ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF MEDICAL BIOCHEMISTRY

Postsurgical Assessment of Serum Interleukin-6 and C-reactive protein in Breast Cancer Patients before Receiving Adjuvant Chemotherapy

BY: Ketsela Yirdaw (BSC)

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

June, 2016

ADDIS ABABA, ETHIOPIA

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This is to certify that a thesis prepared by **Ketsela Yirdaw Woldehawariat** entitled on: "postsurgical assessment of serum interleukin-6 and C-reactive protein in breast cancer patients before receiving adjuvant chemotherapy" is submitted in partial fulfillment of the requirements for the Master of Science Degree in Medical Biochemistry.

Signed by the Examining Committee:

External examiner	Signature	Date
Internal examiner	Signature	Date
Advisor	Signature	Date
Advisor	Signature	Date

Chair of Department or Graduate Program Coordinator

Statement of Declaration

I, the undersigned, declare that this MSc thesis is my work in collaboration with the AAU breast cancer project, has not been presented for a degree in this and any other university and that all sources of materials used for this thesis have been duly acknowledged. All scholarly matter that is included in this thesis has been given recognition through citation. I affirm that I have cited and referenced all sources used in this document. Every effort has been made to avoid any plagiarism in the preparation of this thesis.

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Signature: _____

This thesis has been submitted with my approval as University advisor

Name: Daniel Seifu, PhD

Signature: _____

Acknowledgement

I want to express my sincere thanks to Dr. Daniel Seifu my research advisors, whose help from the beginning to the end of this project was very substantial. I am very much indebted for his maximum cooperation for providing research facilities and for their encouragements.

My sincere appreciation is also extended to University of Gondar, the School of Graduate Studies, Addis Ababa University (AAU) and department of Medical Biochemistry for their financial supports.

I express my deepest regards for Mr. Feyissa Challa (Ethiopia public health institution, National HIV Laboratory Clinical Chemistry Unit), for analyzing the blood samples.

I would like to express my thanks to Mr. Yhonas Mulugeta and Mr. Mohamed Mehdi for there all rounded assistance and their time during data collection and laboratory analysis.

Last but not least, I would like to thank members of my family and my class mates, for their moral supports as well as for their constructive ideas and comments.

Abstract

***Background**: Breast cancer is one of the most common cancers in females. Several studies have shown proinflammatory biomarkers can facilitate tumor growth and metastasis by altering tumor cell biology and activating stromal cells in the tumor microenvironment. They have been also associated with poor survival of breast cancer patients.*

***Objective:** The aim of this study was to evaluate postsurgical serum interleukin 6 and C reactive protein in breast cancer patients before receiving chemotherapy in Tikur Anbessa specialized teaching hospital, Ethiopia.*

***Methodology:** A hospital based cross sectional study design and a convenient non-probability sampling technique were applied in this study. Forty-four breast cancer patients visiting oncology chemotherapy unit were identified for the study and were subjected to assessment. Serum interleukin-6 and c –reactive protein values were measured before initiation of adjuvant chemotherapy in breast cancer patients using an ElectroChemiLuminescencetechnology (ELC) assay technique and immunoturbidimetric respectively.*

***Result:** A total of 44 cases were included in the study. The median age was 42 years, the majority of patients had invasive ductal carcinoma, and did present with positive nodes involvement. Postsurgical elevated levels of serum CRP and IL-6 were observed in most of the patients. Progress in breast cancer staging were associated with increases in serum IL-6. IL-6 level correlated with stage ($P < 0.026$), CRP has borderline correlation with stage ($p = 0.05$). However, IL-6 and CRP have shown insignificance correlation with tumor size and lymph node involvement. Serum IL-6 levels were correlated positively with CRP levels ($rho = 0.530$, $p < 0.01$).*

***Conclusion:** These results suggest that proinflammatory status of the breast cancer patient have high values of interleukin-6 and C– reactive protein. Progressive elevation of serum interleukin 6 observed as stage of cancer advanced.*

***Key words:** interleukin-6, C - reactive protein, breast cancer, proinflammatory*

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Acronyms and Abbreviations

BMI	Body Mass Index
BRCA 1/2	Breast cancer gene 1 /2
CAD	Cardiovascular disease
CRP	C – reactive protein
CSC	Cancer stem cell
ECL	ElectroChemiLuminescence
ER	Estrogen receptor
IL	Interleukin
HER2	Human Epithelial Growth Factor Receptor 2
CRP	C – Reactive Protein
IQR	Interquartile range
JAK	Janus Kinase
M2	Macrophage 2
MDA-MB-231	M.D. Anderson - metastatic breast
MCF-7	Michigan Cancer Foundation-7
MRM	Modified radical mastectomy
NF-κB	Nuclear Factor kappa light chain enhancer of activated B cells
PR	Progesterone receptor
TGF-β	Transforming Growth Factor beta
TNBC	Triple negative breast cancer
TNM	Tumor size (T), Number of Lymph node (N), Metastatic (M)
STAT	Signal transducer and activator of transcription

1. Introduction

1.1. Background

Breast cancer is the most frequent malignant tumor and the second most commonly diagnosed cancer in women behind non-melanoma skin cancer and the second leading cause of death in women behind lung cancer in developed regions(Ferlay *et al.*, 2015).

Numerous risk factors have been found to increase a woman's risk of developing breast cancer. The common risk factors are gender, advanced age, early age at menarche, nulliparity, a first degree relative with breast cancer, exposure to estrogen, exposure to radiation and smoking, alcohol intake, physical inactivity, as well as adult and postmenopausal obesity (Kushi *et al.*, 2012).

Breast cancers can be divided in two types according to their origin; sporadic cancers which are largely related to estrogen exposure, and hereditary cancers which associate to family history of first degree relative with breast cancer or germline mutations. Hereditary breast cancers account for 5-10 % of all breast cancers(Lacroix & Leclercq, 2005). The most common cause of hereditary breast cancer is an inherited mutation in the tumor suppressor's gene in Breast cancer gene 1 (BRCA1) and Breast cancer gene2 (BRCA2).

Breast cancers are defined according to the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Gene-expression profiling has further defined the molecular subtypes of breast cancers according to their gene-expression profile. Molecular profiling identified five main categories of breast cancer subtypes. They are described as luminal A, luminal B, HER2/neu/ERBB2 positive, basal and normal breast like (Perou *et al.*, 2000; Sørlie *et al.*, 2001; Sørlie *et al.*, 2003).

Luminal A and luminal B are commonly ER positive, and possess expression patterns typical for luminal type cells. Patients with luminal tumors generally have better prognosis(Sørlie *et al.*, 2001) . The HER2/ErbB2 positive types of breast cancers are characterized by over expression of HER2 genes. These tumors are mostly ER negative and indicate aggressive breast carcinoma(Kumar *et al.*, 2010). Basal-like type tumors are derived from basal cells and largely correspond to triple-negative cancers (ER-, PR- and HER2-)(Chavey *et al.*, 2007; Nielsen *et al.*, 2004). These type of breast cancers are often associated with BRCA1 mutations. Basal like and HER2 positive tumors are considered as the most aggressive breast carcinoma types and decreased patient survival and high susceptibility to disease relapse (Sørlie *et al.*, 2003).

Understanding of breast cancer subtypes and hormone receptor status and over expression of HER2 have implications for systemic treatment in breast cancer patients as targeted treatment options are now available(Lin & Winer, 2008). Treatment for breast cancer includes often surgery, radiation therapy, and chemotherapy depending on disease stage. In addition, therapies targeted at tumor expressed proteins such as HER2 and ER have become available for breast cancer patients (Romond *et al.*, 2005).

Carcinogenesis is understood to be a multistep process during which cells progressively acquire a number of neoplastic characteristics or “hallmarks”. Initially, this concept included six hallmarks of cancer and described the key mutations in cell physiology that underlie malignant progression: tumor cell proliferation; escape from growth suppressors; invasion and metastasis; replicative immortality, angiogenesis and resisting cell death(Hanahan & Weinberg, 2000, 2011).In the last decade, understanding of carcinogenesis has evolved and two hallmarks have emerged-deregulation of cellular metabolism and evasion of cancer cells from immune recognition and destruction(Hanahan & Weinberg, 2000, 2011).

Inflammation in the tumor microenvironment stimulates tumor growth, invasion, and metastasis. It seems to favor invasion and metastasis more than to mount an effective host antitumor response. Many tumors arise at sites of chronic inflammation or they trigger inflammatory responses that result in the formation of an inflammatory microenvironment around the tumor(Allin *et al.*, 2011; Balkwill, 2002).

Inflammation is associated with poor prognosis and decreased survival in many types of cancer. As a marker of persistent inflammation, elevated concentrations of the inflammatory cytokine interleukin-6 (IL-6) have been shown to be associated with shorter survival periods in patients with various cancers, including colorectal and pancreatic cancers, melanoma, head and neck squamous cell carcinoma, soft tissue sarcoma, and diffuse large-cell lymphoma(Nikiteas *et al.*, 2005).

Inflammatory status may also be a prognostic factor for breast cancer. Clinical and experimental data suggest that chronic inflammation promotes mammary tumor development through mechanisms involving chronic activation of humoral immunity and infiltration of T helper 2 cells and polarized innate inflammatory cells(DeNardo & Coussens, 2007). Breast cancer patients have elevated concentrations of C-reactive protein(CRP) before surgery, more so in women with advanced disease, suggesting that CRP may be related to tumor burden or progression (Pierce *et al.*, 2009).

