PhD), Yonas Mulugeta (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 ‘‘to evaluate changes in cardiac and inflammatory biomarkers level following chemotherapy treatment among female breast cancer patients’’ attending at TASH

Participants to be included: All breast cancer patients who are admitted for chemotherapy treatment and meet the inclusion criteria will be included in the study.

Risks and discomfort: There is no risk in participating in this study. You will be asked to give one or two small blood samples, taken from your arm by the routine safe method for drawing blood by a health care professional. Risks from this are minimal. There could be minor pain and change in color of your skin following the blood drawing and which would disappear in short duration. The amount of blood taken from each volunteer throughout the study period is not more than 5ml; which will not affect your health.

Benefits and incentives: The result of the finding may, if it is helpful, be communicated to your physician for use in the management of the disease. You will have the chance to know your test result. You will not be provided with any direct incentives for your participation in the research.

Confidentiality: All information about the participants will be kept confidential. Logbooks 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 investigators.

Participant Rights: Your participation is entirely voluntary and up to you to decide. There is no penalty if you do not agree to participate. Also you have the right not to answer any questions you do not want to. You have full right to withdraw from participating in this study at any time before and after consent without explaining the reason. Your decision will not affect your right to get health service that you are otherwise entitled to.

Persons to contact: If you have any question, you can ask at any time. If you have additional questions about the study, you can contact the:

Principal investigator: Muluabay Getie

Cell phone: 0913728361,

E-mail: muluabayget@gmail.com

Thank you for your cooperation.

10.2. Annex 2: Informed consent (English version)

Department of medical 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 changes in cardiac and inflammatory biomarkers level following chemotherapy treatment among female 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 III

Socio-demographic and Clinical data recording Format

Admission Date _____/_____/_____

(To be filled by the data/sample Collector nurse)

Section I. Review on General Information

1.1. Initials (code) _____

1.2. Age (in years) _____

1.3. Sex Male ———— Female ————

1.4. Ethnicity _____

1.5. Current weight (Kg) _____

1.6. Height (m) _____

1.7. BMI (kg/m^2) _____

Section II. Breast cancer related information

2.1.1. Menopausal status Premenopausal ———— Postmenopausal ————

2.1.Histological type of the cancer:

2.1.1. Infiltrating ductal ————

2.1.2. Intraductal ————

2.1.3. Lobular ————

2.2.Tumor size at diagnosis ————

2.3.Degree of differentiation:

2.3.1. Well differentiated ————

2.3.2. Moderately differentiated ————

2.3.3. Poorly differentiated ————

2.4.Nodal involvement

2.4.1. Number of lymph nodes positive_____

2.4.2. Number of lymph nodes removed_____

2.6. TNM stage of the tumor _____

2.7. Site of tumor: on Left breast ———— on Right breast ————

2.8. Patient Habits of smoking Yes ———— No ————

2.9. Chat chewing status Yes ———— No ————

2.10. Alcohol drink status Yes ———— No ————

2.11. Presence of severe concomitant disease Yes ———— No ————

Section III. Treatment related information

3.1 Type of the current chemotherapy setting (Protocol)

Neo-adjuvant ————

Protocol CMF / AC / FEC / FAC / (others specify_____

Adjuvant ————

Protocol CMF / AC / FEC / FAC / (others specify_____

Metastatic ————

Protocol CMF / AC / FEC / FAC / (others specify_____

3.2. For patients on adjuvant chemotherapy (in Q3.1), was the patient put on primary (neo-adjuvant) therapy previously?

Yes ——— No ——— date _____

3.3. For patients on adjuvant chemotherapy (in Q3.1), was surgery done before this chemotherapy?

Yes ———— No ————

3.4. Type of surgery _____

3.5. Co-medications (if any, including premedication) (Name, dose, etc)

_____

3.6. Is the patient on other medical conditions (if any specify?) _____

3.7. Did the mother, father, sister or brother have breast cancer? Yes_____ No_____

3.8. Have you had radiation treatment to your chest? Yes_____ No_____

3.9. Have you had any of the following treatments for your cancer yet? If so, please can you estimate the date this treatment started? Please tick all that apply.

a) Surgery Yes ____ No_____ Date of first treatment _____

b) Chemotherapy Yes ____ No_____ Date of first treatment _____

c) Others / Please specify _____

3.10. Which panel of chemotherapy regimen is prescribed?

CMF: cyclophosphamide, methotrexate, and 5-fluorouracil _____

FAC: 5-fluorouracil, doxorubicin, cyclophosphamide _____

TC: Taxotere (docetaxel) and cyclophosphamide _____

AC: Adriamycin (doxorubicin) and cyclophosphamide _____

Others specify ———————————————————————————

IV. Clinical and/or Lab parameters

4.1. Time of blood sample taking

T1 _____ T3_____

T2 _____ T4_____

_____

Completed by: Approved by:

Name _____ Name _____

Qualification _____ Qualification_____

Signature _____ Signature_____

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፡፡

የመረጃና የስምምነት ቅፅ

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፡፡

‘‘To evaluate changes in cardiac and inflammatory biomarkers level following chemotherapy treatment among female breast cancer patients attending at Tikur Anbessa specialized hospital’’

የጥናቱ ርእስ ሲሆን አላማውም የጡት ካንሰር ያለባቸው የ ኬሞቴራፒ ታካሚዎች በደማቸው ውስጥ ያለውን የልብ የጤንነት ሁኔታን የሚያመላክቱ ነገሮችን እንዲሁም ሌሎች ከልብ 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 በጎላ 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

ሞባይል፡ **0913728361** ሙሉዓባይ ጌቱ ፣ኢሜል አድራሻ፡muluabayget@gmail.com

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 ኑሮባቸው የኬሞቴራፒ ህክምና ተከታታ ታካሚዎች በደማቸው ውስጥ ያለውን የልብ የጤንነት ሁኔታን የሚያመላክቱ ነገሮችን እንዲሁም ሌሎች ከልብ 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 እንደማይባክንና እንደሚያዝም ተነግሮኛል፡፡

በተጨማሪም ጥናቱ ዉስጥ ላለመሳተፍ ከፈለኩኝ መብቴ የተጠበቀ እንደሆነና በማንኛዉም 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፡፡

ማንኛዉንም ያልገባኝን ነገር የመጠየቅ እድል ተሰጥቶኝ በሚገባኝ ቋንቋ መልስ አግኝቻለሁ፡፡ በተጨማሪም የሁሉም የላብራቶሪ ምርመራ ዉጤቶች በጊዜዉ ለሀኪሜ 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 _____ _____