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Assessment of Serum Iron Profiles and Associated Factors in Newly Diagnosed Female Breast Cancer Patients Attending Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by MELAKU TSEGAYE, entitled: **“Assessment of Serum Iron Profiles and Associated Factors in Newly Diagnosed female Breast Cancer patients Attending Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia, 2024.”** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Clinical chemistry track) complies with the regulations of the University and meets the accepted standards concerning originality and quality.

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Abbreviations

AAU	Addis Ababa University
BRCA1	Breast Cancer gene 1
BRCA2	Breast Cancer gene 2
DNA	Deoxyribo Nucleic Acid
EPHI	Ethiopian Public Health Institute
Fe ³⁺	Ferric iron
GLOBOCAN	Global Cancer Incidence, Mortality and Prevalence
HER2/neu	human epidermal growth factor receptor 2
NCRNDF	National Cancer Registry and National Death File
NTBI	Non-Transferrin-Bound Iron
OPD	Outpatient Department
ROS	Reactive Oxygen Species
SST	Serum Separating Tube
SPSS	Statistical Package for the Social Sciences
TASH	Tikur Anbessa Specialized Hospital
TDLU	Terminal Duct- Lobular Unit
TIBC	Total Iron Binding Capacity
TF	Transferrin
TNM	Tumor lymph Node Metastasis

Abstract

Background: - Breast cancer is the leading cancer among females globally, with high morbidity and mortality rates. Iron in cells is vital for oxygen transport as part of haemoglobin, stored in ferritin, and carried by transferrin, have role in numerous biological processes, including DNA and RNA synthesis. Maintaining iron concentrations in the cells and in the body so crucial, since both iron deficiency and iron overload can impair cellular function also can raise cancer risk, including breast cancer.

Objective: To assess serum iron profiles and their associated factors in newly diagnosed female breast cancer patients attending Tikur Anbessa Specialised Hospital, Addis Ababa, Ethiopia.

Methods: A comparative cross-sectional study was carried out between March and November 2023. In all, 74 breast cancer patients were used as cases, and 74 samples from people who were apparently healthy were used as a control group at Tikur Anbessa Specialised Hospital. After collecting socio-demographic and anthropometric data, 5 ml of blood was collected from each participant. Then serum iron, ferritin, TIBC, and percent transferrin saturation levels were determined by using the COBAS 6000 machine at EPHI laboratory departments. After cleaning, checking for completeness, and entering it into MS Excel, the data was analysed using SPSS version 27.0 software. The data were analysed using descriptive statistics (mean $\pm$ SD) and assessed using Chi-square, student t-test, ANOVA, and P-value within a 95% confidence interval. Binary logistic regressions were employed to determine the association between dependent and independent variables among breast cancer patients. Finally, the findings were presented through text, tables, charts, and graphs.

Results: The age of study participants ranged from 25 to 75 years, and the mean ages of breast cancer patients and the control study group were 44.66 ± 11.78 and 46.0 ± 12.05 years, respectively. The mean $\pm$ SD of serum ferritin levels were significantly increased ($p < 0.05$), and significant decreases in serum iron, TIBC, and TS% were observed in the case groups in comparison to the normal groups ($p < 0.05$). Factors like age, BMI, alcohol consumption, and a diet rich in meat were significantly linked with abnormal serum iron profile results. In breast cancer patients, the ferritin level was higher in postmenopausal (274.01 ± 127.72 ng/ml) compared to premenopausal (107.49 ± 83.52 ng/ml) patients and even in their healthy counterparts. Ferritin levels in advanced-stage disease exhibited a significant rise in comparison to patients at an earlier stage.

Conclusion: According to the study, women with breast cancer generally have deficient iron levels, apart from increased ferritin levels. Higher ferritin levels in patients with breast cancer might serve as an additional parameter for diagnosis and staging. Iron metabolism is strongly involved in the pathogenesis of breast cancer and can serve to assess the severity of the disease.

