ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF MEDICAL BIOCHEMISTRY

Assessment of Malnutrition Using Biochemical Markers among Female Breast Cancer Patients Attending Tikur Anbessa Specialized Hospital, Ethiopia

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A Master's thesis submitted to the department of medical Biochemistry, school of Graduate Studies, Addis Ababa University in partial fulfillment of the requirements for the degree "Master of Science in Biochemistry" in the department of medical Biochemistry.

June, 2017

Addis Ababa, Ethiopia

Addis Ababa University

School of Graduate Studies

This is to certify that the dissertation prepared by Kibrom G/meskel, entitled: - "**Assessment of Malnutrition Using Biochemical Markers among Female Breast Cancer Patients Attending Tikur Anbessa Specialized Hospital, Ethiopia"** and Submitted in partial fulfillment of the requirements for the degree "Master of Science in 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: **Assessment of Malnutrition Using Biochemical Markers among Female Breast Cancer Patients Attending Tikur Anbessa Specialized Hospital, Ethiopia,** 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.

Kibrom Gebremeskel

Signature……..

Date……

ACKNOWLEDGEMENT

First of all, I am deeply indebted to Almighty God and His Mother Saint Virgin Marry for giving me wisdom, patience and strength during this research project and indeed throughout my life; may their names be honored, glorified and exalted.

Besides, it is an extraordinary privilege for me to sincerely articulate my deep rooted sense of gratitude and thank to my venerated advisors and live mentors, Dr. Solomon Genet and Dr. Wondemagegnhu Tigeneh. Their meticulous guidance, intriguing motivation and unflinching encouragement in every detail of the thesis contributed a lot to the successful realization of the thesis. Above all and the most needed, their truly scientist intuition have made them constant oasis of ideas and passions in science which inspired and enriched my growth as a researcher and scientist want to be.

Words fail me to overstate my appreciation to everybody who was supporting me during the research work in various ways, and I want to express my apology that I could not mention personally one by one. Needless to say, the errors are all mine.

Lastly and most importantly, I would like to thank Biochemistry department for giving me this opportunity. My thank also goes to Addis Ababa University for funding this study, Arba Minch University for sponsoring my postgraduate education. Without them, this study could not have been pursued.

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ABBREVIATIONS

ANOVA	Analysis Of Variance
APR	Acute Protein Response
BCG	Bromocresol Green Method
BMI	Body Mass Index
CRP	C – Reactive Protein
FAA	Free Amino Acid
FFA	Free Fatty Acid
IFN	Interferon
IL	Interleukin
PEM	Protein-Energy Malnutrition
PFAA	Plasma Free Amino Acid
REE	Resting Energy Expenditure
SE	Standard Error
SPSS	Statistical Package for the Social Sciences
TASH	Tikur Anbessa Specialized Hospital
STAT3	Signal Transducers and Activators of Transcription 3
TLC	Total Lymphocyte Count
TNF	Tumor Necrosis Factor
UCP	Uncoupling Protein
WHO	World Health Organization

ABSTRACT

Introduction: Breast cancer is the most common cancer worldwide. Malnutrition occurs frequently in cancer patients. Malnourished cancer patient responds poorly to therapeutic interventions, such as chemotherapy, radiotherapy and surgery with increased morbidity and mortality.

Objective: The aim of the present study was to evaluate malnutrition in breast cancer patients through biochemical markers at Tikur Anbessa Specialized Hospital, Ethiopia.

Materials and methods: Hospital based cross-sectional study was conducted in 50 breast cancer patients and 50 healthy individuals from January to April 2017 at Tikur Anbessa Specialized Hospital, Ethiopia. The required amount of blood was withdrawn from both group by well trained nurses in the hospital and serum stored at -80^0c refrigerator until analysis. Data were collected on socio demographic factors, biochemical, anthropometric and hematological parameter.

Result: The mean age of the study and control group was 43.06 year. Comparison of mean between study and control group shows 3.89 ± 0.04 g/dl and 4.34 ± 0.17g/dl for albumin, 3.92 ± 0.08 g/dl and 3.35 ± 0.04 g/dl for globulin, 1.73 x 10^3 ± 0.29 cells / mm^3 and 2.35 x 10^3 ± 1.12 cells /mm^3 for total lymphocyte count, 0.72 ± 0.03 mg/l and 0.96 ± 0.03 mg/l for creatinine, 25.19 ± 1.22 mg/l and 21.62 ± 1.01 mg/l for urea for study and control group respectively. All the above mean values show statistically significant difference between study and control groups with p value < 0.05. There were no statistically significant difference mean value levels of total protein and body mass index in our study. Prevalence of malnutrition assessed through albumin was 32%, TLC 46% and BMI 36%. Pearson correlation analysis revealed positive correlation between globulin and total protein level ($r = 0.84$, $P < 0.0001$) and negative correlation between albumin and globulin level ($r = -0.48$, $p < 0.0001$) in the breast cancer patients. Albumin positively correlated with TLC ($r = 0.51$, $p= 0.03$) in the breast cancer patients.

Conclusion: The present study revealed serum albumin, globulin, creatinine, urea level were reliable biochemical markers combined with TLC for assessment of malnutrition in breast cancer patients.

Key words: breast cancer, Malnutrition, Albumin, Globulin, creatinine and total lymphocyte Count.

1. INTRODUCTION

1.1. Overview of breast cancer

Breast cancer is the commonest malignancy of females all over the world (Liu *et al.*, 2015). All women regardless of their racial, ethnic origin or heritage are at risk of developing breast cancer (Fatima *et al.*, 2013). Eventhough it is the most common cancer in women of high income countries, there is a trend of increasing incidence and mortality from breast cancer in lower income countries (Romieu, 2011). According to the National Cancer Institute one woman in eight will develop breast cancer in her Life time (Ledesma *et al.*, 2013).

1.2. Literature review

1.2.1. Malnutrition

Malnutrition is defined as acute or chronic state of nutrition in which a combination of varying degrees of excess or deficiency of nutrients, imbalance of energy and inflammatory activity led to a change in body composition, impairment in function and clinical outcome (Sauer *et al*., 2012). Malnutrition is common in cancer patients and may be due to several mechanisms including cancer, host response to tumor and anticancer therapies as shown in (**Figure 1**) below (Righini *et al*., 2013).

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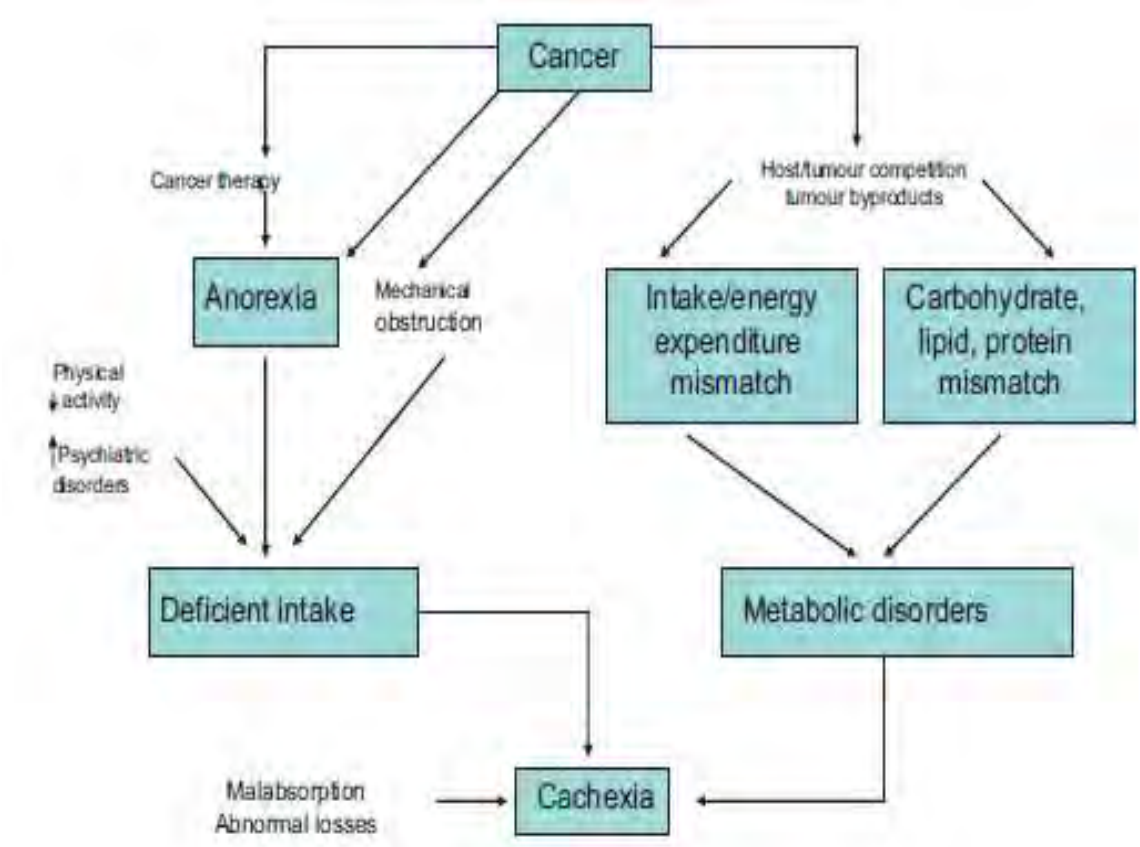

Figure 1: Multifactorial etiology of malnutrition in cancer patients (Righini *et al*., 2013).

Malnutrition is a possible complication in patients with cancer and can be the first symptom to reveal the presence of the disease. It is a major cause of morbidity and mortality. Even before starting anticancer treatment patients can experience profound metabolic and physiological alterations with increased needs of macro and micronutrients. Malnutrition impairs the immune status and reduces the body's defense against infectious diseases (Santarpia *et al*., 2011). The incidence of malnutrition in cancer patients ranges between 40% and 80%, the prevalence ranges from 50% to 80% worldwide depending on the tumor type, tumor location, stage of disease and the type of nutritional assessment method used (Lis *et al.*, 2012).