Cytokines play a role in human breast carcinogenesis. In fact many cytokines have been found expressed by cancer cells or produced in the microenvironment of the primary or metastatic tumor(Carpi *et al.*, 2009).IL-6 and CRP have related roles in the inflammatory response: IL-6 induces CRP production in the liver by activating Janus kinases. Signal transducers and activators of transcription subsequently switch on the CRP gene expression, leading to the production of CRP (Heikkilä *et al.*, 2007). Inflammatory status plays a pivotal role in the immunomodulation of the tumor environment, as cytokines can exert a stimulatory or suppressive effect, affecting prognosis and the response to therapy (Herrera *et al.*, 2012).

1.2. Statement of the Problem

Cancer is a major cause of morbidity and mortality, with approximately 14 million new cases and 8 million cancer-related deaths in 2012, affecting populations in all countries and all regions(Ferlay *et al.*, 2014; Stewart & Wild, 2014).

Prevalence estimates for 2012 indicate that there were 8.7 million people (older than 15 years) alive who had had a cancer diagnosed in the previous year, 22.0 million with a diagnosis in the previous 3 years, and 32.6 million with a diagnosis in the previous 5 years (Ferlay *et al.*, 2014; Stewart & Wild, 2014).

Among men, the five most common sites of cancer diagnosed in 2012 were the lung (16.7% of the total), prostate (15.0%), colorectal (10.0%), stomach (8.5%), and liver (7.5%). Among women, the five most common incident sites of cancer were the breast (25.2%), colorectal (9.2%), lung (8.7%), cervix (7.9%), and stomach (4.8%)(Ferlay *et al.*, 2014; Stewart & Wild, 2014).

Breast cancer is the second most common cancer in the world and, by far the most frequent cancer among women with an estimated 1.7 million new cancer cases diagnosed in 2012. This represents about 12% of all new cancer cases and 25% of all cancers in women. A slight majority of cases occur in women in less developed regions. Incidence rates vary nearly four-fold across the world regions, with rates ranging from 27 per 100,000 in Middle East, Africa and Eastern Asia to 96 in Western Europe(Ferlay *et al.*, 2015; Ferlay *et al.*, 2014).

Breast cancer was the most commonly diagnosed cancer and the second leading cause of cancer death among women in 2008 in Africa(Jemal *et al.*, 2012). Among women, Sub-Saharan Africa is the only region where cervical cancer is equivalent to breast cancer in terms of incidence (each constitutes approximately a quarter of the total burden) and is the most common cause of cancer death in women (23.2% of the total) (Stewart & Wild, 2014).

Breast cancer as a public health problem is growing throughout the world, but especially in developing regions, where the incidence has increased by as much as 5% per year. The mortality: incidence ratio is much higher in developing countries than in developed countries: only half of global breast cancers are diagnosed in the developing world, but they account for three-fourths of total deaths from the disease(Anderson *et al.*, 2008).

In Ethiopia, an estimated age-standardized incidence rate of 19.5 per 100,000 annually and an estimated age-standardized death rate of 11.8 per 100,000 females are reported. Women with breast cancer account for 19% of the total cancer patients(Kantelhardt *et al.*, 2014).

Guidelines from the 2000 National Institutes of Health Consensus Development Conference stated that "because adjuvant polychemotherapy improves survival, it should be recommended to the majority of women with localized breast cancer regardless of lymph node, menopausal, or hormone receptor status," and that "at the present time, there are no convincing data to support the use of any known biologic factor in selecting a specific adjuvant chemotherapy regimen in breast cancer." These recommendations clearly took no account of potential heterogeneity of tumor biology, which has been extensively studied in the interim (Coates *et al.*, 2012).

In Ethiopia, the majority of patients receive adjuvant treatment according to the Breast Health Global Initiative (BHGI) guidelines, which recommend anthracyclines (patients with chemotherapy received anthracyclines) and tamoxifen (for positive and unknown hormone receptor status) for this setting with limited resources. Adjuvant therapy is done in a standardized manner at Addis Ababa University Radiotherapy center for the majority of the patients. Surgery is mainly modified radical mastectomy (Kantelhardt *et al.*, 2014).

1.3. Literature Review

It has become increasingly clear that inflammation plays a major role in breast cancer pathogenesis. Some studies show evidence that chronic inflammation is linked to breast cancer recurrence and that elevated biomarkers of inflammation are associated with reduced survival among breast cancer patients (Kamel *et al.*, 2012).

1.3.1. Tumor Microenvironment and Breast Cancer

The tumor microenvironment refers to the biochemical and cellular composition of the tissue encompassing a tumor and comprises a heterogeneous population of cancer cells, a variety of non-cancer cells, soluble proteins, blood vessels, peritumoral lymphatic vessels, and a supporting structural matrix(Chen *et al.*, 2015).

Bidirectional communication between cells and their microenvironment is critical for both normal tissue homeostasis, and for tumor growth. In particular, interactions between tumor cells and the associated stroma represent a powerful relationship that influences disease initiation, progression and patient prognosis(Joyce & Pollard, 2009).

Macrophage colony-stimulating factor promotes the differentiation of monocytes to resident macrophages, whereas IL-6 and IL-10 polarize macrophages towards macrophage 2 (M2) phenotype (Sica *et al.*, 2008). Results from clinical and experimental studies suggest that the functional properties of M2 favor tumor progression. Macrophages 2 possess limited antigen-presenting capacity and suppress Th1adaptive immunity while actively promoting angiogenesis and tissue remodeling processes.

Fibroblasts constitute the predominant cell type in tumor stroma of carcinomas and are referred to in the literature as “cancer-associated fibroblasts” (Bhowmick *et al.*, 2004; Hanahan & Weinberg, 2011). It has been recognized in the last decade, that stromal fibroblasts play a tumor promoting and even tumor inducing role in carcinogenesis(Bhowmick *et al.*, 2004). Cancer cells have the ability to induce transcription factors that are involved directly in the activation of fibroblasts or indirectly through secretion of chemokines/cytokines, which are mediators of a

reactive inflammatory microenvironment(Bhowmick *et al.*, 2004; Bissell & Radisky, 2001; Coussens & Werb, 2002).

Primary cancer-associated fibroblasts derived from invasive breast carcinomas had greater potential to promote tumor growth and angiogenesis than the normal tissue-associated fibroblasts derived from non-cancer breast regions of the same patients(Orimo *et al.*, 2005).

Cytokines are low-molecular-weight proteins that mediate cell-to-cell communication. Depending on the tumor microenvironment, cytokines can modulate an antitumoral response, but during chronic inflammation, they can also induce cell transformation and malignancy, conditional on the balance of pro- and anti-inflammatory cytokines, their relative concentrations, cytokine receptor expression content, and the activation state of surrounding cells(Zamarron & Chen, 2011).

Breast cancer evolves out of multifactorial and dynamic processes, which are greatly influenced by the tumor microenvironment. Dominated by inflammatory traits, the tumor milieu in breast cancer is enriched with inflammatory cytokines that very often fail to induce immune protective mechanisms but rather skew the balance toward tumor-promoting events. Thus, multifaceted activities exerted by proinflammatory cytokines on stroma cells, leukocytes, and the tumor cells themselves lead to increased angiogenesis, tumor growth and progression, and eventually aggravate disease course(Allavena *et al.*, 2008; Colotta *et al.*, 2009; Goldberg & Schwertfeger, 2013; Jiang & Shapiro, 2013).

1.3.2. Interleukin-6 and Breast Cancer

Interleukin-6 is a pluripotent cytokine produced by a variety of cell types including lymphoid cells, endothelial cells, fibroblasts, and adipose tissue and at sites of acute and chronic inflammation, where it signals to induce an inflammatory response that includes autocrine stimulation and upregulation of other proinflammatory cytokine(Madeleine *et al.*, 2011).

IL-6 is typical proinflammatory cytokine with tumor growth effect, mainly by activating JAK tyrosine kinases and the transcription factor STAT3, as seen in lung, kidney, and breast cancer in which a high expression of STAT3 has been identified (Hodge *et al.*, 2005). It also facilitates tumor development by promoting conversion of non-cancer cells into tumor stem cells. In particular, IL-6 secretion by non-cancer stem cells in low attachment culture conditions up regulates Oct4 gene expression by activating the IL-6R/JAK/STAT3 signaling pathway(Kim *et al.*, 2013).

Circulating IL-6 levels are positively associated with clinical tumor stage, lymph node infiltration, and number of distant metastases in breast cancer patients(Dethlefsen *et al.*, 2013; Salgado *et al.*, 2003). The transient induction of IL-6 by monocyte-derived monocyte chemotactic protein 1**(**MCP1) drives a feed-forward inflammatory signaling pathway that leads to constitutive IL-6 production and breast cancer cell transformation and tumorigenesis, revealing a novel mechanistic link between IL-6 and breast cancer initiation(Rokavec *et al.*, 2012).

In triple negative breast cancer (TNBC), autocrine expressions of IL-6 is critical for their anchorage-independent growth and resistance to apoptosis(Hartman *et al.*, 2013). IL-6 not only regulates breast cancer stem cell(CSC) self-renewal(Marotta *et al.*, 2011), but also promotes CSC survival and proliferation through the activation of Notch, Wnt, Hedgehog, and TGF-β signaling pathways(Dethlefsen *et al.*, 2013). IL-6 also promotes breast cancer metastasis through the induction of epithelial to mesenchymal transition (EMT) (Hwang *et al.*, 2013; Korkaya *et al.*, 2012; Xie *et al.*, 2012). These studies suggest that IL-6 may promote breast cancer progression, metastasis, and resistance to treatment by acting on the CSC population and initiating EMT.