Keywords: breast cancer, ferritin, serum iron, TIBC, Transferrin saturation percent

1. Introduction

1.1 Background

Cancer is a hereditary disease primarily resulting from mutations in the genes that regulate our cells' growth and division. Factors such as errors during cell division, DNA damage from harmful environmental agents, and inherited genetic changes can lead to cancer [1].

Breast cancer is the most prevalent cancer among women. It is a devastating disease that affects millions of women worldwide each year. Understanding the serum iron profiles of newly diagnosed female breast cancer patients is crucial for developing targeted treatment strategies and improving overall patient outcomes. Iron is an essential micronutrient involved in various cellular processes, including DNA synthesis and cell proliferation, both of which are critical factors in cancer development and progression. By assessing serum iron levels, transferrin saturation, total iron binding capacity, and ferritin levels in these patients, we can learn key aspects about how iron metabolism contributes to the development of breast cancer [2].

It is clear that there is not a single gene that is mutated in all cases of familial breast cancer. One of the first abnormalities to be identified was a mutation in the tumour suppressor gene p53 (also seen in sporadic breast cancer) on the short arm of chromosome 17. Li-Fraumeni syndrome is a cancer family syndrome with a germ-line mutation in p53 causing breast cancer but also sarcomas, various childhood malignancies, and gliomas. This autosomal dominant inherited condition is, however, associated with less than 5% of familial breast cancers. However, breast cancer-associated gene 1 (BRCA1) and subsequently gene 2 (BRCA2) have been cloned [3, 4].

Staging describes the extent of cancer in the body, considering factors such as tumour size, location, and spread to other areas. In Stage 0, the disease is non-invasive. It has not spread beyond the breast ducts. In Stage I, cancer cells have extended to the surrounding breast tissue. Stage II: The tumour is either under two centimetres in diameter and has spread to underarm lymph nodes or over five centimetres in diameter but has not spread to underarm lymph nodes. Stage III: The cancer has spread beyond its initial location, commonly referred to as locally

advanced breast cancer. Stage IV: Cancer has metastasized to distant areas like the bones, liver, lungs, or brain. Stage IV breast cancer is also called metastatic breast cancer [5-7].

Numerous pathways involved in the genesis of cancer depend heavily on iron. However, iron can also play a crucial role in mediating programmed cell death, known as ferroptosis [8]. Iron might, therefore, serve as a tumour suppressor. In fact, iron's capacity to switch between its oxidised and reduced forms helps produce free radicals that accelerate the onset of tumours [9]. The loss of the cell's antioxidant capability and the increase in intracellular iron levels appear to be the two processes that cause ferroptosis, a cell death pathway [8]. Iron also affects angiogenesis and metastatic development via iron metalloproteases. Through the destruction of the iron matrix and cancer spread, iron has the capacity to modify the microenvironment [10-12].

Two possible roles for iron in cancer have been hypothesized. First, iron can act as a tumour initiator by increasing the generation of oxygen radicals that damage DNA and cause mutations that can cause cancer. Secondly, iron serves as a vital growth factor; tumours have a high need for iron, and changes to the pathways by which iron is acquired and used may be among the major metabolic modifications that characterise cancer [13].