Cancer-associated malnutrition occurs when the nutritional needs of the patient are not met due to poor food intake, absorption and increased nutrient losses due to the tumor metabolism. At the time of diagnosis, approximately 75% of cancer patients are malnourished. Metabolic changes associated with cancer affect the metabolism of protein, fat and carbohydrate (Buncic, 2009). Malnutrition has been associated to several clinical consequences, including quality of life impairment, decreased treatment response, increases the risk of infections, high risk of chemotherapy induced toxicity, length of hospital stay, hospitalization costs and increased morbidity and mortality (Geirsdottir & Thorsdottir, 2008; Beghetto *et al.,* 2009; Kuzuya *et al*., 2005). It may affect performance of organ systems and even the whole organism (Andreoli *et al*., 2013). The consequences of cancer-associated malnutrition are presented in the (**Figure 2)** (Buncic, 2009).

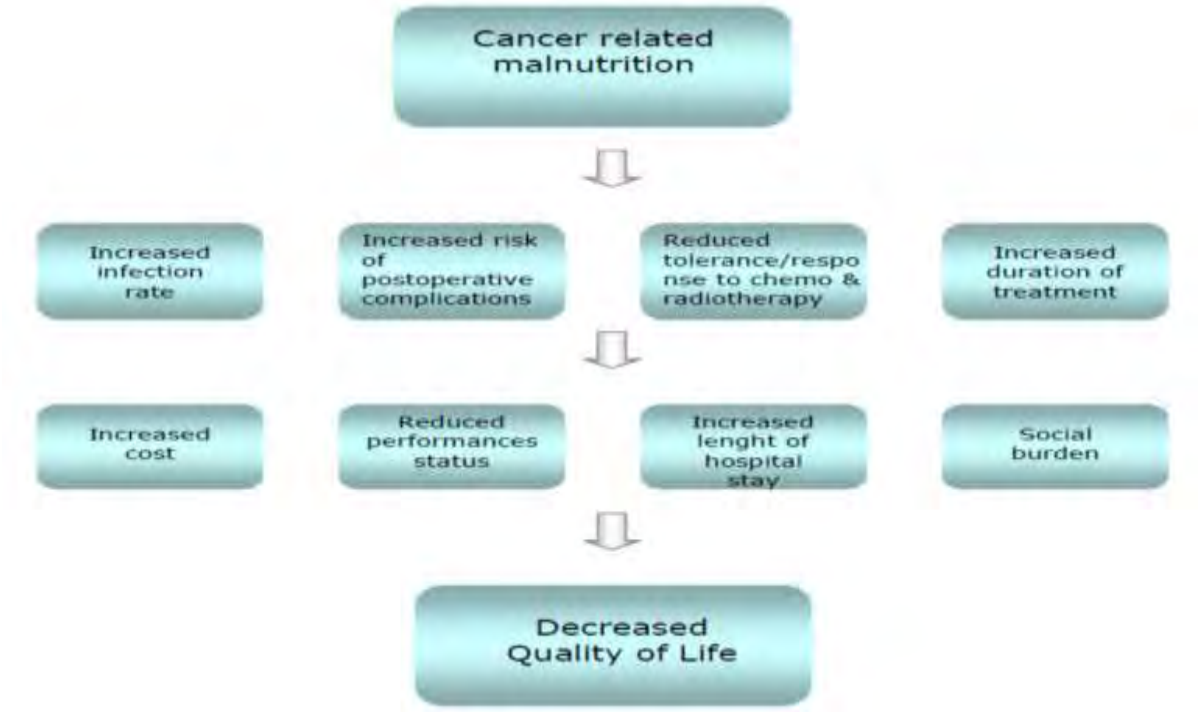

Figure 2: Impact of malnutrition (Buncic, 2009).

1.2.1.1. Protein-energy malnutrition in breast cancer

Protein-energy malnutrition is caused by an imbalance between intake and the body's requirements. This imbalance causes tissue loss in particular of muscle tissue with harmful functional consequences. The prevalence of protein-energy malnutrition increases with age. It is 4 to 10 % in elderly persons living at home, 15 to 38 % in those in institutional care and 30 to 70 % in hospitalized elderly Patients (Guidelines, 2007). Protein deficiency produces a fall in serum protein levels, especially serum albumin. Serum protein levels may be maintained for a considerable period of time, despite limited protein intake. Patients with a serum albumin less than 3.5 g/dl and a TLC less than 1,500 cells per mm^3 have a PEM (Robinson, 2015).

1.2.2. Cancer Cachexia

Cancer cachexia is characterized by systemic inflammation, negative protein and energy balance, and an involuntary loss of lean body mass, with or without wasting of adipose tissue. In addition to rapid loss of skeletal muscle mass, cardiac muscle is also depleted in the cancer cachexia but muscle of other visceral organs tends to be preserved. Cachexia occurs in the majority of terminal cancer patients and responsible for the death of 22% of cancer patients (Aoyagi *et al*., 2015).

Approximately half of all patients with cancer experience cachexia. Death usually occurs when there is 30% weight loss. Cancer patients who have lost a significant percentage of their body-weight before surgical treatment are subject to a much greater risk of postoperative mortality and morbidity (Douglas & Shaw, 1990).

1.2.2.1. Pathophysiology of cancer cachexia

Systemic inflammation is a hallmark of cancer cachexia, indicated by the production of acute-phase response proteins such as CRP. These phenomena increase muscle catabolism and transfer amino acids from muscle anabolism toward the amino acid pool required for APR protein anabolism. Signaling through cytokines pathways has a role in cancer cachexia and anorexia (**Figure 3**) (Aoyagi *et al*., 2015).

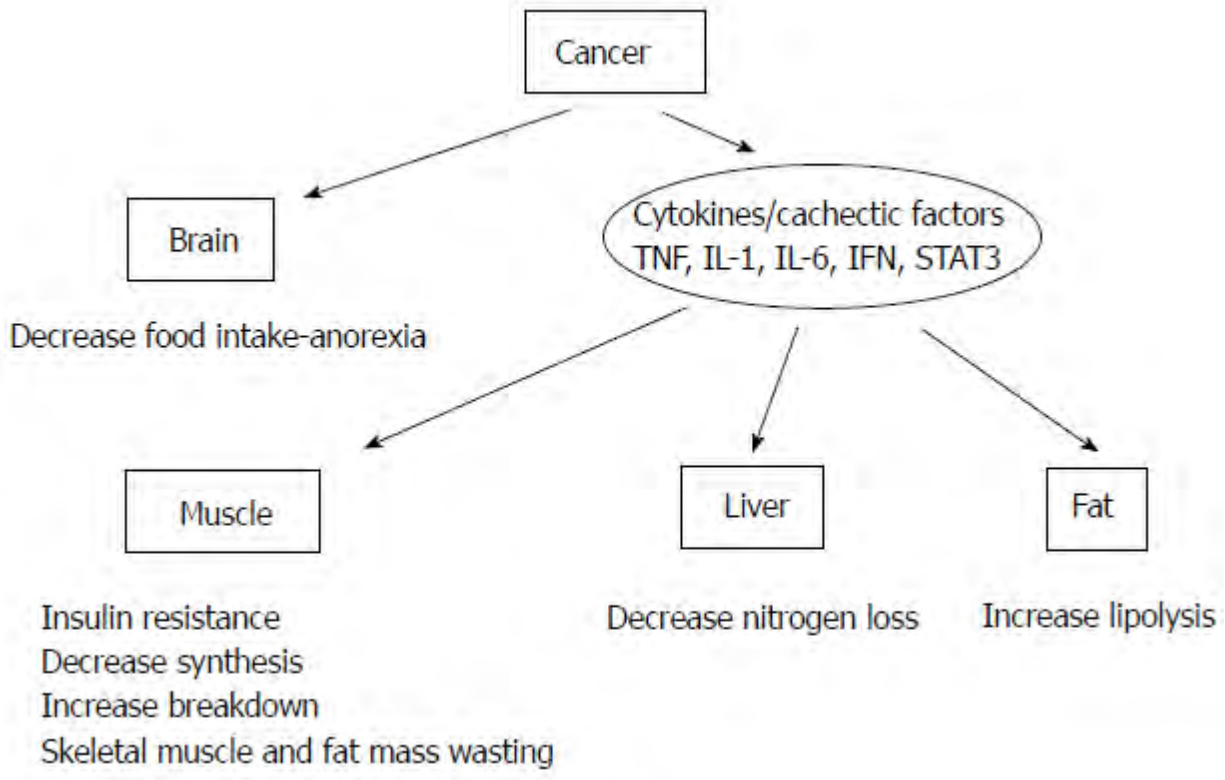

Figure 3: Role of tumor-induced systemic inflammation with metabolic pathways in organs affected by cancer cachexia (Aoyagi *et al*., 2015).

TNF-α increases gluconeogenesis, lipolysis, proteolysis, decreases the synthesis of proteins, lipids and glycogen, induces the formation of IL-1, and stimulates the expression of UCP 2 and

UCP3 in cachectic skeletal muscle. Cytokines are transported across the blood brain barrier where they interact with the luminal surface of brain endothelial cells causing release of substances that affect appetite. IL-1 induces anorexia in cachectic patients as it causes an increase in plasma concentrations of tryptophan, leading to increased serotonin production from the hypothalamus causing early satiety and suppressing hunger (Aoyagi *et al*., 2015). Either tumor cell production of pro-inflammatory cytokines or the host inflammatory cell response to tumor cells is the source of the APR proteins seen in many malignancies (Douglas & Shaw, 1990).

1.2.2.2. Metabolic disturbance in cancer cachexia

In malnourished patients near death there is an increase in REE and protein catabolism which could relate to the utilization of the last skeletal muscle mass (Aoyagi *et al*., 2015). Nutritional demand in the tumor-bearing state is increased due to alterations either by the neoplasm itself or by the stressed host (Younes & Noguchi, 2000). Resting energy expenditure is increased in the cachectic state (Douglas & Shaw, 1990), with futile metabolic cycling and contributes to the wasting process. Increased energy expenditure can result from uncoupling of oxidative phosphorylation especially in brown adipose tissue. UCP 2 and UCP 3 shunt energy from oxidative phosphorylation into heat rather than ATP. Energy expenditure also increased *via* increased activity of the Cori cycle (Siddiqui *et al.*, 2006).

In the cachectic cancer patient there is an accelerated mobilization and oxidation of energy substrates and loss of nitrogen. These changes are a consequence of alterations in intermediary metabolism associated with cancer (Douglas & Shaw, 1990). Tumor consumes abundant amounts of glucose, even under aerobic conditions, releasing large amounts of lactate into the

host's circulation and then converting lactate to glucose by the liver. In addition to lactate, glucose synthesis from alanine and glycerol is increased**.** Hence, proteolysis and lipolysis occur at accelerated rates in order to maintain a high level of glucose synthesis **(Figure 4)**. Cancer patients experience insulin resistance in adipose tissue, skeletal muscle, and liver (Esper & Harb, 2005).