Although data concerning the actual levels of IL-6 in breast tumors are surprisingly scarce, Chaveyet al.*(Chavey et al., 2007)* convincingly demonstrated that breast tumors frequently contain high concentrations of IL-6 protein relative to normal breast tissue. Furthermore, they show that its expression is particularly high in tumors with unfavorable clinical features such as ER-negativity and high grade.

Several groups have measured IL-6 in human serum and observed high levels in the blood of breast cancer patients versus healthy controls. Serum IL-6 also appears to be positively correlated with advanced disease stages and poor prognosis (Knüpfer & Preiß, 2007). Although intratumoral leukocytes are an important source of IL-6 in breast cancer, IL-6 can also be produced by malignant breast cells. This is exemplified by the MDA-MB-231 breast carcinoma cell line, which maintains ER suppression via autocrine IL-6 production(D'Anello *et al.*, 2010).

Similarly, Benoyet al.(Benoy *et al.*, 2002) found that the serum IL-6 concentration is significantly higher in patients with breast cancer compared with healthy controls (n=26; $P<0.001$). Median IL-6 serum levels were nearly 10 times higher in patients with metastatic breast cancer (n=73) as compared to those with locoregional disease (n=31) (6.0 pg/ml versus 0.7 pg/ml respectively).

Furthermore, Yokoe and colleagues (Yokoe *et al.*, 2000), in a small preliminary study, reported that continuously elevated serum IL-6 levels correlate to poor survival in patients with hormone-refractory metastatic breast cancer (n=12). They also reported that pretreatment IL-6 levels are predictive indicators of response to therapy and prognosis of patients with recurrent breast cancer.

1.3.3. C-reactive Protein and Breast Cancer

C-reactive protein is a nonspecific, acute-phase, hepatic protein secreted in response to cytokines including IL-1, IL-6, and tumor necrosis factor. CRP has several immune-related functions, such as opsonization for phagocytosis. This biomarker is also a measure of low-grade chronic inflammation and potential predictor of cancer risk and/or survival(Pierce *et al.*, 2009).

Elevated plasma levels of C-reactive protein may be associated with poor prognosis of breast cancer. CRP is a classical acute-phase protein displaying rapid and pronounced rise of its plasma concentration in response to acute inflammation, infection, and tissue damage (Johnson AM, 2006; Pepys and Hirschfield, 2003)

Circulating levels of CRP are also moderately elevated during chronic inflammatory diseases and cancer. CRP is produced in the liver, predominantly under transcriptional control by the cytokine interleukin-6 originating from the site of pathology (Allin *et al.*, 2011).

1.3.4. Magnitude of Proinflammatory IL- 6 and CRP in Breast Cancer

An experimental study conducted on MDA-MB-231 indicated that plasma IL-6 promote breast cancer cell growth as aggregates and induce adhesive recruitment of breast cancer cells on E-selectin coated surfaces under flow. The experiment also shown that IL-6 concentrations in blood may regulate the recruitment of breast cancer cells to the inflamed endothelium. This results suggest that therapeutic approaches targeting cytokine receptors and adhesion molecules on cancer cells may potentially reduce metastatic load and improve current cancer treatments (Geng *et al.*, 2013).

Study conducted in 30 breast cancer patients in National Cancer Institute in Egypt indicated that a significant increase ($p<0.0001$) in CRP and IL-6 in breast cancer patients with or without metastasis as compared to the healthy group. Also, there was positive correlation ($p<0.0001$) between those biomarkers and the tumor grades. The study also showed an association between CRP and IL-6(Ahmed *et al.*, 2015).

Another study conducted in 59 female patients admitted for breast cancer showed that increases in cancer invasion and staging are generally associated with increases in preoperative serum IL-6 levels. This study also found that IL-6 and CRP levels correlated with lymph node metastasis ($P < 0.001$, $P < 0.001$) and TNM stage ($P < 0.001$, $P < 0.001$). However, in the study CRP evidenced no significance with regard to patient's overall survival levels and serum IL-6 levels were correlated positively with CRP levels ($r^2 = 0.579$, $P < 0.01$) (Ravishankaran & Karunanithi, 2011).

A clinical study conducted on a total of 30 histologically confirmed cases of breast cancer indicated that the mean values of CRP in patient with breast tumor decreased after three cycles of chemotherapy and this decrease was highly significant in patients with partial/complete response to chemotherapy. Similarly, levels of hsCRP were high in patients with estrogen receptor positive status than in patients with estrogen receptor negative status. No significant correlation was observed between levels of hsCRP with progesterone receptor status and Her-2/neu status (Sharma *et al.*, 2014).

A cohort study conducted in 535 non diabetic women diagnosed with T1-3, N0-1, M0 breast cancer show that hsCRP was significantly correlated with body mass index ($r = 0.60$), but not T or N stage, grade or estrogen receptor/progesterone receptor(Tibau *et al.*, 2013).

A research conducted to evaluate the association between the optimal times to initiation of adjuvant chemotherapy and survival on breast cancer patients, showed that initiation of chemotherapy 61 days after surgery was associated with adverse outcomes. Patients with TNBC tumors and those with HER2 –positive tumors treated with trastuzumab who started chemotherapy 61 days after surgery had worse survival compared with those who initiated treatment in the first 30 days after surgery (De Melo Gagliato *et al.*, 2014).

1.4. Significant of the Study

There are studies done in Ethiopia on breast cancer but none of these researches were done about inflammatory status of cancer patients. The aim of this study was to assess the status of proinflammatory activity of breast cancer patients so that it may generate some useful information for clinicians and patients during the management of subsequent treatment.

Early identification of proinflammatory status is very important for establishing successful cancer treatment and reduce risk, that are related with inflammation like oxidative stress due to inflammatory activities induce the production of oxidative stress.

C-reactive protein is commonly used to predict the risk of cardiovascular disease and monitor acute inflammation. Recently, there are reports indicating the risk of cardiovascular disease in breast cancer survival patients is associated with an elevated level of CRP.

Currently there are studies that link the worse outcome of the breast cancer patients and elevated levels of proinflammatory interleukin-6 and CRP in the circulation. Therefore, monitoring of these inflammatory activities and use of anti-inflammatory drugs enable to improve quality health of cancer patients. This research also provide a baseline information that can be used as a reference for further study on this area.

2. Objective

2.1. General Objective;

- To assess postsurgical serum interleukin-6 and C -reactive protein in breast cancer patients before receiving chemotherapy in Tikur Anbessa specialized teaching hospital, Addis Ababa, Ethiopia.

2.2. Specific Objectives;

- Assess socio-demographic characteristics of breast cancer patients
- Assess common hematologic and bio-chemical profile in breast cancer patients.
- Determine value of serum IL-6 and CRP in breast cancer patients.
- Evaluate the correlation of IL-6 and CRP
- Correlate postsurgical serum IL-6 and CRP with histopathology characteristics of breast cancer.

3. Materials and Methods

3.1. Study Area

The study was conducted in Tikur Anbessa specialized teaching hospital which is found in Lideta sub city of Addis Ababa, capital city of Ethiopia. Addis Ababa is situated in the central Ethiopia at an elevation of about 2440 meters (about 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 in Ethiopia. In 1998 Tikur Anbessa Hospital, was given to Addis Ababa University (AAU) by the Ministry of Health (MoH) for the faculty of medicine and health sciences as a main teaching hospital. The hospital provides a tertiary level referral medical service for over 3 million people of Addis Ababa and 90 million people referred from other parts of the country. It is open 24 hours a day for emergency services. According to the report of the Ethiopian Ministry of Health in 2010, the hospital has 700 beds with over 300,000 annual patient visits. Over 200 physicians and 627 nurses are currently working in Tikur Anbessa specialized hospital.

3.2. Study Design and Period

Institutional (hospital) based cross sectional study design was conducted. The study was conducted in Tikur Anbessa specialized Hospital in Addis Ababa from November – May 2015. In this period blood sample collection and patient card review of breast cancer patients greater than eighteen years old conducted. The required analytes were measured prior to initiation of chemotherapy

3.3. Source and Study population

The source population includes all breast cancer patients greater than eighteen years old who were visiting radiotherapy center at Tikur Anbessa specialized teaching hospital during the study period.

The study population have included voluntary breast cancer patients who were greater than eighteen years old visiting oncology clinic during the study period.

3.4. Sample Size

When calculating the sample size requirements for study, a number of factors are taken into consideration including effect size, cooperation and attrition, practical constrains such as time, subject availability and finance, subgroup analysis and sensitivity of the measurement used(Miles, 2003).

Many studies are based on relatively small sample size due to practical constraints such as time and subject availability, which often limits sample size. Examining the sample size of other comparable studies carried out internationally assessing cytokines level in breast cancer women sample size varies from 30 to 174 in these studies. However, power analysis and sample size calculation was not reported in any of these studies.

By considered the above conditions, we have conventionally included 44 participants in our study by considering the availability of the case, finance, and the study period.

3.5. Sampling Technique

A convenient non probability sampling technique were applied in this study. Individuals were selected for the study considering their demography, BMI, IL-6 and CRP levels, and histopathology of breast cancer.

3.6. Variables

3.6.1. Dependent Variables

- IL-6
- CRP

3.6.2. Independent Variables

- Age
- BMI
- Histology grade
- Lymph involvement
- Menopausal status
- Tumor size
- Tumor stage

3.7. Inclusion and Exclusion Criteria

3.7.1. Inclusion Criteria

After informed consent, volunteer breast cancer patients greater than eighteen years old who were visiting oncology clinic to take adjuvant chemotherapy for breast cancer during the study period were included.

3.7.2. Exclusion Criteria

Patients with associated diseases (e.g. infectious diseases, inflammatory bowel disease, autoimmune conditions, allergy, asthma, etc.) capable of raising the serum levels of IL-6 and CRP were excluded from this study.