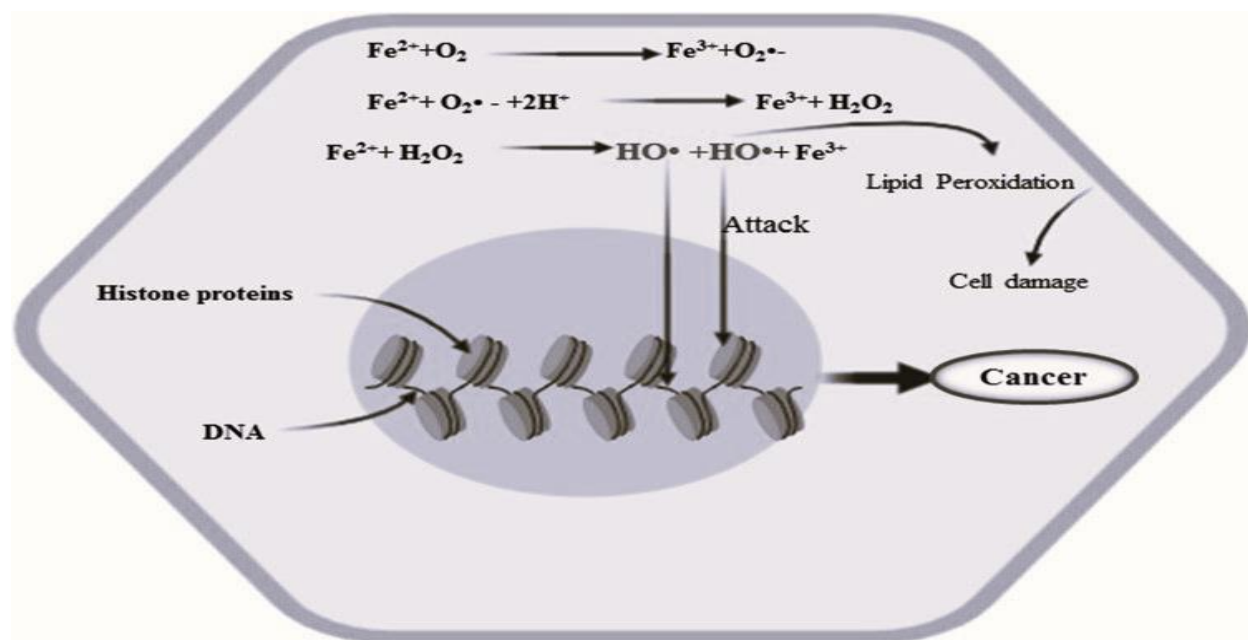


Figure 1: The Fenton/Haber-Weiss reaction: Iron drives the generation of ROS, leading to cell damage and DNA mutation, ultimately causing aggressive tumor growth [14].

Due to its acute-phase reactant nature, serum ferritin can be raised in various medical diseases, such as liver disease, infections, inflammation, and cancer. Iron and serum ferritin exhibit acute-phase reactions to inflammation; therefore, ferritin may increase and iron may decrease without regard to the amount of iron stored in the marrow. Hyperferritinemia is frequently observed in neoplasia or chronic inflammatory conditions. However, a number of factors affect ferritin levels and iron status, which can make data interpretation more difficult. Patients with breast cancer frequently have anaemia, and high ferritin levels brought on by the malignancy can hide an iron deficiency [15].

Breast cancer is the leading cancer among females globally, with high morbidity and mortality rates. Anaemia is a frequently observed condition in cancer patients, impacting their quality of life and treatment outcomes. Despite advancements in diagnostic and therapeutic strategies, the connection between breast cancer and serum iron levels is still not fully understood. The pathophysiology of anaemia in cancer is multifactorial, involving inflammation, chemotherapy-induced bone marrow suppression, and iron deficiency. Understanding the serum iron status of newly diagnosed female breast cancer patients is crucial for optimising their treatment plans and improving clinical outcomes. By evaluating the serum iron profiles and associated factors in this population, healthcare professionals can tailor interventions to address potential deficiencies and enhance the overall management of breast cancer patients. Further research is crucial to understanding the intricate relationship between iron metabolism and the progression of breast cancer [2, 15].

Levels of plasma iron, TIBC, and ferritin are measured directly by assay. An equation using transferrin and plasma iron is used to determine the iron (transferrin) percent saturation. The parameter TIBC can also be computed using the transferrin plasma concentration [16].