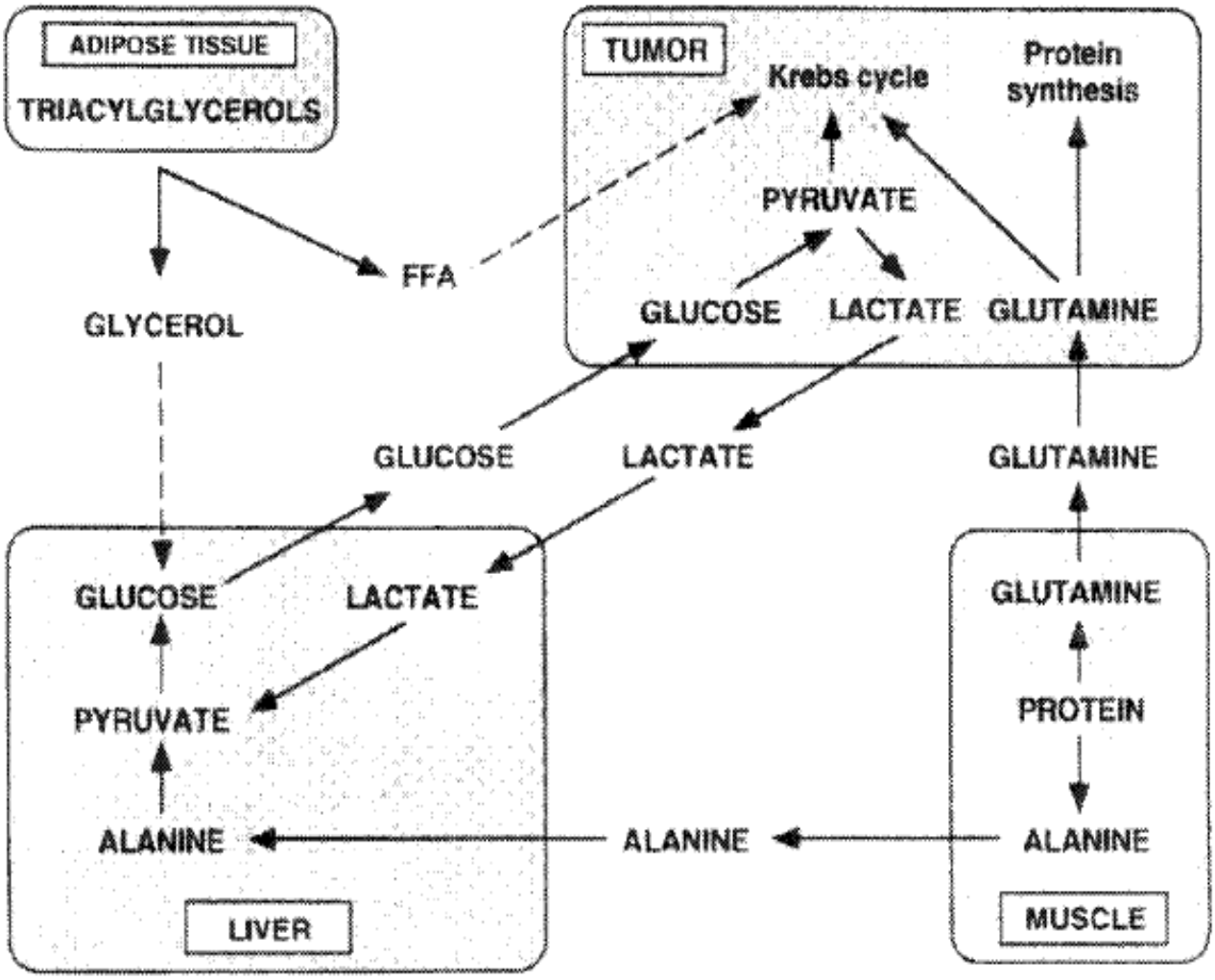

Figure 4: Metabolic interactions between tumor and host. The 3 main metabolic disturbances associated with cancer cachexia are (1) lactate recycling, (2) lipid mobilization and lipolysis, and (3) enhanced protein degradation (Esper & Harb, 2005).

FFA, free fatty acids

Abnormalities associated with cancer cachexia include, inappropriate increases in energy expenditure, muscle protein loss and atrophy, and accompanying defects in carbohydrate, lipid, and protein summarized below (**Table 1**).

Table1: Intermediate metabolism alteration in cancer cachexia (Delano & Molder, 2006).

Carbohydrate metabolism
Up-regulated glucose transporters GLUT 1 and GLUT 3
Increased expression hexokinase 2
Increased Cori cycle activity
Increased hepatic gluconeogenesis
Increased glucose turnover
Insulin resistance
Lipid metabolism
Decreased adipose tissue activity
Increased host adipose tissue lipolysis
Tumor directed adipose tissue lipolysis
Increased hepatic lipogenesis
Hyperlipidemia
Increased brown adipose tissue thermogenesis
Protein metabolism
Tumor directed proteolysis
Increased hepatic protein synthesis
Increased muscle protein breakdown
Increased whole body protein turnover
Decreased muscle amino acid uptake

GLUT, glucose transporter protein

1.2.2.3. Protein metabolism in cancer patients

Tumors are “glucose eaters” as well as “nitrogen sinks”, depleting the host protein mass and resulting in characteristic alterations in protein metabolism. Redistribution or translocation of peripheral proteins to support visceral or tumor protein synthesis is an essential feature of amino

acid metabolism in cancer cachexia (Younes & Noguchi, 2000). In patients with cachexia there is an increase in muscle protein catabolism leading to a net loss of muscle mass. This imbalance of protein synthesis and degradation is obvious aspects of metabolism disruption in cancer cachexia (Aoyagi *et al*., 2015). Loss of body protein in patients with cancer cachexia is manifested clinically as skeletal muscle atrophy and hypoalbuminaemia, and is associated with an impaired tolerance of treatment procedures (Douglas & Shaw, 1990). Patients with advanced malignancy or stage IV cancer had significantly greater protein turnover, synthesis, and catabolism than patients with localized disease. There is also significant correlation between alterations in protein metabolism and cancer stages (Younes & Noguchi, 2000).

1.2.2.4. Muscle protein degradation in cancer cachexia

Wasting is accelerated by the proteolysis of skeletal muscle and consumption of body fat. Accelerated mobilization and consumption of host protein stores from peripheral tissues occurs to support gluconeogenesis and acute phase protein synthesis (Younes & Noguchi, 2000). Removal of certain amino acids by the tumor would lead to a depression of host protein synthesis since normal protein synthesis requires the full complement of amino acids (Smith & Tisdale, 1993).

1.3. Assessment of malnutrition in breast cancer

Since nutritional status affects a patient's response to illness, nutritional assessment provide integral part for patient care. These assessments should allow for the early detection of both nutrient deficiencies and excesses in a non-invasive, inexpensive and feasible way (Maqbool *et al.*, 2012). A number of tools are used to assess protein energy malnutrition in breast cancer

based on standard references value presented in **(Table 2)** below. The most frequently used malnutrition assessment tool includes biochemical parameters, anthropometric and physical examination (Lis *et al.*, 2012). The breast, lung and colon are leading sites of cancer in westernized countries. These cancers are commonly treated with chemotherapy, which often has adverse effect on the nutritional status of the affected patient. Nutritional status cannot be evaluated by single parameters and supports the need for several measurements (Geirsdottir & Thorsdottir, 2008). The early detection of malnutrition is important in order to implement an adequate nutritional therapy with the purpose of maintaining the nutritional status and avoiding the progression of malnutrition and its complications (Norte *et al.*, 2015).

Table 2: Reference value for biochemical markers used for full nutritional assessment of breast cancer patients.

Nutritional parameter	Categories	Reference value	references
Albumin (g/dl)	Normal	Above 3.5	Norte et al., 2015
	Mild malnourished	3 -3.5	
	Moderate malnourished	2.4 – 2.99	
	Sever malnourished	< 2.4	
TLC (cells/mm^3)	Normal	Above 2000	Norte *et al*., 2015
	Mild malnourished	1200 - 2000	
	Moderate malnourished	800 - 1999	
	Sever malnourished	< 800	
Total protein (g/dl)	Normal	6.6 – 8.7	Tietz,2008
	Malnourished	< 6.6	
Creatinine (mg/dl)	Normal	0.5 – 1.2	Henry,1984
	Malnourished	< 0.5	
BMI (kg/m^2)	obese	Above 30	Babiarczyk & Turbiarz, 2012
	overweight	25 - 29.99	
	nourished	18.5 – 24.9	
	Mild malnourished	17 – 18.4	
	Moderate malnourished	16 – 16.9	
	Sever malnourished	< 16	

1.3.1. Biochemical method of malnutrition assessment

Biochemical nutritional assessment is important to identify individuals who will benefit from nutritional therapies and to establish baseline values against which to measure the effectiveness

of nutritional intervention. Biochemical methods are more sensitive than other methods in showing recent changes in nutritional status (Omran & Morley, 2000). Among the biochemical parameters, serum albumin levels have long been considered a major measure of protein energy malnutrition and are the most commonly used laboratory tests (Kuzuya *et al*., 2005). But there is a criticism for albumin as a measurement of nutritional status in hospitalized patients. This is due to the fact that albumin is inversely correlated with markers of inflammatory activity and can behave as an acute-phase reactant, with markedly reduced levels in the setting of acute illness (Rosenthal *et al.*, 1998).

1.3.1.1. Nutritional assessment by using serum total proteins

Total human serum protein profile is made up of albumin and globulins. Serum protein levels are important markers of the body protein pool. Proteins with a long half-life are most useful in evaluating chronic nutritional changes in the outpatient setting. Proteins with a short half-life are most useful in the acute or sub-acute settings (Omran & Morley, 2000).