3.8. Data Collection Techniques

Preliminary data were collected by preparing a standardized questionnaire that contains information about the socio-demographic characteristics of patient, family history of breast and ovarian cancer. Blood collection was done by an experienced clinical nurse working at the oncology clinic of Tikur Anbessa specialized teaching hospital. Clinical information, biopsy report and biochemical and hematological profiles were taken from patient cards.

3.9. Data Analysis

The data obtained were coded, entered, processed, edited, and analyzed using SPSS version 21 for analysis. During analysis median, interquartile range (IQR), mean ± SD of the different variables were determined. The Spearman rho correlation coefficient (r) was employed to evaluate the correlation between the IL-6 and CRP levels. P values < 0.05 was considered as clinical significant(S) and P value <0.001 was considered as highly significant (HS). Because the distributions of CRP and IL-6 were skewed, these values were log transformed to provide increased normality. Log-transformed values for CRP and IL-6 are used in all statistical analyses.

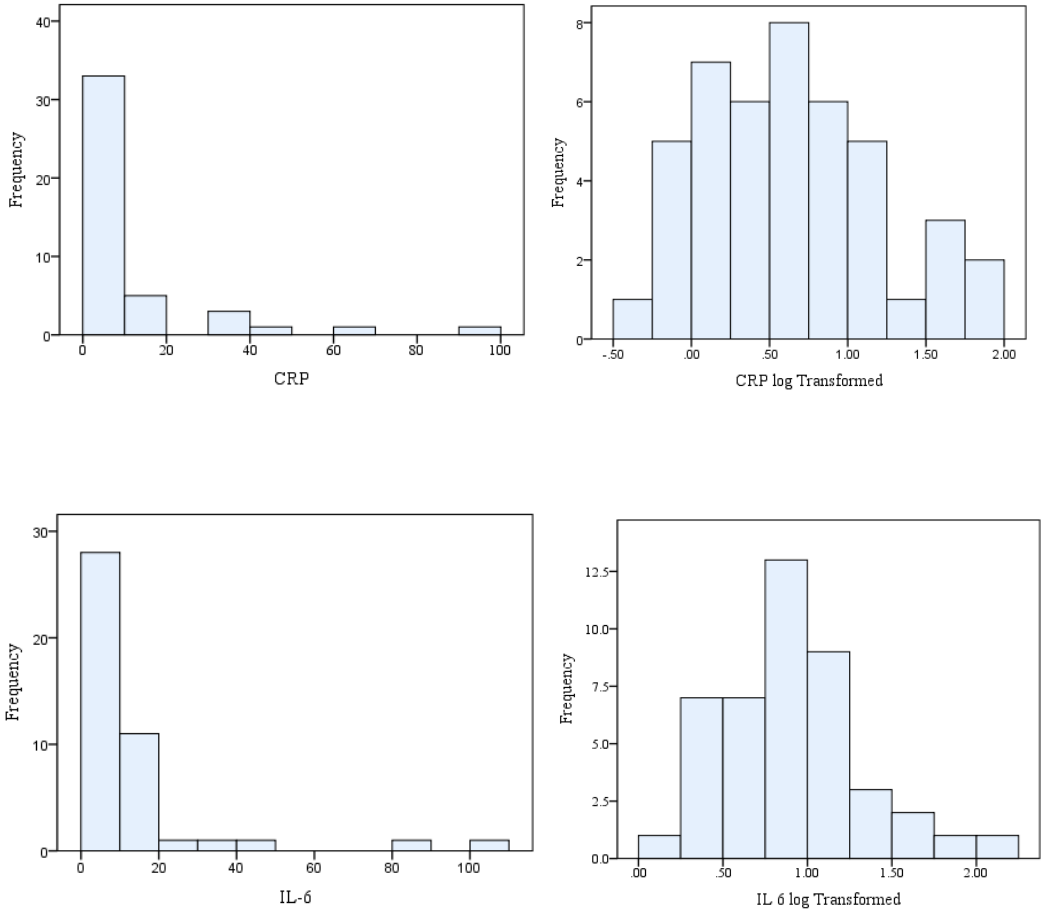

Figure 1. log transform of CRP and IL-6

3.10. Data Quality Assurance

Laboratory results were checked for completeness on a daily basis by the immediate supervisor and the principal investigator. The completed laboratory result was rechecked repeatedly by the principal investigator to maintain the quality of data. The required tests were measured using standardized procedure of kit manual. The blood sample was collected with precaution according to the standard operation procedure of sample collection. Clinical chemistry analyzers were calibrated to adequate temperature and appropriate wavelength to conduct the standard procedure, and also discrepancy results was repeated. The reagents for tests were kept in appropriate temperature according to the kit instruction.

3.11. Ethical Considerations

Ethical clearance was obtained from Addis Ababa University College of Health Sciences Department of Biochemistry after fully review conducted with meeting number DRERC 04/14 attended by the main researcher committee and give approval with protocol number of M.Sc. Thesis 11/14. Prior to data collection, general agreement was asked from Black Lion specialized referral hospital oncology clinic focal person through a letter written from the Department of Medical Biochemistry. The principal investigator explained the purpose of the study for the concerned bodies and obtained written informed consent from oncology clinic focal person and from the study participants. Any activity of conducting this study assumes not to harm the study population medically, morally, culturally and religiously.

3.12. Principle of Measurements of CRP and IL-6

3.12.1. Sample Collection and Preparation

Five milliliter of blood specimen was collected using standard vein puncture techniques before starting of adjuvant chemotherapy. After leaving for minimum of 30minutes at room temperature, to allow clotting to occur, blood was centrifuged at 3000 rpm for 10 minutes to separate serum from clotted blood using serum separator tube. Serum was separated, stored and assayed using clinical chemistry analyze for CRP (Cobas Integra Plus 400) and IL-6 (Cobas e411). Specimens which cannot be assayed within 24 hours of collection was frozen at -80^0C.

3.12.2. Principle of IL-6 Measurements

Method: ElectroChemiLuminescencetechnology assay

Principle: The strong streptavidin-biotin bond is used to affix the antigen/ antibody complex to a paramagnetic microbead. The affinity of streptavidin to biotin is one of the strongest non-covalent interactions known in nature and is resistant to organic solvents, denaturants, detergents, proteolytic enzymes, and extremes of temperature and PH. Paramagnetic microbeads enable a controlled capture and release of the antigen/antibody complex through the application of magnetic forces.

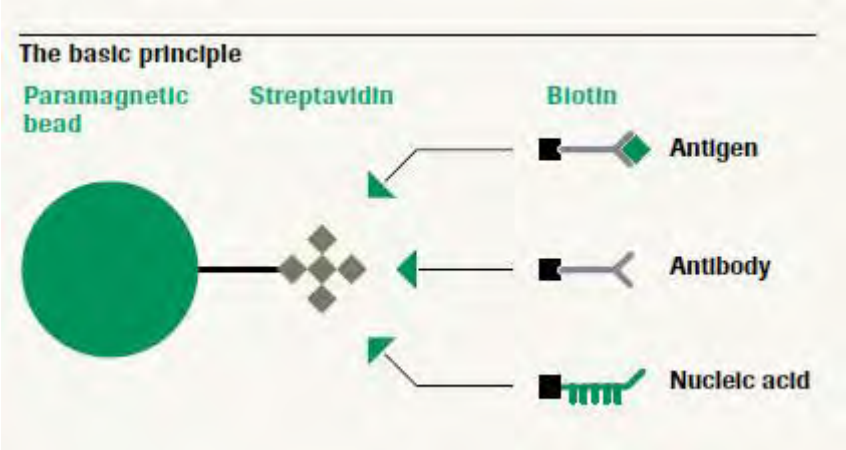

Figure 2. Basic principle of ElectroChemiLuminescence assay (Roche, www. www.cobas.com)

3.12.3. Principle of CRP Measurements

C –reactive protein, a major reactant of the acute phase response, is an established and widely accepted sensitive indicator of the inflammation status for a variety of inflammatory conditions. In healthy adults the concentrations of CRP in serum have been reported to be at 1 mg/L.

Method: **The Tina-quant® C - reactive protein assay**

Turbidimetry is Roche's technology for homogeneous immunoassay detection. Continuous development of the classical antigen-antibody assay design to the patented DuREL (Dual-radius enhanced latex) technology forms the basis for high sensitivity and broad dynamic range detection.

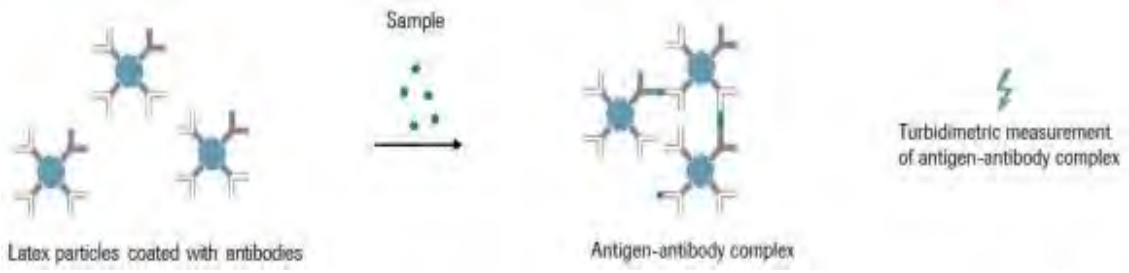

Figure 3. Principle The Tina-quant® C - reactive protein assay (Roche, www. www.cobas.com)

4. Results

4.1. Socio-Demographic characteristics and Breast cancer related information

The socio-demography and clinical characteristics of the study participants who were completed questionnaires and blood samples summarized below (table 1 and 2). The total number of participants included were 44 women, with mean age of 41.98 (range 20-70, SD 9.99) years; Mean (SD) of BMI was 24.3 ± 4.21 kg/m^2. Concerning Ethnicity, 43.2% (19), 31.8% (14) and 25% (11) Amhara, Oromo, and others (Gurage, Kefa, Silt and Tigray), respectively.