This study aims to raise awareness about the significance of considering serum iron levels during diagnosis and to investigate the potential associations between serum iron profiles and various clinical and pathological factors in newly diagnosed female breast cancer patients, ultimately contributing to a better understanding of the disease's aetiology and progression. A balance should be struck for the patient's best interests, regardless of whether high or low levels were observed.

1.2 Statement of the problem

In 2022, cancer was responsible for 20 million new cases and approximately 9.7 million deaths globally, positioning it as the second most common cause of death [17]. Cancer is responsible for one out of every six fatalities worldwide [18]. In Sub-Saharan Africa, including Ethiopia, due to population ageing, expansion, and rising rates of westernised diets, physical inactivity, and smoking, cancer-associated lifestyle choices are becoming a bigger public health burden [19].

The incidence of breast cancer is reported to be 20 times higher in high-income countries than in low- to middle-income countries. In 2016, around 719,000 cases of breast cancer were reported in high-income countries, while only 37,000 cases were reported in low- to middle-income countries [20].

In 2022, globally, there were 2.3 million new cases of breast cancer in women and 670,000 deaths related to the disease. The most prevalent cancer globally by the end of 2022 was breast cancer, with 7.8 million women alive who had been diagnosed within the last 5 years. More women globally lose disability-adjusted life years to breast cancer than any other form of cancer. Among women, breast cancer can occur at any age after puberty and is more prevalent in later years worldwide [21]. Breast cancer is the most prevalent type of cancer in the UK, with 45,000 instances reported annually. It represents 16.0% of total cancer cases and results in 12,000 deaths [22].

In India, cases of breast cancer are often diagnosed at an advanced stage. The quality of care a woman receives there is largely determined by her access to an oncologist [23]. In Bangladesh, a majority of patients are diagnosed with cancer at an advanced stage due to low awareness and limited access to healthcare facilities. Late-stage presentation raises both morbidity and mortality rates [24].

In several sub-Saharan African nations, breast cancer is presently the most commonly diagnosed cancer among women, and its prevalence is expected to rise over the next several decades. Rates of breast cancer are on the rise in Sudan. In 2003, it occurred 610 times (10.01% of all cancer cases reported), and in 2010, it occurred 1015 times (16.7% of all cancer cases reported).

Additionally, a large number of individuals in their late twenties to mid-forties (27% of those aged 41 to 50 and 26% of those aged 31 to 40) were affected by breast cancer [25].

Ethiopia reported 80,334 new cancer cases and 54,698 deaths per 10,000 people in 2022. From these, breast, cervical, and colorectal cancers account for the first dominating malignancies and account for 16,133, 7,445, and 6,048 new cases, respectively. 17.1% of all cancer-related deaths are from breast cancer, which is followed by colorectal cancer (7.15%) and cervical cancer (10.2%) [26]. Age-standardised mortality in Ethiopia is expected to be 11.8 per 100,000 females, while age-standardised incidence is predicted to be 19.5 per 100,000 [27].

Breast cancer is the most prevalent cancer in females, and early detection of biomarkers is critical for this type of tumour [28, 29]. In fact, approximately 30% of individuals diagnosed with early-stage breast cancer will experience a recurrence, often leading to metastasis and showing resistance to treatment [30]. So, the quest for early warning signs for diagnosing breast cancer is an expanding area of study. Research on population-based iron levels and cancer risk has produced inconsistent findings. Patient outcomes in different malignancies may be affected by changes in iron homeostasis.

When there is an excess of iron, transferrin's ability to bind it becomes saturated, which leads to an accumulation of nontransferrin-bound iron (NTBI). Although NTBI has the capacity to accelerate free-radical reactions, its accumulation may pose serious health risks [31]. Hence, an excess of iron may increase the risk of oxidant-mediated breast carcinogenesis [32]. Iron-binding and iron-transporting proteins are frequently dysregulated in breast cancer.

Breast cancer is closely related to anaemia, a condition commonly found in women affected by the illness. The prognosis for breast cancer may be impacted if these individuals receive therapeutic iron to treat their anemia. Only a few published studies have shown a connection between serum iron, TIBC, and transferrin saturation percentage and breast cancer, emphasising the need for further research. As far as we are aware, Ethiopia has no published studies.