1.3. 1.2. Nutritional assessment by using serum albumin

Albumin is a serum hepatic protein that has a long half-life approximately with 14–20 days. It functions is maintain oncotic pressure in the capillaries, transport substances in plasma such as minerals, hormones and fatty acids. Serum levels of albumin reflect the net result of hepatic synthesis (12–15 g/dl), plasma distribution and protein loss (Bharadwaj *et al*., 2016; Omran & Morley, 2000). Out of total pool albumin, 50% to 60% present in extravascular compartment and can be mobilized to the intravascular space in periods of stress due to surgery or infection. The functional catabolic rate of albumin is proportional to the size of the extravascular pool, which allows the concentration in the serum to remain relatively constant (Omran & Morley,

2000).Despite protein consumption have no any effect on the patient's albumin level; its pool is affected by a Number of inflammatory conditions and drugs, especially those that affect liver function (Bharadwaj *et al.,* 2016).Malnutrition and inflammation suppress albumin synthesis. In an adult the normal range of serum albumin is 3.5-5.0 g/dl. levels < 3.5 g/dl of albumin indicate malnutrition (hypoalbuminemia) (Kuzuya *et al.,* 2005). Serum albumin assesses the nutritional status, severity of disease, disease progression and prognosis (Gupta & Lis, 2010). Cytokines such as TNF-α, IL-2 and IL-6 inhibit albumin production by inhibiting albumin gene expression and cause a vascular endothelial leak, resulting in an increase plasma clearance rate of albumin (Omran & Morley, 2000).

1.3.1.3. Nutritional assessment by using serum Creatinine

Creatinine is an endogenous substance generated from the non-enzymatic cyclization of creatine and phosphocreatine, 95% of which is found in muscle (Thongprayoon *et al.,* 2016; Zhang *et al*., 2015; Patel *et al.*, 2013). Serum creatinine synthesized from the amino acids glycine and arginine in liver, pancreas, and kidneys and serves as a rapidly mobilizable reserve of high-energy phosphates in skeletal muscle (Ostermann, 2016; Rosa *et al*., 2016). Creatinine production is determined by the amount of creatine generated in liver, pancreas, and kidneys, creatine ingested (intake of red meat) and muscle function. A decrease in muscle mass could decrease serum creatinine levels, and increased with higher muscle mass (Rosa *et al.,* 2016). In normal subjects under the steady state and stable kidney function, creatinine is usually produced at a relatively constant rate by the body depending on the absolute amount of muscle mass (Patel *et al*., 2013; Rosa *et al*., 2016). Due to the correlation between serum creatinine levels and muscle mass, creatinine levels in the steady state has been used as a surrogate of muscle mass measurements. Protein malnutrition could result in low creatinine levels (Thongprayoon *et al.,* 2016).

Low creatinine excretion measure low muscle mass (Patel *et al.,* 2016). Absolute creatinine production declines with age in line with decreasing muscle mass.

1.3.1.4. Nutritional assessment by using serum Urea

Urea is the primary metabolite derived from dietary protein and tissue protein turnover**.** Malignant tumor in appropriately metabolize both dietary and host proteins, resulting negative nitrogen balance.

1.3.2. Nutritional assessment by using Total lymphocyte count

Since abnormalities in immune function have been associated with malnutrition, measures of total lymphocyte count may be useful on initial evaluation of the newly diagnosed patient.

Lymphocyte count reflects the response of cellular immunity in a cancer patient and its alteration influences the disease progression (Chauhan *et al.,* 2016).The higher lymphocyte count and lower the platelet- lymphocyte ratio, the better the overall survival of breast cancer patient (Ruffell *et al.*, 2012). TLC decreases with progressive malnutrition and correlates with morbidity and mortality in hospitalized patients (Omran & Morley, 2000). Malnutrition leads to suppression of cellular immunity and a delayed hypersensitivity reaction (Kuzuya *et al*., 2005).

1.3.3. Anthropometric methods of malnutrition assessment

Anthropometry measurements such as weight, height and body mass index, generally considered as the single most easily obtainable, inexpensive, and noninvasive method by which to assess nutritional state (Ojoawa *et al*., 2015). BMI is defined as weight in kilograms divided by height

in meters squared (Rosenthal *et al.,* 1998). Diagnosis of malnutrition is considered when BMI less than 18.5 kg/m^2 (Kuzuya *et al*., 2005)**.**

1.4. Statement of the problem

Breast cancer is the most common cancer in women worldwide, with nearly 1.7 million new cases diagnosed in 2012. This represents about 12% of all new cancer cases and 25% of all cancers in women (WCRF, 2012). Currently Breast cancer is the most commonly diagnosed cancer in women in several sub-Saharan African countries and its burden increases in the coming decades. An age-standardized incidence rate of 19.5 per 100,000 and an estimated age-standardized death rate of 11.8 per 100,000 females are estimated in Ethiopia (Kantelhardt *et al*., 2014). The prevalence of Malnutrition in people with cancer is estimated up to 30–50% and 85% in long-term care facilities. However, the actual prevalence in the population is unknown due to historical inconsistencies in defining and identifying malnutrition. Furthermore, malnutrition has been associated with increased healthcare associated costs including longer hospital length of stay and increased rates of major and minor complications (Bharadwaj *et al.,* 2016).

In a study done by Laky *et al*., 2008 of people dying from cancer 50% are malnourished and up to 20% die from the effects of malnutrition rather than from the cancer it self. Thirty to sixty of cancer patients are diagnosed with protein- calorie malnutrition (Kumar, 2012). The problem is that there is no standard method for screening and diagnosing cancer patients with malnutrition, so that the patients take a therapy (surgery, chemotherapy and radiotherapy) without knowing their nutritional status. In addition therapies also exaggerate the malnutrition resulting increase in morbidity and mortality of the patients. So the patient negatively response to therapy, increases

the incidence of treatment-related side effects and can decrease survival. Hence early assessment of nutritional status is important for nutritional therapy in order to reduce the above complications. Moreover reversing the nutritional status will have a profound effect in addition to the therapeutic regimens. To our knowledge no previous research was done in Ethiopia. This paper, therefore, attempts to assess Malnutrition Using Biochemical Markers among Female Breast Cancer Patients Attending Tikur Anbessa Specialized Hospital, Ethiopia.

1.5. Significance of the study

Early assessment of malnutrition in breast cancer patients reduces patients' response to therapy, treatment related side effects and guide for planning of nutritional support to the patient. In addition, early nutritional intervention is cost effective, as it reduces complication rates and length of hospital stay. The development and use of screening and assessment tools is essential for effective nutritional intervention and management of patients with cancer.

2. OBJECTIVES OF THE STUDY

2.1. General Objective

- To assess malnutrition in breast cancer patients through biochemical markers, anthropometrics and Total lymphocyte count.

2.2. Specific objectives

- To quantify serum albumin, globulin, total protein, creatinine, urea level and determine total lymphocyte count of breast cancer patients.
- To determine body mass index as one anthropometric parameter of breast cancer patients
- To determine the prevalence of malnutrition in breast cancer patients.
- To compare feasibilities of the different parameters for diagnosis of malnutrition.
- To compare the biochemical and anthropometric measurement methods for diagnosis and recommend utilization of both for better diagnosis of malnutrition due to breast cancer.

3. MATERIALS AND METHODS

3.1. Study area and period

The study was conducted from January, 2016 to April 2017 at Tikur Anbessa Specialized Hospital, Addis Ababa.

3.2. Study design

Hospital based cross-sectional study was conducted to evaluate the serum levels of biochemical profiles and anthropometric parameters among new breast cancer patients attending Tikur Anbesa Specialized Hospital, Addis Ababa with healthy individual as a control group.

3.3. Population

3.3.1. Source population

The source population for this study was all female breast cancer patients attending at Tikur Anbessa Specialized Hospital.

3.3.2. Study population

The study population for this study was all newly diagnosed Female breast cancer patients attending at Tikur Anbessa Specialized Hospital, Addis Ababa in the time interval of the study period.

3.4. Inclusion and exclusion criteria

3.4.1. Inclusion criteria

All newly diagnosed female breast cancer patients attending at the oncology outpatient clinic of the Tikur Anbessa Specialized Hospital and age and sex matched healthy individuals during data collection period were included in the study.

3.4.2. Exclusion criteria

The excluded patients were those with diagnosed renal and liver failure, surgery**,** chemotherapy**,** radiotherapy and dialysis, using immunosuppressive medication, or those who were not willing to fill the informed consent form.

- Age > 65 years

3.5. Sampling method and sample size determination

Convenient sampling method was performed until I get 50 breast cancer patients.

3.6. Variables

3.6.1. Dependent variables

- Serum total protein
- Serum albumin
- Serum globulin

- Serum creatinine
- Serum urea
- Total lymphocytes count
- Body mass index

3.6.2. Independent variables

- Age
- Appetite status
- Mode of feeding
- Cancer stage
- Residence
- Economic status
- Marital status
- Alcohol consumption
- Smoking status

3.7. Blood sample and data collection procedures

After the study participants had been asked for their consent to be interviewed and to give sample blood, about 5 ml blood was withdrawn from the study participants. The sample was collected by qualified professional nurses in the hospital for further analysis of the blood sample. In addition, the questionnaire was filled by face to face interview and some anthropometric indicators were also measured following standard procedures. Blood collected in appropriate

tubes was allowed to stand for 30 minutes at room temperature to allow complete clotting and clot retraction. Samples were then centrifuged at 4000 rpm for 10 min to extract serum. The serum extracted was then used to determine the levels of albumin, total protein, creatinine and urea. About 2ml of the blood was collected in EDTA coated tubes and hematological profiles were determined for all samples using a hematological analyzer. Safety precautions were taken while handling blood and disposing it.

3.7. Test principles of the laboratory analytes

3.8.1. Serum albumin concentration

Serum albumin level was measured by the method of bromocresol green (Doumas *et al.* 1971). The method is based on the specific binding of bromocresol green, an anionic dye, and the albumin at acid pH producing a color change of the indicator from yellow – green to blue - green with the resulting shift in the absorption wavelength of the complex. The absorbance of the color produced was measured in a spectrophotometer at 632nm wave lengths. The intensity of the color formed is proportional to the concentration of albumin in the sample. The normal albumin levels were considered to be 3.5 - 5.1 g/dl. The reaction and its calculation were as follows:

BCG + Albumin $\xrightarrow{\text{PH 4.3}}$ **BCG - albumin complex (blue – green)**

$$\text{Albumin (g/dl)} = \frac{\text{Absorbance of unknown concentration}}{\text{Absorbance of standard concentration}} \times \text{standard concentration}$$

3.8.2. Serum total protein concentration

Total protein was determined by using an automatic chemistry analyzer in the diagnostic Center, Tikur Anbesa Specialized Hospital. The measurement was performed by a Biuret reaction using a total protein reagent kit. Serum protein form a violet colored complex in the presence of copper salt in alkaline solution, the intensity of the color is proportional to the protein concentration. The reaction and calculation is shown below.

$$\mathbf{Cu^{2+} + serum\ protein \xrightarrow[25\text{-}37\ ^{o}c]{PH\ \ 12} copper\text{-}protein\ complex\ (violet\ colored\ complex)}$$

$$\text{Total Protein (g/dl)} = \frac{\text{Absorbance of Unknown concentration}}{\text{Absorbance of standard concentration}} \times \text{standard concentration}$$

3.8.3. Serum globulin concentration

Total serum globulins were determined by subtracting the values of albumin from total protein (Fatima *et al*., 2013).