Table 1. Socio-Demographic Characteristics

Characteristics	**Frequency**	**Percent (%)**
Age		
Mean (Range)	41.98(20 – 70)	Median 42
BMI		
<25	26	50.09
25+	18	40.91
Ethnicity		
Amhara	19	43.2
Oromo	14	31.8
Others	11	25
Religious		
Catholic	1	2.3
Muslim	6	13.6
Orthodox	31	70.5
Protestant	4	9.1
Unknown	2	4.5

BMI = Body Mass Index

From participant 47.7% (21) were premenopausal. Of the participants, 20.5 % (9) had breast cancer history and 2.3 % (1) had ovarian cancer history. With regard to habit of drinking alcohol, smoking cigarette and chewing chat, 2.3% (1) had drinking alcohol, 2.3% (1) had smoking and 4.5% (2) had chewing chat.

Table 2. Breast Cancer Related Information

Characteristics	**Frequency**	**Percent (%)**
Menopausal status		
Pre	21	47.7
Post	20	45.5
Unknown	3	6.8
History of breast cancer		
Yes	9	20.5
No	34	77.3
Unknown	1	2.3
History of ovarian cancer		
Yes	1	2.3
No	40	90.9
Unknown	3	6.8
Radiation exposure to chest		
No	43	97.7
Unknown	1	2.3
Chat chewing		
yes	2	4.5
no	42	95.5
Smoking		
yes	1	2.3
no	43	97.7
Alcohol		
yes	1	2.3
no	43	97.7

4.2. Histopathological Characteristics of the Breast Cancer Patients

The patients were classified by their pathologic characteristics, including degree of differentiation, histologic type, tumor size, status of lymph node involvement, and staging (table 3). 75% (33) of patients had clinically or pathologically confirmed lymph node involvement at diagnosis. From 44 patients, 15.9% (7) patients belong to TX, 13.6% (6) patients belong to T1, 31% (14) patients belong to T2, 20.5% (9) patients belong to T3, and 18.2% (8) belongs to T4 and. All patients had modified radical mastectomy. Histological grades of cancer patient at the time of diagnosis were 15.9% (7) grade I, 43.2% (19) grade II, 22.7% (10) grade III and 18.2% (8) unknown. Of the participant, 84.1% (37) had advanced (invasive) ductal carcinoma, 6.8 % (3) lobular carcinoma, 4.5 % (2) mixed ductal and 4.5 % (2) other were histological types of patient at the time of diagnosis.

Table 3. Histopathological Characteristics of Breast Cancer Patient

	Frequency	Percent (%)
Histological Grading		
I	7	15.9
II	19	43.2
III	10	22.7
Unknown	8	18.2
Histological Type		
Invasive ductal	37	84.1
Invasive lobular	3	6.8
Mixed	2	4.5
Other	2	4.5
Tumor Size		
T1	7	15.9
T2	17	38.6
T3	9	20.5
T4	8	18.2
TX	3	6.8
Lymph Node Involved		
Yes	33	75.0
No	8	18.2
Unknown	3	6.8
Tumor Site		
On the left	17	38.6
On the right	24	54.5
Unknown	3	6.8
Type of Primary Therapy		
Modified Radical Mastectomy	44	100

T = tumor size, TX= unknown size

4.3. Interval from Definitive Surgery

The interval from definitive surgery that the patient received chemotherapy after surgery; 23.8% (10) were received chemotherapy ≤ 60 days from surgery, 34.1% (15) were received chemotherapy 61 to 90 days after surgery, and 36.4% (16) were received chemotherapy ≥ 91 days after surgery (table 4).

Table 4. Interval from Definitive Surgery

Interval from Definitive Surgery	Frequency	Percent (%)
≤ 60days	10	22.7
61- 90 days	15	34.1
> 90 days	16	36.4
Unknown	3	6.8

4.4. Biochemical Profile of the Patients

Renal and liver function assessment measured before the initiation of chemotherapy (table 5). Mean (SD) value of renal function tests such as creatinine and BUN were 0.9 mg/dL (range 0.5-1.2, SD 0.186) and 17.46 mg/dL (range 7-42, SD 6.24) respectively. Additionally, ALT, AST and ALP tests were done to assess liver function of the patients, and their values were 32.23 U/L, 37.62 U/L and 303 U/L, respectively.

Table 5. Renal and liver Function Tests before Receiving Adjuvant Chemotherapy

	Minimum	**Maximum**	**Mean ± SD**	**Reference value***
Cr (mg/dL)	0.50	1.20	0.90 ± 0.186	0.6-1.1
Ur (mg/dL)	7	42	17.46 ± 6.24	6-20
ALT (U/L)	9	172	32 ± 27	6-37
AST (U/L)	16	147	38 ± 26	5-30
ALP (U/L)	35	940	303 ± 209	30-90

BUN = Blood Urea Nitrogen, Cr = Creatinine, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, ALP = Alkaline phosphatase, * (Bishop *et al.*, 2013)

As part of routine test prior to initiation of chemotherapy, renal and liver function tests were performed in order to check the fitness of the patients to take treatment. The maximum value of urea was exceed upper limit of the reference value. On the other hand, the maximum value of liver enzymes were surpassed above the reference values.

4.5. Common Electrolytes Status of the Patients

Common electrolytes measured prior to initiation of chemotherapy are indicated below (table 6). The Mean value of sodium, potassium and calcium ions were 132 mmol/L, 3.78 mmol/L and 1.1 mmol/L, respectively. From the total, 47.73% of the patient's sodium were below the reference range and 11.36% were above the range. Additionally, potassium and calcium were 27.27% and 59.1% were below the range respectively.

Table 6. Common electrolytes before Receiving Adjuvant Chemotherapy

	Minimum	**Maximum**	**Mean ± SD**	**Reference values***
Na^+ (mmol/L)	84	163	132 ± 14.5	136 -145
K^+ (mmol/L)	2.60	4.7	3.78 ± 0.5	3.5-5.1
Ca^{++} (mmol/L)	0.75	1.33	1.1 ± 0.13	1.16-1.32

Na^+ = sodium, k^+ = potassium, ca^{++} = ionized calcium, * (Bishop *et al.*, 2013)

Even though the means value of electrolytes were in the reference range, most of patients had low sodium, potassium, and calcium. It was only sodium that have values above the reference range. Therefore, it is very important to monitory electrolytes values prior as well as during therapeutic intervention due to the effect of the chemotherapy.

4.6. Hematological Parameters of the Patients

Hematological profile obtained from 44 patients are summarized below (table 7). The mean(SD) of WBCs were 6.73 x 10^3 ± 2.49, platelet (PLT) were 296 ± 92.22, neutrophil were 54.05 ± 8.24, and hemoglobin (Hgb) were 14.27 ± 4.49 mg/dl were before chemotherapy started.

Table 7. Common Hematological Parameters before Receiving Adjuvant Chemotherapy

	Minimum	**Maximum**	**Mean ± SD**
WBC(X10^3/µL)	3.60	16.3	6.73 ± 2.49
Neu(X10^3/µL)	1.33	10.50	3.67 ± 1.74
PLT(X10^3/µL)	74	586	296 ± 92.21
Hgb(mg/dL)	11.3	40.90	14.27 ± 4.5

WBC = White Blood Cell, Lym = lymphocytes, Hgb = hemoglobin, PLT = platelets, Neu = neutrophils

The minimally acceptable pretreatment levels of complete blood count(CBC) required to begin chemotherapy as follow WBC 4x10^3/µL, absolute neutrophils greater than 2x10^3 /µL and platelet count 100x10^3/µL (Bae *et al.*, 2009). However, in our assessment the minimum value of WBC was 3.6x10^3/µl, ANC was 1.33x10^3/µL and platelet count was 74x10^3/µL, these minimal values were below the recommendation values. Most of delay in the chemotherapy due to the effect of treatment in the CBC. The minimum value of the hemoglobin was 11.3 mg/dL, approximately close to the normal value.

4.7. Serum IL-6 and CRP Levels in Breast Cancer Patients

In this study, 44 (100%) of patients had detectable IL-6 and CRP levels. The median (IQR) of IL-6 and CRP were 8.78 (3.73-12.34) pg/mL and 4.02 (1.64-10.49) mg/L respectively. Accordingly, minima and maxima of IL-6 and CRP were 1.5 pg/mL and 0.55 mg/L (table 8).

Table 8. Serum IL-6 and CRP levels in Breast Cancer Patients

	CRP(mg/L)	**IL-6 (pg/mL)**
Minimum	0.55	1.5
25%	1.64	3.73
Median	4.02	8.76
75%	10.49	12.34
Maximum	98.53	108.30
Mean	11.23	13.89
SD	19.44	20.25

The postoperative assessed CRP level in serum was in the majority of patients (54.55%) within the normal reference interval, conventionally defined as ≤5 mg/L, while a subset of patients (45.45%) displayed an elevated CRP level (> 0.5 mg/L). In other hand, serum level of IL-6 in the majority of the patients (63.64%) was more than normal value expected of healthy individual which is 7 pg/mL (Ravishankaran & Karunanithi, 2011).