1.3 Significance of the study

Breast cancer is among the most common cancers globally and typically affects individuals in their prime years. Breast cancer patients experience numerous abnormalities.

It was anticipated that this study would draw attention to the need to take serum iron levels into account when diagnosing breast cancer. For the patient's best interests, a balance should be achieved regardless of high or low levels were found. As a result, it will help doctor's better monitor patients with breast cancer and predict cancer outcomes.

Assessing the iron status of female patients who initially present with a cancerous breast lump can provide valuable insights into their diagnosis and prognosis.

In order to enhance the outcomes of the patients, this study concentrated on the iron status of breast cancer patients by giving information for accurate and fast diagnosis, medication, and monitoring. The results of this study will be useful to other scholars who are interested in the topic as a starting point for more research.

Identifying supportive tests can help lower mortality rates due to their predictive or diagnostic applications.

2. Literature review

2.1 Physiological locations of iron

Iron is distributed all through the body, with a large portion being biologically active and the rest reserved for future needs. The liver and bone marrow's intracellular compartments hold most of the body's stored iron. Iron is primarily stored as ferritin, consisting of iron and a protein known as apo ferritin. Hemosiderin is the iron storage form in the absence of apo ferritin [33].

2.2 Iron metabolism in cancer

Iron metabolism plays a crucial role in the development and progression of breast cancer. The dysregulation of iron homeostasis can contribute to tumour growth and metastasis. Cancer cells often exhibit an increased demand for iron due to their rapid proliferation rate. They achieve this by upregulating the expression of transferrin receptors (TfR1), which facilitate the uptake of iron-bound transferrin from the bloodstream. Once inside the cell, iron is utilised for essential processes such as DNA synthesis, cell proliferation, and metabolism. Cancer cells can modulate ferritin expression to regulate intracellular iron levels based on their metabolic needs. Ferroportin is the sole known iron exporter in mammalian cells. Its expression can be regulated by various factors, including hepcidin, a hormone that inhibits ferroportin function. Cancer cells may downregulate ferroportin expression to retain iron intracellularly [34].