Concentration globulin (g/dl) = Concentration Total Protein (g/dl) – Concentration Albumin (g/dl).

3.8.4. Serum creatinine concentration

Serum creatinine reacts with picric acid in alkaline solution yielding a yellow- orange colored compound. The intensity of the color is directly proportional to creatinine concentration present in the sample and is measured at an absorbance between 490-500nm.

Creatinine + picrate $\xrightarrow{\text{NaOH}}$ **Yellow-red complex**

Creatinine (mg/dl) = Absorbance un known concentration X Standard concentration (mg/dl)
Absorbance standard concentration

3.8.5. Serum urea concentration

The enzyme urease converts urea to ammonia and carbonic acid. Glutamate dehydrogenase catalyzes the reaction of ammonia with α- ketoglutarate and oxidizes NADH in to NAD^+. The decrease of absorbance of NADH, measured at 340 nm when the ammonia reacts with ketoglutarate, is proportional to the urea in the sample. The reaction sequence & its calculation are as follows:

Urea + $2H_20$ $\xrightarrow{\text{urease}}$ **$2NH^+_4$ + $2HCO3^-$**

2- ketoglutarate + NH^+_4 + NADH $\xrightarrow{\text{GLDH}}$ **L- Glutamate + NAD^+ + H_2^o**

Urea (mg/dl) = Absorbance un known concentration x standard concentration of urea
Absorbance standard concentration

3.8.6. Determination of total lymphocyte count

Automated hematology analyzer, was used to accurately count and size cells by detecting and measuring changes in electrical resistance when a particle such as a cell in a conductive liquid passes through a small aperture. Each cell suspended in a conductive liquid acts as an insulator.

As each cell goes through the aperture, it momentarily increases the resistance of the electrical path between the submerged electrodes on either side of the aperture. This causes a measurable electronic pulse. For counting, the vacuum used to pull the diluted suspension of cells through the aperture must be at a regulated volume. The number of pulses correlates to the number of particles. The height of the electrical pulse is proportional to the cell volume.

3.8. Anthropometrical measurement procedure

The weights of the breast cancer patients and the control were measured using a standard balance, and the height was measured by using a height measuring device attached to the balance. Body Mass Index was then calculated from the body weight (kg) and height (meter) as follows: BMI = Weight (in kg) / (Height in m)^2. According to (Norte et al., 2015),four categories of BMI can be identified as follows: <18.5 kg/m2 (underweight); 18.5 to 24.9 kg/m2 (normal); 25 to 29.9 kg/m2 (overweight); and ≥30 kg/ m2 (obesity).The participants' ages were also recorded.

3.9. Data quality control and management

- The data collection questionnaire was well prepared and all variables were filled on the data extraction format daily.
- All the laboratory procedures were handled by professional laboratory technologists.
- All the tests were standardized and automated.

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 into Epi Data statistical software version 3.1 and then to SPSS software version 23 package and the different variables were tested and analyzed. Simple descriptive statistics was used to present the socio demographic and clinical characteristics of the study subjects. Continuous variables were presented as mean ± standard error and compared using the student t-tests and one way analysis of variance (ANOVA). Other associations were 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 reference number SOM/DRERC/BCHM060/2009 was obtained from the Departmental Research and Ethics Review Committee, Department of Biochemistry, College of Health Sciences, Addis Ababa University. The objective of the study was briefly clarified and explained for each participant, before enrolling any of the eligible study participants. Samples and data were collected after informed consent had been obtained from the study participants. The findings of the study will be disseminated for health care professionals and other concerned bodies for better care of the breast cancer patients.

3.12. Operational definitions

Macronutrient: - a chemical element or substance, such as carbohydrates, proteins, and fats, required in relatively large quantities in the diet.

Micronutrient: - organic compound, such as a vitamin, or chemical element essential in minute amounts in the diet.

Nutritional assessment: - A detailed more specific and in-depth evaluation of a patient's nutritional state, typically by an individual with nutritional expertise.

Anthropometry: - measurement referred to as body composition analysis.

Outpatient: - A patient who attends a hospital for diagnosis or treatment but does not occupy a bed.

Nutritional support: - Nutritional support includes food, oral nutritional supplements, tube feeding and parenteral nutrition.

Nitrogen balance: – difference between the amounts of nitrogen ingested and that excreted.

Cachexia: - ultimate form of malnutrition

4. RESULTS

4.1. Socio demographic characteristics of the breast cancer patients and control groups.

This study enrolled 50 female breast cancer patients and 50 healthy female as controls which meet the inclusion and exclusion criteria. The average age of the breast cancer patients and control groups were 43.06 years ranging from 21 to 56 years as they were matched by age. Most of the breast cancer patients in the study were of middle economic status (58%), rural resident (58%), married (74%), moderate loss of appetite (48%), self-feed without difficulty (70%). In addition only 12 % of the breast cancer patients drink alcohol, and 2% did smoke cigarette. Whereas the control groups were of middle economic status (72%), urban (84%), married (72%), with good appetite. They had no habit of smoking and alcohol consumption **(Table 3).**

Table 3: Socio demographic characteristics of the breast cancer patients and control groups

Variables		Breast cancer patients Total (50) N (%)	Controls Total (n=50) N (%)
Age [a]		43.06 ± 1.72	43.06 ± 1.72
Socio economic status[b]	Low	20 (40)	6 (12)
	Middle	29 (58)	36 (72)
	High	1 (2)	8 (16)
Residence	Rural	29 (58)	8 (16)
	Urban	21 (42)	42 (84)
Marital status	Married	37(74)	36 (72)
	Single	2 (4)	12 (24)
	Widow	7 (14)	0 (0)
	Divorce	4 (8)	2 (4)
Appetite Status	No Loss of appetite	21 (42)	50 (100)
	Moderate Loss of appetite	24 (48)	0 (0)
	Severe Loss of appetite	5 (10)	0 (0)
Mode of feeding	Self-feed without difficulty	35 (70)	50 (100)
	Self-feed with Difficulty	15 (30)	0 (0)
Alcohol consumption	Yes	6 (12)	0 (0)
	No	44 (88)	50 100)
Smoking status	Yes	1 (2)	0 (0)
	No	49 (98)	50 (100)

[a] Age, continuous variable, is expressed as mean ± standard error; [b] for the rest of the variables, qualitative, the numbers are in percent out of the total 50 patients and 50 controls.

With regards to cancer stage, stage III (36 %) were the highest proportion whereas stage I (18%) found in least proportion of the breast cancer patients (**Figure 5)**.

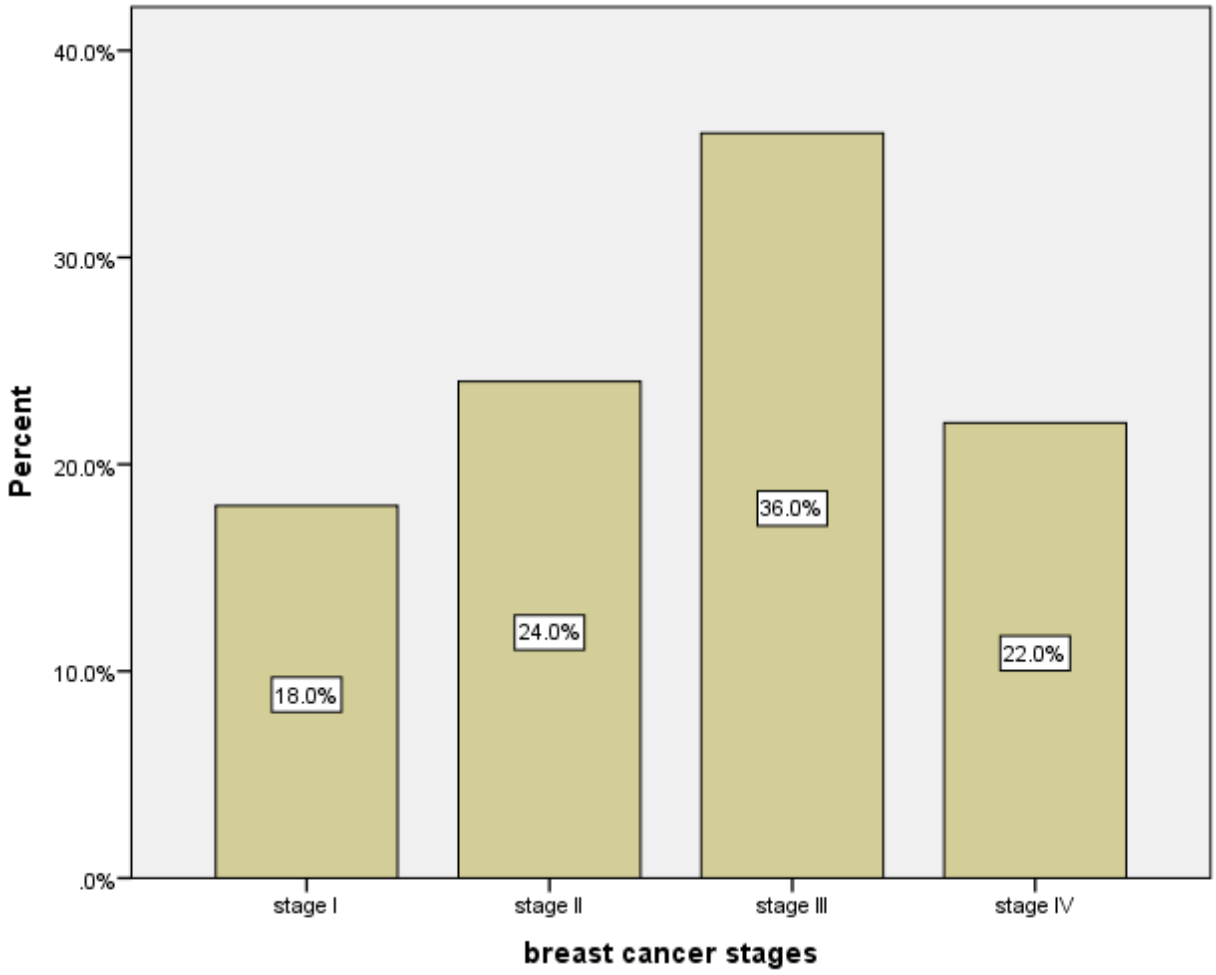

Figure 5: Proportion of breast cancer patients' enrolled in the study.