4.8. Correlation between CRP and IL-6

Serum IL-6 levels correlated positively with that of CRP (rho = 0.530, $p < 0.01$) thus proving a positive association between the two variable (Figure 1)

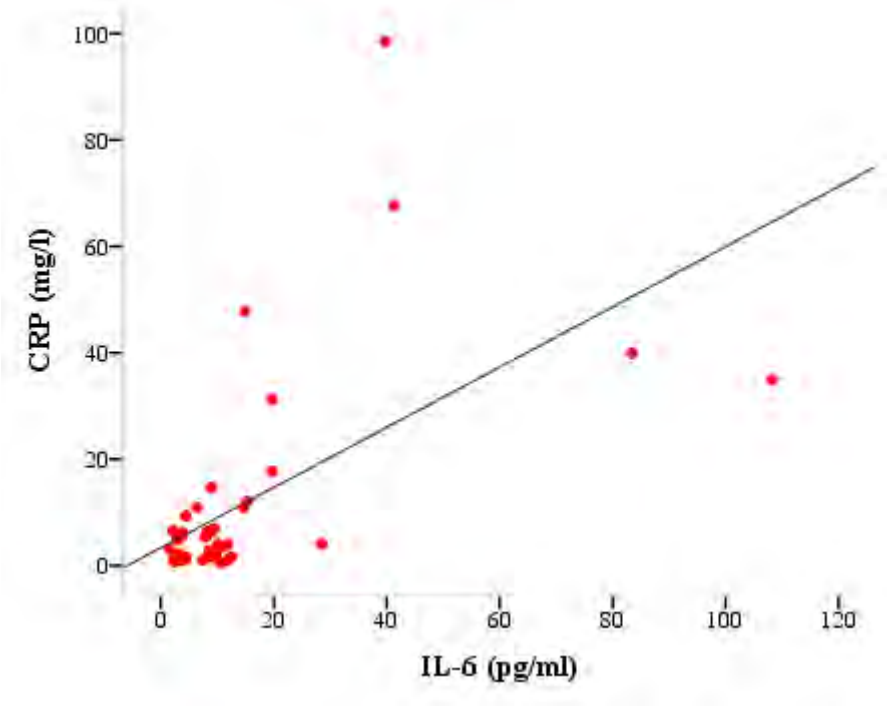

Figure 4. Correlation of CRP and IL-6

4.9. Association of Interval from Definitive Surgery with CRP and IL-6

Initiation of systemic chemotherapy after modified radical mastectomy is grouped in to three depending on timing of initiation of treatment (two months, three months and more than three months). Levels of CRP concentration varies across the group even though there were no statistical difference (*P*= 0.245).

Table 9. Association of Interval from Definitive surgery with CRP and IL-6

	CRP(mg/L) Median (IQR)	***P value***	***IL-6(pg/ml)* Median (IQR)**	***P value***
Interval from Definitive surgery				
≤ 60days	1.95(0.89-7.96)	0.245	8.71(2.86-9.81)	0.114
61- 90 days	5.37(2.19-12.07)		7.96(2.80-14.86)	
>90 days	4.72(1.57-25.69)		10.39(5.19-26.28)	

Starting of systemic chemotherapy after MRM grouped in three depending on timing of initiation of treatment (two months, three months and more than three months). Levels of IL-6 concentration slightly different across the group even though there were no statistical difference (*P* = 0.114).

4.10. **Association of Age, BMI, and Menopausal Status with CRP and IL-6**

Serum IL-6 and CRP levels were not significantly associated with age, menopausal status and BMI. Median of CRP in age <50; premenopausal and BMI < 25 indicates greater value than their respective groups. However, median of IL-6 show greater value in age ≥50, postmenopausal, and BMI ≥ 25 (table 9)

Table 10. Association of Age, BMI, and Menopausal Status with CRP and IL-6

	CRP (mg/L)	**P value**	**IL-6 (pg/mL)**	***P value***
	Median (IQR)		**Median(IQR)**	
Age				
< 50	5.37(1.63-11.52)	0.203	8.27(3.42-14.76)	0.555
≥ 50	1.96(1.64-5.39)		9.36(4.26-11.88)	
Menopausal status				
Pre	5.37(2.20-13.35)	0.244	7.79(2.81-15.07)	0.356
Post	3.77(1.57-6.85)		9.44(4.31-12.35)	
BMI				
<25	3.57(1.27-10.96)	0.653	8.71(3.59-13-09)	0.926
25+	5.38(2.20-9.71)		8.80(3.66-11.24)	

IQR = Interquartile range, BMI = Body Mass Index

The BMI of the majority of breast cancer patients was normal weight, accounting for 59.9% of total patients and 40.1% of breast cancer patients had a BMI classifying as overweight (≥25 kg/m^2). BMI was no significantly relation with CRP and IL-6 levels of the patients (P> 0.05).

4.11. **Serum CRP and IL-6 levels with Histopathologic Characteristics**

The association of circulating levels of CRP concentration with histopathologic characteristics of breast cancer are presented (table10). CRP levels were varied in all histopathological characteristics of breast cancer patients even though there were no statistical difference. We noted that postoperative CRP levels borderline significant with stage (P = 0.05). CRP different across histological grade, but there was no significant differences with grade (well = 3.09(1.99-.44) mg/L, moderate = 4.00(1.07-6.99) mg/L, poor = 5.20 (2.03-25.98) mg/L; P>0.05). Similarly, the CRP levels did not differ significantly in patients with different tumor size (P = 0.357), lymph node involvement (p = 0.417), and histological type (P = 0.328).

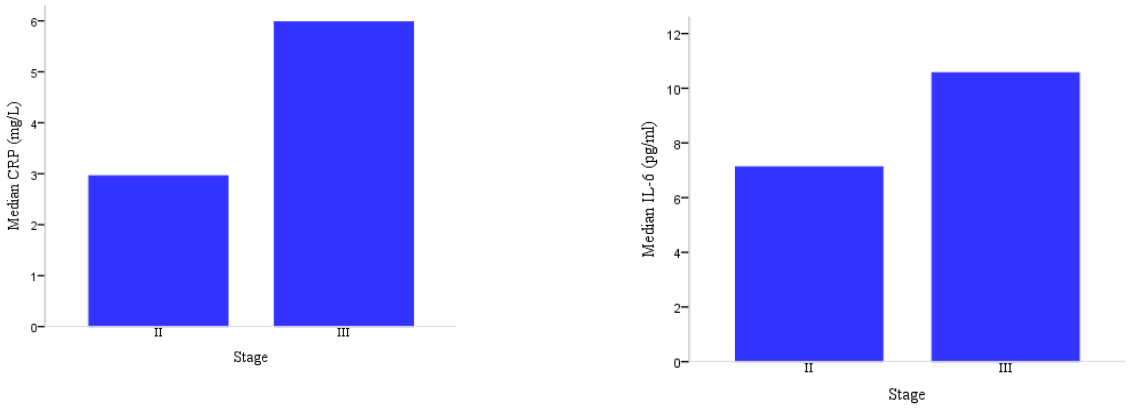

Figure 5. Correlation of serum CRP and IL-6 with stages II and III

However, IL-6 levels were higher in all histopathological characteristics of breast cancer patients even though there were no statistical difference with most of histopathological characteristics. We noted that pretreatment of IL-6 level unlike that of CRP differ significantly with stage (P = 0.026). In the other hand, the median levels of IL-6 increased across histological grade in the study, but there were no significant differences in grade (well = 9.04 (2.91-11.72) pg/mL, moderate (4.00 7.79 (2.80-10.72) pg/mL, poor = 10.20 (4.14-21.38) pg/mL; P> 0.05). Similarly, the IL-6 levels did not differ significantly in patients with different tumor size (P = 0.115), and lymph node involvement (p = 0.395).

Table 11. Association of serum levels of CRP and IL-6 with histopathologic characteristics of 44 consecutive patients who underwent modified mastectomy

	CRP (mg/L) **Median(IQR)**	***P value***	***IL-6*****(pg/mL)** **Median(IQR)**	***P value***
Stage				
II	2.96(1.47-6.66)	0.056	7.13(3.17-9.40)	**0.026**
III	5.98(1.69-32.11)		10.57(4.25-24.76)	
Histological Grading				
I(well)	3.09(1.99-.44)	0.403	9.04(2.91-11.72)	0.708
II(moderate)	4.00(1.07-6.99)		7.79(2.80-10.72)	
III (poorly)	5.20(2.03-25.98)		10.20(4.14-21.38)	
Tumor size				
T1	4.00(0.97-6.42)	0.439	9.04(7.79-10.10)	0.115
T2	5.39(1.48-10.92)		4.44(3.03-10.12)	
T3	4.93(1.64-51-35)		9.31(2.66-40.48)	
T4	4.78(2.13-25.69)		11.77(5.32-26.28)	
Lymph node				
Yes	4.04(1.65-11.48)	0.417	8.97(3.77-14.76)	0.395
No	4.01(1.44-6.85)		8.41(3.80-10.42)	

5. Discussion

To our knowledge, this is the first study that evaluate the status of proinflammatory condition of postoperative breast cancer prior to chemotherapy in Ethiopia. We assessed proinflammatory status using inflammatory markers IL-6 and CRP on the postoperative breast cancer patients. We observed that serum IL-6 and CRP levels varies among the patients.

We have shown that age has no significant association with CRP and IL-6 ($p > 0.05$). The age of the participants in the study ranged from 20 to 70 years old, and the mean age was 41.98 years, which is younger than the mean age among Hong Kong (55.4 years), Norway (57.5 years) and china (47.3 years)(Chen *et al.*, 2013; Chow *et al.*, 2005; Emaus *et al.*, 2010).