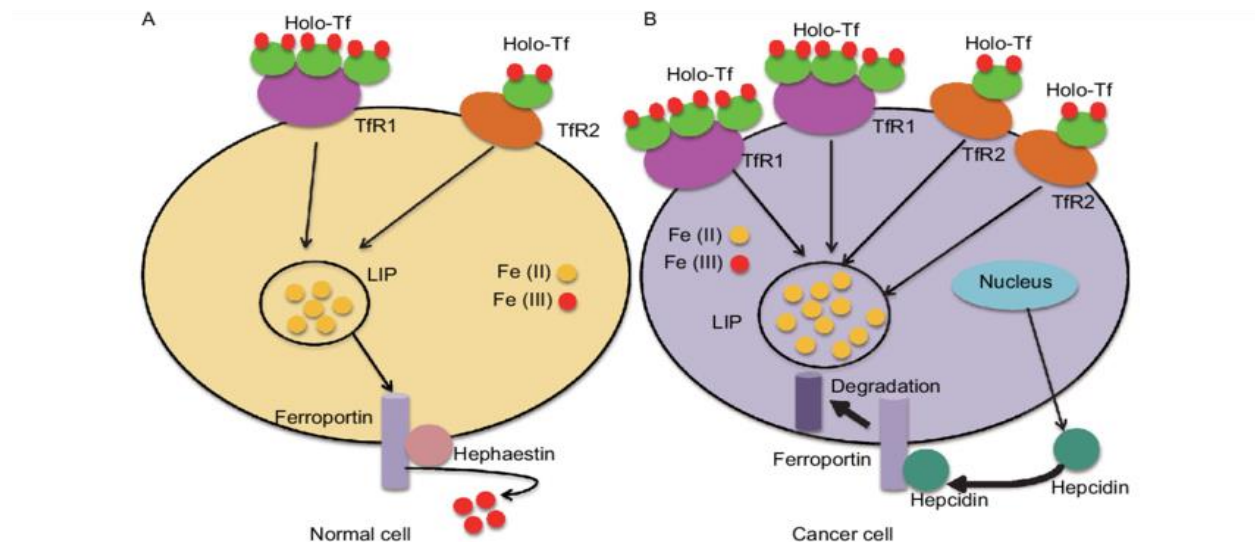


Figure 2: Iron metabolism in normal cell and cancer cell [35].

(A) The expression of Tf, TfR1, TfR2, and hepcidin is low, whereas the expression of the iron exporter gene FPN is high in normal cells, leading to a small pool of labile iron. (B) Cancer cells exhibit increased expression of TfR1 and hepcidin but low levels of FPN, leading to an increased labile iron pool [35].

2.3 Genetics of breast cancer

Oncogenes and tumour suppressor genes with anomalies in their structure or function are linked to the onset of malignancy. Many genes, like those in the tyrosine kinase family of growth factors such as HER2, c-myc, the tumour suppressor gene p53, and ras family members, have been identified to show abnormalities in invasive breast cancer. Many of these irregularities are observed in instances of sporadic breast cancer. While just 5–10% of cases of breast cancer are hereditary, certain families have a much higher chance of developing this disease compared to the general population [5].

2.3.1 BRCA1 and BRCA2 genetic mutations

Most cases of inherited breast cancer are associated with mutations in the BRCA1 and BRCA2 genes, known as breast cancer genes one and two. Everyone possesses both of these genes. Repairing cell damage and maintaining proper growth of breast, ovarian, and other cells are the functions of BRCA genes. However, inherited mutations in these genes can result in dysfunction and increase the chance of breast, ovarian, and other cancers. Approximately 10% of all cases of breast cancer, or one in every ten instances, could potentially result from mutations in the BRCA1 or BRCA2 genes [5].

2.4 Risk factors of breast carcinoma

Epidemiologic studies have identified the causes of breast cancer. Some etiological and predisposing factors are associated with the disease, while others are not. These include geographic and ethnic influences, genetic predisposition, age, reproductive life length, parity, age at first childbirth, obesity, and dietary factors.

2.5 Previous studies on the level of iron profiles

According to a study conducted in the USA, higher baseline levels of blood iron and TS in both males and females are linked to an increased risk of cancer-related mortality. In general, women had a greater tendency to be associated with cancer, serum iron, and TS [36].

The results of various studies indicate a connection between high levels of serum iron and breast cancer. In an Indian case-control study, metal ion concentrations in the serum of female breast cancer patients were investigated. 54 female breast cancer patients and 54 female control subjects took part in the research. The study revealed that breast cancer patients exhibited statistically significant increases in their serum iron levels compared to the control group [37].

In an Indian study, Dhankhar et al. found that out of 90 women in the research (30 in a healthy control group and 60 recently diagnosed with untreated, histopathologically confirmed breast cancer), the iron, ferritin, and TIBC analysis results were compared in 30 early-stage breast cancer patients and 30 advanced-stage breast cancer patients before therapy with those of 30 healthy controls. Breast cancer patients exhibited notably elevated levels of all three parameters in comparison to healthy controls, with values being higher in advanced stages than in early-stage disease. According to their research, serum tests for iron, ferritin, and TIBC could assist in determining the severity and prognosis of the illness [38].

Furthermore, blood iron levels were assessed at the time of enrollment in cohort research conducted by Pang et al. on 309,443 Taiwanese people without a history of cancer. Linking individuals to the National Cancer Registry and the National Death File allowed for associating initial iron levels with future cancer risk. The connection between serum iron levels and cancer risk showed a J-shaped pattern, indicating increased risk at levels below 60 mg/dL or above 120 mg/dL. According to these results, elevated blood iron levels are both a frequent condition and a sign of a higher risk for a number of cancers [39].