4.2. Anthropometric and biochemical characteristics of the breast cancer patients and control groups.

Assessment of malnutrition among the breast cancer patients and control groups was performed using anthropometric techniques and biochemical tests using standard kits. The age group ranged between 21 and 56 years. There was a good matching with regards to age between the control and study groups. There was no statistically significant difference between the mean ages of patients with breast cancer and the control groups.

Serum albumin level was measured for controls and study subjects and the results showed that there was a statistically significant difference between the two groups. The study group had lower mean albumin level (3.89 ± 0.04 g/dl) than the control group (4.34 ± 0.17 g/dl) with a p -value < 0.0001. Serum total protein level was also measured for the two groups and the results showed that there was no significant difference between them. The study group had almost similar mean average total protein level (7.81 ± 0.07 g/dl) to the control group (7.7 ± 0.05 g/dl) with a p -value 0.19. Serum globulin was calculated from the total protein for the breast cancer patient & control groups. The study group had higher mean globulin level (3.92 ± 0.08 g/dl) than the control group (3.35 ± 0.04 g/dl) with a p-value < 0.0001.

Serum creatinine level was also measured. The study groups mean value of serum creatinine level was (0.72 ± 0.03 mg/l) and the control mean value level was (0.96 ± 0.03 mg/l) with a p-value < 0.0001. This shows that the creatinine level had really gone down for the breast cancer patients than control groups. Urea mean value level for study group was (25.19 ± 1.22 mg/l) and

for control group (21.62 ± 1.01 mg/l) with a p-value 0.033. In addition total lymphocytes count was determined for both groups and its mean value for the study group (1.73 x 10^3 ± 0.29 cells/mm^3) & the control group (2.35 x 10^3 ± 0.15 cells/mm^3) with a p-value < 0.0001. Anthropometric parameter such as body mass index was determined by measuring weight and height of both groups using standard measuring procedures. The mean body mass index value was 17.97 ± 0.6 kg/m^2 for study group and 20.43 ± 0.64 kg/m^2 for control group. The results of the two groups is shown in **(Table 4)** below.

Table 4: Comparison of mean value of anthropometric and biochemical measurements of the breast cancer patients and control groups.

Parameters	Breast Cancer (n=50) Mean ± SE	Control (n=50) Mean ± SE	P-Value
Age	43.06 ± 1.72	43.06 ± 1.72	1.000
Albumin[a]	3.89 ± 0.04	4.34 ± 0.17	< 0.0001
Globulin[a]	3.92 ± 0.08	3.35 ± 0.04	< 0.0001
Total protein[a]	7.81 ± 0.07	7.7 ± 0.05	0.190
lymphocyte count[b]	1.73 ± 0.29	2.35 ± 1.12	< 0.0001
Creatinine[c]	0.72 ± 0.03	0.96 ± 0.03	< 0.0001
Urea[c]	25.19 ± 1.22	21.62 ± 1.01	0.033
Body mass index[d]	17.97 ± 0.6	20.43 ± 0.64	0.069

Values bearing different superscripts a, b, c, d; represents units, [a]g/dl, [b]10^3 x cells/ mm^3, [c]mg/dl, [d]kg/m^2

4.3. Prevalence of malnutrition in breast cancer patients and control groups

Serum albumin, total lymphocyte count, total protein, creatinine and body mass index were measured to see the prevalence of malnutrition between control group and study subjects. The results obtained are shown in **(Table 5)** below. The overall prevalence of malnutrition was 32%, with 12% and 20% cases of moderate and mild malnutrition, respectively according to serum albumin. According to total lymphocyte count prevalence of malnutrition were 46%; with 4%, sever; 4%, moderate and 38% mild malnutrition in the study group. In addition 2% were, severely; 34%, mildly malnourished; 14% were over - weight and 12% obese breast cancer patients with regard to body mass index. There was no statistically significant prevalence of malnutrition using serum total protein and creatinine level in the study group. There was almost no prevalence of malnutrition in the control group through all biochemical nutritional markers assessed in the study.

Table 5: Prevalence of malnutrition in breast cancer patients and control individuals.

Variables	Breast cancer (50) N (%)	Control (50) N (%)	P – value
Albumin			
Severely malnourished	0 (0)	0 (0)	
Moderately malnourished	6 (12)	0 (0)	0.03*
Mildly malnourished	10 (20)	0 (0)	
Normal	34 (68)	50 (100)	
Total lymphocyte count			
Severely malnourished	2 (4)	0 (0)	
Moderately malnourished	2 (4)	0 (0)	0.01*
Mildly malnourished	19 (38)	3 (6)	
Normal	27 (54)	47 (94)	
BMI			
Sever malnourished	1(2)	0(0)	
Mild malnourished	17(34)	2(4)	0.01*
Normal	19(38)	34(68)	
Overweight	7(14)	14(28)	
Obese	6(12)	0(0)	
Total protein	1(2)	0(0)	0.99
Malnourished	49(98)	50(100)	
Normal			
Creatinine			
Malnourished	11(22)	0(0)	0.07
Normal	39(78)	50(100)	

***P** value < 0.05 is statistically significant

Pearson correlation analysis for anthropometric and biochemical measurements for the study and control group were done and are shown in **(Table 6).** Albumin positively correlated to TLC (r = 0.51, p = 0.03) in study group and serum total protein (r = 0.56, p < 0.0001) in the control group. Albumin also negatively correlated with nutritional indicators of serum globulin (*r* = -0.48, P < 0 .0001) in study group. Globulin showed a statically significant positive correlation with total protein in both study (r = 0.84, p < 0.0001) and control groups (r = 0.86, p < 0.0001). Creatinine positively correlated to urea (r = 0.33, p = 0.02) and body mass index (r = 0.43, p < 0.0001) in the study group. But there was no correlation in the control group. Total lymphocyte count positively correlated with body mass index (r = 0.47, p = 0.04) in study group.

Table 6: Pearson correlation co-efficient between anthropometric and biochemical indices for breast cancer patients and control groups.

			AGE	GLOB	TPRO	TLYMC	CRE	UREA	BMI
Albumin	Breast Cancer	R	-0.21	-.48**	0.06	0.51*	0.02	0.05	0.1
		P	0.14	<0.0001	0.65	0.03	0.88	0.73	0.48
	Control groups	R	-0.11	.05	.56**	0.02	0.26	0.02	0.05
		P	0.45	0.71	<0.0001	0.16	0.06	0.89	0.74

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

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

TPRO; total protein, GLOB; globulin, TLYMC; total lymphocyte count, CRE; creatinine, BMI; body mass index

			AGE	ALB	TPRO	TLYMC	CRE	UREA	BMI
Globulin	Breast Cancer	R	0.21	-.48**	.84**	0.04	-0.2	0.01	0.07
		P	0.15	<0.0001	< 0.0001	0.78	0.33	0.9	0.59
	Control groups	R	0.12	0.05	.86**	-0.15	0.15	0.07	0.06
		P	0.42	0.71	< 0.0001	0.3	0.3	0.65	0.68

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

ALB; albumin, TPRO; total protein, TLYMC; total lymphocyte count, CRE; creatinine, BMI; body mass index

			AGE	GLOB	TPRO	TLYMC	ALB	UREA	BMI
Creatinine	Breast Cancer	R	-0.2	0.14	0.15	0.26	0.02	0.33*	0.43**
		P	0.17	0.33	0.31	0.08	0.88	0.02	<0.0001
	Control groups	R	-0.05	0.15	0.26	0.23	0.26	0.08	0.12
		P	0.73	0.3	0.07	0.1	0.07	0.56	0.43

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

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

ALB; albumin, TPRO; total protein, GLOB; globulin, TLYMC; total lymphocyte count, BMI; body mass index

			AGE	ALB	TPRO	CRE	UREA	BMI	GLOB
TLYMC	Breast Cancer	R	-0.14	0.08	-0.09	0.26	0.19	0.47*	0.04
		P	0.35	0.56	0.52	0.07	0.18	0.04	0.78
	Control groups	R	-0.13	0.2	0.04	0.06	0.67	0.04	0.17
		P	0.35	0.16	0.79	0.64	0.64	0.77	0.23

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

ALB; albumin, TPRO; total protein, GLOB; globulin, TLYMC; totallymphocyte count, CRE; creatinine, BMI; body mass index

4.4. Socio demographic characteristics and the dependent variables of breast cancer patients and control groups.

Bivariate, Pearson correlation analysis, showed that age negatively correlated with all biochemical and body mass index in both study and control groups (p >0.05) (**Table 7).**

Table 7: Pearson correlation co-efficient between age and biochemical indices for breast cancer patients and control groups.

			ALBU	TPRO	GLOB	UREA	TLYMC	CRE	BMI
Age	Breast Cancer	R	-0.21	-0.01	-0.20	-0.08	-0.23	-0.15	-0.09
		P	0.13	0.46	0.14	0.57	0.09	0.46	0.51
	Control groups	R	-0.11	-0.04	-0.11	-0.11	-0.09	-0.05	-0.08
		P	0.45	0.78	0.41	0.44	0.5	0.73	0.56

ALB; albumin, TPRO; total protein, GLOB; globulin, TLYMC; totallymphocyte count, CRE;creatinine,BMI; body mass index.