In this study, CRP and IL-6 have not shown any association with BMI. It contradict with previous reports that CRP was positively correlated with BMI (Alokail *et al.*, 2013). However, they conducted in breast cancer patients with BMI greater than 30 kg/m^2, because of production of cytokine in adipose tissue that trigger the production of CRP due to cytokine such as IL-6 and TNF-α. On the other hand, the BMI of the majority of breast cancer patients was normal BMI (< 24.9 kg/m^2), accounting for 59.9% of total patients, which is higher than the patients in Western countries; only 40.1% of breast cancer patients had a BMI classifying them as overweight (BMI ≥ 25), which is lower than in Western countries (Berstad *et al.*, 2010; Chen *et al.*, 2013; Stead *et al.*, 2009), and difference in life style and diet may contribute to this difference.

In this study we demonstrated that interval from definitive surgery to chemotherapy initiation has no significant association with CRP and IL-6. However, different studies suggests that patients with more advanced stages experience worse outcomes when initiation of adjuvant chemotherapy is delayed. Large retrospective cohort study demonstrated that delaying the initiation of adjuvant chemotherapy was associated with worse breast cancer survival outcomes and that the clinical impact varies according to the stage and the breast cancer subtype (De Melo Gagliato *et al.*, 2014).

C-reactive protein is a non-specific acute-phase protein that rises on acute infection as well as tissue trauma, chronic inflammatory disease, myocardial infarction, surgery and cancer. A systemic inflammatory response as shown by an elevated concentration of circulating CRP has frequently been associated with worse outcome in numerous types of cancer (Allin *et al.*, 2009).

In the present study, all of patients had detectable CRP levels. In contrast, elevated CRP levels were detected in only 18% of the total patient in study done soft tissue sarcoma which comprised different cancer including breast cancer (Nakamura *et al.*, 2012). This might be due to high inflammatory status of the patients and used of high sensitive method used to detect CRP.

In our study, the mean value of CRP in postoperative was 11.23 mg/L. In previous study, Allin *et al.* stated that women with CRP levels >3.24 mg/L had increased risk of all-cause mortality, an increased risk of death due to breast cancer, and a suggested increased risk of disease recurrence, independent of age, tumor characteristics, lymph node status, presence of distant metastases, lifestyle factors, BMI and CVD (Allin *et al.*, 2011). Similarly, Pierce *et al.* reported that women with $\geq$ 3.9 mg/L of CRP concentrations had increased risk of all-cause mortality and decreased disease-free survival (Pierce *et al.*, 2009). This finding suggests that the inflammatory status of patients in our study is very high. This might be due to the disease associated with breast cancer or inflammatory condition of the patients.

Elevated CRP level as biomarkers of systemic inflammation may merely reflect the aggressiveness of the tumor and therefore represent a consequence of established prognostic factors, such as tumor size and grade (Allin *et al.*, 2009). A systemic inflammation may not only be a consequence of tumor burden, but actively contribute to tumor progression (Pierce *et al.*, 2009).

In this study we have shown that there was no significant correlation between the level of CRP and the tumor grade, tumor size, present of lymph node and histological grade. On the contrary, previous studies mentioned that elevated CRP levels were associated with tumor grade, tumor size and presence of distant metastases (Ahmed *et al.*, 2015; Allin *et al.*, 2011). However, Ravishankaran and Karunanithi stated that the levels of CRP correlated only with lymph node metastasis and not with tumor size and

distant metastasis(Ravishankaran & Karunanithi, 2011).This components might explain the absent of single consensus on the association of CRP and histopathological characteristics of the breast cancer.

Chronic inflammation plays an important role in the pathogenesis of several cancer forms including breast cancer. The pleiotropic cytokine IL-6 is a key player in systemic inflammation, regulating both the inflammatory response and tissue metabolism during acute stimulations(Dethlefsen *et al.*, 2013).

In this study, the postoperative level of serum IL-6 which measured one to three months after modified radical mastectomy before initiation of chemotherapy was 13.89 pg/mL. This has been considered very high compared to normal value and other studies in normal control value expected of healthy individual which is 7 pg/mL(Kozłowski *et al.*, 2002). This result agree with Ahmed et al. found that the serum levels of IL-6 in breast cancer patients with or without metastasis were significantly increased as compared to the healthy control (Ahmed *et al.*, 2015). They also indicated that after surgical treatment, IL-6 showed an increase in its level in patients who suffered from breast cancer with or without metastasis.

Our result demonstrated that increased circulating levels of serum IL-6 were positively correlated with pathological tumor stage, but not with lymph node involvement. However, several studies show that IL-6 correlated with stage, lymph node infiltration and distant metastases(Fuksiewicz *et al.*, 2010; Gupta *et al.*, 2012). This might be as the result the study conducted after the removal of tumor and in none metastasis patients.

In this study, stage III patients had higher median of IL-6 (10.57 pg/mL) compared to stage II (7.13 pg/mL). This result supported by Jablonska et al. that serum levels of IL-6 in breast cancer patients in stage III and VI had increase serum levels of IL-6 compared with stage II, and they concluded that changes in values of certain cytokines could have a diagnostic and prognostic role in cancer disease(Jabłońska *et al.*, 2000). Furthermore, study showed that there was progressive increase in IL-6 levels as the stage of disease progresses(Goswami *et al.*, 2013; Kozłowski *et al.*, 2002).

However, other studies indicated that the levels of IL-6 correlate with all the aspects of breast cancer like tumor size, lymph node involvement, distant metastasis, and the final TNM staging of the disease(Ahmed *et al.*, 2015; Dethlefsen *et al.*, 2013). They also described elevated level of IL-6 affect the overall survival of the patients. In our study, we did not find any correlation of IL-6 with tumor size and lymph nodes involvement.

Circulating CRP is acutely elevated in response to proinflammatory cytokines (e.g., interleukin-6), and also moderately elevated with low-grade inflammation (Allin *et al.*, 2011; Ravishankaran & Karunanithi, 2011). In this study we observed a significant association between the levels of IL-6 and CRP ($p < 0.01$).

Assessment of common hematological parameters (WBC, neutrophils, platelets, and hemoglobin) and biochemical test such as liver function tests (ALT, AST, and ALP) and renal function tests (creatinine and urea) took place to see the fitness of patients before receiving chemotherapy. In this study, hematological assessment shown the mean values of WBC, neutrophils, and platelet count fell in the normal value. However, some patients had CBC values below the normal values, this might prolong the time of treatment as well recurrence of the disease. On the other hand, liver enzymes such as ALT and AST shown that the absence of the preexist abnormality of the liver disease.

In this study, we assessed the level of electrolyte in the patients and we found that the mean value of sodium, potassium and calcium ions were in the normal range. However, monitoring of the level of electrolytes during chemotherapy important due to the underlying disease and the therapeutic interventions that can contribute to the development of acid-base disturbance(Miltiadous *et al.*, 2008)).

Based on the present study overall inflammatory status of the patients have high values of IL-6 and CRP than expected values. This finding suggests that patients that had high values might have develop risks related with inflammation and subsequent treatment unless intervention implemented. Additionally, it has been demonstrated that staging correlates with the prognosis of patients with breast cancer. In this study, IL-6 has a direct correlation with the stage of the disease, it might be link with the prognosis of the patient contrasting to CRP.

6. Conclusion

This study include 44 patients of breast cancer. These patients had histologically proven disease and eligible to take adjuvant systemic chemotherapy. Our purpose was to find out inflammatory status and correlation of postoperative serum levels of CRP and IL-6 with histopathological characteristics of breast cancer patient.

The present study indicates that postoperative levels of serum interleukin 6 and C – reactive protein concentrations were elevated in breast cancer patients and also correlated with pathological stages of disease. Our findings provide evidence for association between breast cancer pathological stage and systemic inflammation. Evaluation of the inflammatory status of patients important prior to initiation of chemotherapy to reduce influence of inflammation in the subsequent treatment.

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7. Recommendation

- Because chemotherapy may induced the production of proinflammatory activities, assessment of proinflammatory status of breast cancer patients enable to reduce its effect by anti-inflammatory intervention.
- Analysis of CRP is not expensive as IL-6, so CRP may easily available and used to evaluate the inflammatory status of the patient.
- This research might act as baseline in further studies to assess the link between proinflammatory and cancer in our country.
- Finally, we recommend assessment the proinflammatory activities performed at the baseline of the treatment.

8. Limitation

- Due to the constraint of finance and access of the reagents, this study did not include control. Therefore, it is very important to use control in the next study.
- Hormonal receptors such as estrogen (ER) and progesterone receptors (PR) and epidermal growth factor (HER2) are used in treatment to decide treatment options. However, mostly these tests are not done in the hospital. As the result we didn't compare the proinflammatory activity with these receptors.
- We did the research after the tumor removed. We recommend evaluation of inflammatory status from beginning.
- Because of limited resource available, we performed the study using small sample size. As the result, we recommend large sample size for the next study.

9. Reference

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10. Annexes

10.1. Socio-Demography and Clinico-Pathology Questioner

Please answer every question in the questionnaire by marking "X" in the space or filling the necessary information.