In addition appetite status was also assessed in the study groups. There was statically significant difference in mean value of serum albumin (p = 0.027), with appetite status of breast cancer patients but non-statistical significant difference in total protein (p = 0.643), globulin (p = 0.891), Creatinine (p = 0.570), urea (p = 0.135), TLC (p = 0.179) and body mass index (p = 0.786) (**Table 8).**

Table 8: One way ANOVA appetite status effect on biochemical, TLC and BMI parameters, in breast cancer patients.

	no loss of appetite (n =21)	Moderate loss of appetite (n =24)	Sever loss of appetite (n = 5)	p-value
Albumin[a]	4.01 ± 0.09	3.98 ± 0 .05	3.67 ± .05	0.027*
Total protein[a]	7.67 ± 0.1	7.89 ± 0.1	7.89 ± 0.1	0.643
Globulin[a]	3.76 ± 0.1	3.87 ± 0.1	3.98 ± 0.1	0.891
Creatinine[b]	0.77 ± 0.04	0.70 ± 0.03	0.61 ± .06	0.570
Urea[b]	28 ± 1.92	23.5 ± 1.86	22.2 ± 2.96	0.135
Total lymphocyte count[c]	1.83 ± 2.1	1.67 ± .28	1.67 ± .33	0.179
Body mass index[d]	18.49 ± 1.06	17.79 ± .88	17.14 ± 1.36	0.786

***P** value < 0.05 is statistically significant, data are expressed as Mean ±SE; values bearing different superscripts a, b, c, d, represents units, [a]g/dl, [b]mg/dl, [c]10^3 x cells/ mm^3, [d]kg/m^2

4.5. Clinical features and the dependent variables of breast cancer patients

Effect of cancer stage on biochemical nutritional parameters was assessed through one way ANOVA. There was statically significant difference in mean value of serum albumin (p = 0.001), Creatinine (p = 0.001) and total lymphocyte count (p = 0.010) with clinical stage of breast cancer but non-statistical significant difference in total protein (p = 0.922), globulin (p = 0.963), urea (p = 0.088) and body mass index (p = 0.144) (**Table 9)** below**.**

Table 9: One way ANOVA cancer stage effect on biochemical, TLC and BMI parameters, in breast cancer patients.

	stage I (n = 9)	stage II (n = 12)	stage III (n = 18)	stage IV (n = 11)	P- value
Albumin[a]	4.16 ± 0.08	4.01 ± 0.06	3.85 ± 0.05	3.55 ± 0.17	0.001**
Total protein[a]	7.71 ± 0.16	7.83 ± 0.10	7.85 ± 0.18	7.79 ± 0.14	0.922
Globulin[a]	3.75 ± 0.24	3.94 ± 0.10	3.99 ± 0.17	3.91 ± 0.20	0.963
Creatinine[b]	0.80 ± 0.05	0.86 ± 0.03	0.70 ± 0.05	0.55 ± 0.06	0.001**
Urea[b]	20.22 ± 1.75	23.53 ± 2.23	25.6 ± 2.92	30.8 ± 2.58	0.088
lymphocyte count[c]	1.95 ± 0.11	1.70 ± 0.06	1.77 ± 0.08	1.52 ± 0.02	0.010**
Body mass index[d]	19.66 ± 1.20	17.98 ± 0.92	17.17 ± 1.36	16.77 ±0.6	0.144

****P** value ≤ 0.01 is statistically significant, data are expressed as Mean ±SE; values bearing different superscripts a, b, c, d represents units, [a]g/dl, [b]mg/dl, [c]10^3 x cells/ mm^3, [d]kg/m^2

5. DISCUSSION

The present study evaluated the serum biochemical parameters (albumin, total protein, globulin, creatinine & urea), anthropometric parameters like body mass index as well as hematological parameters (total lymphocyte count) in newly diagnosed breast cancer patients and healthy subjects. A total of 100 subjects (50 breast cancer patients & 50 healthy) were involved in the study. Breast cancer patients were found to have elevated levels of globulin and urea, lower level of albumin and ceatinine than the control group. Hematological parameter such as TLC was significantly lower in the patients. Anthropometric indicator like BMI was also lower than the control subjects.

5.1. Levels of biochemical profiles in breast cancer patients and the control subjects

The present study showed statistically non-significant increase in the mean value of serum total protein in breast cancer patients as compared to healthy subjects, similar to several other authors (Fatima *et al.,* 2013; Zainal *et al*., 2009; Dhakar *et al*., 2013; Gao *et al.,* 2005). This increase in serum total protein level is due to the reason that cancer patient synthesize different kinds of proteins such as globulins, immunoglobulin, enzymes and positive acute Phase Reactants (Hasan *et al*., 2010). Lymphocytes produce globulins to the levels that are high enough to compensate the lowered albumin levels in the serum. Another reason for increased in total serum protein level was as the plasma circulates through the tissues, it collects proteins that are released from their original locations due to certain physiological events, such as tissue remodeling, trauma and cell death (Muhtaseb, 2014).

Total protein level did not show significant prevalence of malnutrition as compared with the control. This may be due to compensation of negative acute phase protein by positive acute phase protein. So for assessment of malnutrition prevalence it is better to assess' the fraction of total protein rather than the whole protein.

Our study also indicated that there was statistically significant decrease in mean value of albumin in breast cancer patients as compared to healthy subjects. There result agrees with (Fatima *et al*., 2013; Muhtaseb, 2014; Joudi, 2005). The reduction in serum albumin concentration in our study could be that due to malignancy condition liver synthesizes significantly large amount of positive acute Phase Reactants proteins leaving behind the synthesis of negative acute Phase Reactants proteins (Pastore *et al.,* 2013). Synthesis of inflammatory cytokines such as TNF-α and IL and CRP also reduce serum albumin concentration (Dawood and Hasan, 2013). These inflammatory mediators are produced by tumor and host cells in malignancies. TNF - α and IL6 may act both by increasing the local trans capillary escape of albumin in the tumor bed and by decreasing the hepatic synthesis of albumin (Richter *et al*., 2000). The other cause of the observed reduced albumin level in serum of breast cancer patient may be due to the role of albumin as extracellular antioxidant scavenger; mainly for carbon-centered free radicals (Lawal *et al*., 2010).

Albumin constitutes up to 49% of total plasma antioxidant status and acts as sacrificial antioxidant by inhibiting the generation of free radicals through an immediate attacks of albumin molecule itself, so the radical reaction continue on albumin surface and cause damage to albumin molecule. Such damage is probably biologically insignificant, because albumin is present in plasma in high concentration (Roche *et al*., 2008).

There was 32% prevalence of malnutrition in breast cancer patients with 12% and 20% of moderate and mild malnutrition, respectively according to serum albumin. Albumin positively correlated to TLC in study groups in line with (Norte *et al*., 2015). The low albumin level in the patients increases susceptibility to infection reduces quality of life and increases mortality in the patients. Inaddition albumin negatively correlated with globulins i.e. low albumin to globulin ratio. The low albumin to globulin ratio was predicting long term mortality in breast cancer patients. Moreover, the low plasma albumin concentration is a reflection of poor diet or poor appetite that minimizes the raw material availability for plasma protein biosynthesis.

There was statistically significant higher serum globulin in the study group than the control individuals. This agrees with (Fatima *et al.,* 2013; Dawood and Hasan, 2013). In response to reduced levels of serum albumin in breast cancer patients, albumin to globulin ratio is lowered due to an increase in globulins; mainly immunoglobulin's synthesized by lymphocytes to compensate for the reduced serum albumin. Failure of lymphocytes to raise globulins to levels that is high enough to compensate for the reduced albumin may indicate advanced disease, where protein synthesis is reduced and protein catabolism is accelerated (Inui, 2002; Joudi, 2005).

There was statistically significant lower serum creatinine level in the study group than the control individuals. The low mean serum creatinine value in our study may be a result of decreased muscle mass/wasting of breast cancer patients. A large proportion of the breast cancer patients in our study were stage III and above, they may have lost muscle mass as a result of increased breakdown of muscle protein to provide the essential amino acids required for protein synthesis and energy metabolism /gluconeogenesis for the tumor cells (Giltay *et al*., 1999;

Lecker *et al*., 1999). Decrease in protein synthesis and increase in muscle protein degradation in cancer patients imply that tumors are able to mobilize muscle proteins (Fearon *et al*., 1988). In contrast to decreased protein synthesis in muscle cells, tumor cells exhibit increased protein synthesis in liver (Zhoughbi *et al*., 2014). Removal of certain amino acids by the tumor would lead to a depression of host protein synthesis since normal protein synthesis requires the full complement of amino acids (Smith & Tisdale, 1993). The condition of sarcopenia in an individual with otherwise normal body weight would result in a disproportionately low contribution of muscle derived metabolites to the metabolome overall (Stretch *et al.,* 2012).

Twenty percent or more of patients with cancer may have sarcopenia, i.e. significant loss of muscle mass, and thus will have lower than expected serum creatinine levels (Cohen *et al*., 2015). Muscle catabolic rate increase in the presence of tumor, results a negative nitrogen balance on the muscle due to translocation of nitrogen from host to the tumor. Statistically significant elevated mean urea value in breast cancer was also found as compared to healthy subjects. The elevated serum urea level in our study probably arises from an increased mobilization of body proteins for the production of glucose for use by the tumor. In the presence of cancer the ability of an organism to regulate the synthetic and catabolic processes involving numerous and different proteins with the goal of maintaining relatively constant bodies are disturbed. The malignant tumor seems to inappropriately metabolize both dietary and host proteins, resulting in the wasting of lean body mass. Nitrogen mobilized from tissues represents a potential source of building blocks for rapidly growing tumors (Campos & Andrade, 2014). Thirty to hundred percent of all patients with advanced cancer have negative nitrogen balance (Blackburn *et al.,* 1977).

5.2. Level of lymphocyte count and body mass index in breast cancer patients and control groups.

The present study revealed statistically significant decrease in mean value of total lymphocyte count in breast cancer patients as compared to normal subjects, which is in line with the works of (Shrivastava *et al*., 2017; Ali, 2014; Rufelle *et al.,* 2012). Reduction may be due to cancer directed depletion of albumin or malnutrition may contribute to compromised immunity/ general immunosuppression. Accordingly other investigators reported significant reduction in the number of helper CD4+ cells and depressed natural killer cell activity (Lawal *et al.,* 2010). The reduction level of total lymphocyte count could be due to decrease in Interleukin, and Zinc amounts and/or thymus atrophy (Gunarsa *et al*., 2011). There was 46% prevalence of malnutrition in breast cancer patients with 4%, sever; 4%, moderate and 38% mild malnutrition respectively.

Total lymphocyte count was postively correlated with body mass.Skeletal muscle, which accounts for 40% of body weight and 50% of body protein, plays a vital role in regulating immune function and its loss predispose impaired tissue healing and poor immune function (Thongprayoon *et al*., 2016). The mean values of body mass index obtained showed that the breast cancer patients were below the average value indicating that they were underweight but the control group had a normal value. However, the difference in the average value was not statistically significant. This is in line with the work of (Martins *et al*., 2012). Assesment of malnutrition prevalence in study group through BMI evalated 2%, severe; 34%, mild malnourished; 14% over weight and 12% obese in our study that is agreed with (Irungu, 2015).

5.3. Clinical features and the dependent variables of breast cancer patients

There was statically significant difference in mean value of serum albumin level in breast cancer patients with regard to pathological stages and their value decrease as the stage become advanced that in line with (Irungu *et al.*, 2015; Pan *et al.*, 2015). This is due to incresed degradation and decreased synthesis of albumin with increasing cancer stages. Total lymphocyte count level decrease with increasing in stage of breast cancer in our study agrees with (Rana *et al*., 2015). In addition mean value of serum creatinine also showed statistically significant reduction with advanced clinical stages. There were no differences in mean serum total protein level among breast cancer patients due to the effect of cancer stages agree with (Dhakar *et al*., 2013). The reason for this may be due to despite there is increasing in acute phase protein with advanced stage degradation also increase paralleled. Body mass index non-statistically significant difference with clinical stage of breast cancer patients.

6. CONCLUSION

The results obtained in this study through measurements of BMI, hematological parameters and biochemical parameters showed that breast cancer patients present with different stages of malnutrition. The low albumin concentration was indicative of the malnutrition and unavailability of amino acids for protein synthesis. The high urea level obtained in breast cancer patients is indicative of increased muscle wasting and hence catabolism of proteins resulting in increased urea production. The surge in creatinine level is at par with many other works previously done elsewhere, resulting from shunting of the carbon skeleton of this product to the synthesis of other nitrogen containing molecules. The muscle wasting is reflected through the decreased BMI among breast cancer patients. However, there was no significant difference in total serum protein level since there could be as much new biosynthesis of new proteins from the muscle wasted as there is degradation of muscle proteins. This reflected through a decrease in plasma albumin level but increase in globulins.

Breast cancer patients may be in a state of oxidative stress due to the decline in albumin, which is a useful first line antioxidant molecule in blood. Most of the cancer patients come to the clinics not for early detection but after the cancer reaches its latent stage and when the disease is severe and painful. As a cancer disease progresses but it complicates many issues related to metabolism of nutrients and also perturbs the cellular milieu. As a result there could be many changes in metabolite levels and derangements or alterations of metabolic pathways. The low lymphocyte count also indicates that breast cancer patients may present with immune suppression. As it has been established prior, these patients are in a state of negative nitrogen balance and this has to be considered during treatment through diet supplementation or nutritional therapy has to be

considered as a treatment strategy. It has to be noted that there is a rampant nutritional deficiency in this country that can compound the problem of cancer treatment. Since biochemical markers have their own limitations and their level is affected by different disease, it is advisable to use combination of biochemical markers and lymphocyte count to get feasible assessment strategy and identification of reasons for malnutrition.

Finally it should be noted that early assessment of malnutrition in breast cancer patients allows for a timely intervention i.e. provide nutritional therapy and maximize therapeutic efficiencies (surgery, chemo and radiotherapy) through minimizing their side effects.

7. STRENGTHS AND LIMITATIONS OF THE STUDY

The strength of the study can be that the study was done in the only oncology center in Ethiopia and patients are coming from all over Ethiopia and hence they can represent the whole population. In addition the study includes several demographic and biochemical parameters claimed to be associated with the variables under study. Despite the aforementioned strengths, this study has several weaknesses. As the sample size was small, it was difficult to represent the whole breast cancer patients in the population. Dietary pattern of both the patients and control groups were un known. There are no rigorous previous studies done in this country for comparison and this can be another limitation.

8. RECOMMENDATIONS

The following recommendations are suggested to further investigate and asses malnutrition in breast cancer patients.

- Further studies could be conduct with larger sample size in order for these nutritional parameters to be validated as a means of early assessment of malnutrition in breast cancer patients
- Dietary history should be asses in further studies.
- There should be better public education and care as well as clinical care of the breast cancer patients for better psychological therapy.
- There should be timely evaluation of nutritional status of the patients
- Plan nutritional therapy: amines (branched chain amino acids and creatine) as dietary supplements to counteract skeletal muscle wasting on cancer cachexia.

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10. ANNEXES

10.1. Annex 1: Information sheet (English Version)

Research Project: Assessment of Malnutrition Using Biochemical Markers among Female Breast Cancer Patients Attending Tikur Anbessa Specialized Hospital, Ethiopia.

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

Principal Investigator: Kibrom G/meskel (Bscbiotech, MSc in biochemistry candidate)

Advisors: Solomon Genet (PhD), Wondemagegnhu Tigneh (MD)

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 Assessment of Malnutrition Using Biochemical Markers among Female Breast Cancer Patients.

Procedures

If you agree to take part in the study, the investigator or a health worker will give you verbal and/or written information about the study and you will be given the consent form to sign, the physician or health professional will ask you some questions about your general health and Perform a complete medical examination and assess whether you qualify to participate in the Study. If you are fit for the study about 5 ml of blood samples will also be collected for only the Laboratory examination of complete blood count, albumin, total protein, creatinine, urea and face to face interview for additional questions.

Discomforts and risks and benefits from participation

The degree of discomfort you may encounter in giving the sample is no more than when one does in his/her routine examination. But, there could be cases in which minor pain and change in color of your skin following the blood drawing occur transiently. The blood will be withdrawn by licensed health care professionals in the hospital and appropriate care will also be taken. You will not be provided with any direct incentives for your participation in the research. But the cost

for general medical examination will be covered by the project. In addition, based on the results obtained from the research you will be cared accordingly or the results may serve you as a baseline data. In addition, the result of the study will be beneficial for the better prevention and care of breast cancer patients than before. Hence, you are indirectly benefiting other patients and the society in this aspect.

Confidentiality

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

Contact information: If you have any questions contact: Kibrom G/meskel: 0920867494

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 Assessment Malnutrition Using Biochemical Markers 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_____

10.3. Annex 3: Questionnaire (English version)

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

I. Personal, socio demographic, anthropometric and clinical information

Card no._____

1. Age (in years) _____

2. Height (m) _____

3. Weight (in Kg) _____

4. Body Mass Index (kg/ m^2) _____

5. Marital status:

a. Single b. Married c. Divorced d. Widowed

6. Residential area

a. Urban b. Rural

7. Alcohol consumption:

a. Yes b. No

8. Smoking:

a. Yes b. No

9. Has the patient lost appetite?

a. No b. Moderate loss of appetite c. Severe loss of appetite

10. Mode of feeding

a. Self-fed with some difficulty b. Self-fed without any problem

11. Economic status of the patient

a. Low b. Middle c. Higher

12. Breast cancer stage: …….

13. Localization of the cancer:

a. Left Breast b. Right Breast

10.4. Annex 4: Information sheet (Amharic version)

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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 በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነልዎትን ማንኛዉንም ሃሳብ ይጠይቁ፡፡ Assessment of Malnutrition Using Biochemical Markers among Female Breast Cancer Patients Attending Tikur Anbessa Specialized Hospital, Ethiopia የጥናቱ ርእስ ሲሆን አላማውም በደም ውስጥ ያለውን ፕሮቲንን በመለካት በረጅም ግዜ የሚፈጠረውን የምግብ አለመመጣጠን ማወቅ ነዉ፡፡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 ለካንሰር ማእከል ነርሶች የሚሰጡ ሲሆን የደም ናሙናውን በሚሰጡበትም ሰአት ሁልጊዜ ለምርመራ ከሚሰጡበት የተለየ ህመምና አለመመቸት የለውም ለምናልባት ቢኖር ተገቢውን የጤና እንክብካቤ የሚያገኙ ይሆናል፡፡ በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኃላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋን በዚህ ሆስፒታል የሚሰጠዎ ማንኛዉም አገልግሎት ላይ ተጽዕኖ የለውም፡፡ በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅበዎታል፡፡

ግልጽ ያልሆነልዎ ጥያቄ ካለ

ሞባል፡ 0920867494 ክብሮም ገ/መስቀል

10.5. Annex 5: Informed consent (Amharic version)

የተሳታፊዎች ስምምነት ማረጋገጫ ቅጽ

የሚስጥር ቁጥር ----------

የተሳታፊዉ ስም ----------

እኔ ስሜ ከላይ የተገለጸዉ ግለሰብ በዚህ ጥናት እንድሳተፍ የስምምነት ቃሌን የምሰጠዉ በአጠቃላይ የጥናቱን አላማና ጥቅም በመረዳትና በፍጹም ፈቃደኝነት ነው፡፡ በመጠይቁ ላይ የምሰጠዉ የእኔ መረጃ እንደማይባከን 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፡፡በተጨማሪም የምሰጠዉ የደም ናሙና ለAlbumin,Total protein ,creatinine, urea እና

Total lymphocyte count ምርመራዎች ብቻ እንደሚዉል ተነግሮኝ ተስማምቻለሁ፡፡ 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 _____ _____

10.6. Annex 6: Questionnaire (Amharic version)

መጠይቅ

ውድ ተሳታፊ ቀጥሎ ያለውን መጠይቅ ለመሙላት ስለተባበሩን እናመሰግናለን፡፡

ካርድ ቁጥር_____

1. እድሜ (በአመት) _____

2. ክብደት (በኪ.ግ.) _____

3. ቁመት (በሜትር)_____

4. የሰዉነት ክብደት ልኬት (ኪ.ግ. / ሜ^2) _____

5. የትዳር ሁኔታ: a. ያላገባ……

b. ያገባ…….

c. የፈታ…….

d. የሞተበት……..

6. መኖሪያ ቦታ፡

a. ከተማ b. ገጠር

7. አልኮል ይጠጣሉ:

a. አዎ b. አልጠጣም

8. ሲጋራ ያጨሳሉ:

a. አዎ b. አላጨስም

9. የምግብ ፍላጎት ተዘግታል

a. አዎ b. ኣልተዘጋም

10. አዎ ከሆነ

a. ከፍተ b. መሃከለ

11. የአመጋገብ ዘዴ

a. አርዳታ ይፈልጋለ b. በራሱ ይመገባለ

12. የካንሰር ደረጀ

a. I b. II c. III d. IV