Code No_____

Part 1: Socio-Demographic characteristics

1. Age_____
2. Religion _____
3. Ethnicity _____
4. Region _____
5. Residence area: Rural _____Urban_____
6. Education level: Illiterate _____High school or less _____ college or above_____
7. Marital status: Single _____ Married _____ Widowed _____ Divorced
8. Current Height_____ Weight_____ BSA _____BMI_____
9. Sero status _____

Part 2: breast cancer related information

2.1. Menopausal status premenopausal ☐ postmenopausal ☐

2.2. Histologic type of the cancer

2.2.1.1. Infiltrating ductal

2.2.1.2.Intraductal

2.2.1.3.Lobular

2.3. Tumor size at diagnosis

2.4. Degree of differentiation

2.4.1.1.Well differentiated

2.4.1.2.Moderately differentiated

2.4.1.3.Poorly differentiated

2.5. Node involvement

2.5.1.1.Number of lymph node positive

2.5.1.2.Number of lymph node removed

2.6. Stage of breast cancer

2.7.TNM of the tumor

2.8.Smoking habit Yes _____ No_____

2.9.Alcohol drinking status Yes _____ No_____

2.10. Present of sever concomitant disease Yes _____ No_____

2.11. Do you have family history of ovarian cancer? Yes _____ No_____

10.2. Patient Consent Form

Research title: Postsurgical assessment of serum interleukin 6 and C-reactive protein in breast cancer patients before receiving adjuvant chemotherapy in Tikur Anbessa specialized teaching hospital, Ethiopia.

Principal investigator: Ketsela Yirdaw

Address: mobile – 0920255614

E-mail ketselayirdaw@yahoo.com

PART I: patient information

You are invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take a time to read the following information carefully and discuss it with others if you wish.

Purpose of the study

The aim of the study is to assessment of postsurgical levels of serum interleukin 6 and C-reactive protein in breast cancer patients before receiving adjuvant chemotherapy. This will be important in the monitoring of treatment during chemotherapy in breast cancer. It also used to monitor inflammatory conditions other than breast cancer during treatment.

Procedure

If you agree to volunteer to participate in this study, we will require the following:

- We will collect 5 ml of blood sample from you before chemotherapy treatment and use it to determine the levels of the required tests in your blood.
- We will ask you various questions related the study.

Potential risk

You will not be required to take any new medications other than those you are already taking, and you will not undergo any procedures, except that your blood will be used to determine the desired tests.

Confidentiality

Any information that is obtained from the results and that can be identified specifically with you will remain confidential and will be disclosed only with your permission. We will not use your name in any of the information we get from the study or in any research report that results from the study.

Sharing the result

At the end of the study, the study findings will be published and they will be disseminated to other scientists through publication, and also to your doctor, if needed, so that further medical care can be pursued.

The right to refuse

Please know that your participation in this study is entirely voluntary and you are free to withdraw at any time from the study. Taking your blood sample for this research is completely voluntary. Your decision will not affect your medical care in any way, or right to take medication or any receive health care from any health service facility now or in future.

PART II: consent form

By name below, I confirm that I have read and understood this informed consent. I understand that this is a research study and that my participation is voluntary. I understand that I may change my mind about participating at any time, without my medical care or legal rights being affected. I have had the opportunity to ask questions and my questions have been answered. I have been given adequate explanation and understand the purpose, procedures, risks and benefits of the research study. By signing this form, I give my permission for the researchers to have access to my blood sample for the study.

Patient code number	date	signature
Name of principal investigator	date	signature

10.3. Patient Information and Consent Form Amharic Version

አዲስ አበባ ዩንቨርሲቲ

የሕክምና ፋካልቲ

ባዮኬሚስተሪ ት/ክፍል

የጥናት ተሳታፊዎች የመረጃና የስምምነት ቅፅ

የትናቱ ርዕስ፡- በጥቁር አንበሳ ስፔሻላይዝድ የመማሪያ ሆስቢታል በጡት ካንሰር ህሙማን ከቀዶህ ክምና ብሃላ የሚወሰድ መድሐኒት (adjuvant chemotherapy) እክምና ምላሽ ለመከታተል ሊሆን የሚችል በደም ውስጥ ንጥረነገር (CRP and IL-6) መለካት፡፡

የጥናቱባለቤት፡ ቀፀላ ይርዳው

አድራሻ፡- ሞባይል 0920255614

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ክፍል አንድ፡- የጥናቱ ተሳታፊዎች የመረጃ ቅፅ

መግቢያ፡- በዚህ ጥናታዊ ፅሁፍ ላይ እንዲሳተፉ እየተጋበዙ ነው፡፡ በጥናቱ ላይ ለመሳተፍ ከመወሰን በፊት ጥናቱ ልምን እደሚካሄድና ምንምን ዓይነት ነገሮች እንደሚያስፈልጉት ማወቅ ነው፡፡ ስለዚህ ጥቂት ግዜ ይወስዱና የሚከተለውን ስለጥናቱ በተመለከተ መረጃ ይመልከቱ አስፈላጊ ከሆነም ከሌሎችሰዎች ጋር ይወያዩ፡፡ ማንኛውም ገልፅ ያልሆነ ነገር ካለ ወይም ተጨማሪ መረጃ ከፈለጉ የጥናቱ ባለቤት መጠየቅይችላሉ፡፡

የጥናቱ አላማ፡- የዚህ ጥናት ዋና አላማ በጡት ካንሰር ህሙማን ከቀዶ ህክምና ብሃላ የሚወሰድ መድሐኒት (Adjuvant Chemotherapy) ለመከታተል የሚይስችል ንጥረነገር (CRP and IL-6) መለካት ነው፡፡ ይህን ጥረነገር (CRP and IL-6) በደም ውስጥ በመለካት የሚወስዱቱን መዲሐኒት ምላሽ ለመከታትል ይረዳ ዘንድ የሚካሄድ ምርምር ነው፡

የጥናቱ ሂደት፡- በዚህ ጥናት ለመሳተፍ ፍቃደኛ ከሆኑ ከእርሶ የምጠበቁት የሚከተሉት ናቸው፡፡

1. ምርምሩን ለመስራት የሚያፈልገው የደም መጠን አምስት ሲሲ ኬሞ ቴራቢ ክመጀመሮና እየወሰዳቹ የሚወሰድ መሆኑን፡፡ የተወሰደው ደም ጥናቱን ለመስራት ብቻ እነሚውል፡፡
2. የጥናቱ ባለቤት ጥናቱን በተመለከተ አንዳንድ ጥያቄዎችን ሊጠይቅ ይችላል፡፡

ጉዳት፡- ከዚህ ጥናት ጋርበተያያዘ በጤናም ሆነ በሚያገኙት ተገቢ ህክምና ምንም አይነት ጉዳት ስለማያስከትል አይስጉ፡፡

ሚስጥራዊነት፡- ማንኛውም ከዚህ ጥናት ጋር የተያያዘ የግል መረጃ ሚስጥራዊነት የተጠበቀ ነው፡፡ ስለዚህ የጥናቱ መረጃ ይፋ የሚሆነው ለረሶቢቻ ነው፡፡ ስለሚወሰደው ማንኛውም መረጃዎች ሆነ የጥናቱ ውጤት ለማሰራጨት በስም ሳይሆን በሚስጥር(ኮድ) የሚመዘገብ ይሆናል፡፡

የተሳትፎ መብት፡- በዚህ ጥናት መሳተፍ ሙሉበሙሉ በፅርሶ ፍቃድ የተመሰረተ መሆኑን ልናሳስብ እንወዳለን፡፡ በመሆኑም በማንኛውም ግዜ ምንም ዓይነት ምክንያት ሳይሰጡ ከጥናቱ ራስን የማግለል መብት የተጠበቀ ነው፡፡ የሰጡት ደም ለዚህ ጥናት እንደሚውል ማድረግ በእርሶ ሙሉ ፈቃድ ብቻ ሲሆን በጥናቱ ላይ ለመሳተፍ መወሰን ወይም አለመወሰን መድሐኒት ወይም ሌላ የጤና አገልግሎት የማግኘት መብት አሁንም ሆነ ለወፊቱ ምንም አይነት ተፅኖ አያሳድርቦትም፡፡

ክፍል ሁለት፡ የስምምነት ቅጽ

የሚሰጢርቁጥር----------

□ኔ ስሜ ከላይ የተጠቀሰው የጥናቱ ተሳ□ በጡት ካንሰር ምርመራ ላይ ሊደረግ ለ□ ሰበው ጥናት መረጃ አግኝቻለሁ፡፡ ለዚህ ይረዳ ዘንድ ከሣኔ ላይ የጡትና የደም ናሙና ሣንደሚፈለግ ተረድቻለሁ፡፡ ስለጥናቱ አላማ፤ሣንዲሁም ናሙና ሲወሰድ በሣኔ ላይ መጠነኛ የህመም ስሜት ሊያስከትል ሣንደሚችል ከተመራማሪው ገለጻ ተረድቻለሁ፡፡

በተጨማሪም መጠይቁ ውስጥ በተካተቱት ጥያቄች መሰረት የምሰጣቸው መረጃች በ□ቅላላ በሚስጥር ሣንደሚጠበቁ ተገልጾልኛል፡፡ ሣንዲሁም ሣኔን በተመለከተ የምጠየቀውን መረጃ ያለመስጠት፤ በጥናቱ ያለመተባበርና ከጥናቱ በማንኛውም □□ ራሴን የማግለል መብቴ የተጠበቀ መሆኑ ተገልጾልኛል፡፡

ስለዚህ **ለተመራማሪዎቹ መረጃ ሣና የስምምነት ቃሌን የሰጠሁት በአጠቃለይ ሁኔ□ዉን በመረዳትና በአ□ም □ቃደኝነትነው፡፡ ከሣኔ የሚወሰደው ናሙና ለምርምር ሣንደሚውል ተረድቻለሁ፡፡ በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ፡፡**

የጥናቱ ተሳ□ኝቆች □መረጃና □ስምምነት ቅን

የትናቱ ር□ስ፡- በ□ቁር አንበሳ ስከ ሻ□□ት ሆስ□□ል

የተሳታፊዉ ፊርማ **----------** **ቀን** **----------**

የጥናቱ ባለቤት ፊርማ **----------** **ቀን----------**