A cross-sectional study conducted by Mazdak H1 et al. on 51 patients with solid cancer (including breast cancer) and 58 healthy volunteers after matching for age, sex, and smoking habits. Patients' serum iron levels were found to be significantly lower than those of the control group ($P < 0.001$) [40].

A retrospective study conducted in Croatia involved 46 patients with solid cancer who were tested for ferritin levels in their plasma. The patient plasma's ferritin concentration was analysed, with levels ranging from 5.98 to 850.78 µg/L, averaging at 174.62 µg/L, while the control group comprised 46 individuals. The ferritin level in the control group ranged from 5.94 to 351.54 µg/L, with an average of 63.17 µg/L. Between the two groups, there is a statistically significant difference [41].

A study from the University of Belgrade in Serbia revealed that patients with breast cancer who had advanced disease or had not undergone surgery had significantly higher ferritin levels ($p < 0.05$) compared to healthy premenopausal women. There was no difference in ferritin levels between preoperative patients and healthy postmenopausal women. Patients with the highest ferritin levels were those with advanced disease ($n = 140$, range 18–719). The levels were significantly higher ($p < 0.05$ in all instances) compared to pre-menopausal and postmenopausal healthy individuals and early-stage breast cancer patients. In the control group, the median value identified was 38 (range 9–207). Premenopausal women ($me = 18$, range 9–41) had significantly lower ferritin levels than postmenopausal women ($p 0.05$) [42].

Another study from the Iraqi University of Sulaimani reveals that the mean S.D. of serum iron, ferritin, TIBC, and transferrin in patients with breast cancer was (28.5 9.48 ug/dl), (672.3 570.8 ng/ml), (259.09 54.31 ug/dl), and (153.89 24.26 ug/dl), respectively, while the control group was (91.84 ± 23.25 ug/dl), (54.007 ± 26.15 ng/ml), (441.47 ± 47.45 ug/dl), and (283.4 ± 35.35 ug/dl), respectively, showing a significant variation in iron profiles between breast cancer and control groups [43].

Nevertheless, another study conducted in Sweden found that in menopausal women, serum iron is a minor risk factor (hazard ratio of standardised SI 1.09 (95% CI 1.02-1.015)). Excessive iron stores may increase the risk of cancer-related mortality in postmenopausal women, possibly due to an accumulation of iron after menopause [44].

Another cross-sectional study by Kayode S. Adedapo et al. was conducted in Nigeria. 63 newly diagnosed postmenopausal breast cancer patients and age-matched, apparently healthy women were recruited. Statistically significant differences in mean trace element values were observed among the participants. The average serum iron levels were significantly lower in patients with breast cancer (59.99 ± 4.33) compared to the healthy groups (82.94 ± 1.71) [45].

According to the study conducted in Sudan on 120 samples, 50 cases of breast cancer and 70 non-cancer controls were identified. The mean age of the case group was 47 years, compared to 42 years for the control group. The most common age categories were 45–54 years and 35–44 years, respectively. Both groups had a mean parity number of 4. Mean serum iron levels in stromal sarcoma were 188.00 ($\mu\text{g/dL}$), non-invasive ductal carcinoma was 254.00 ($\mu\text{g/dL}$), and invasive ductal carcinoma was 247.76 ($\mu\text{g/dL}$). They have identified a statistically significant disparity between the case and control groups with regards to serum iron (higher in the case group) and transferrin saturation percentage (lower in the case group) [46].

Several studies have used serum iron and transferrin saturation (TSAT) as indicators of iron status, but their findings have been conflicting. Some studies pointed out an increased chance of breast cancer specifically and cancer overall. At greater TSAT and serum iron levels, while others showed no meaningful correlations [44].

2.5 Conceptual framework

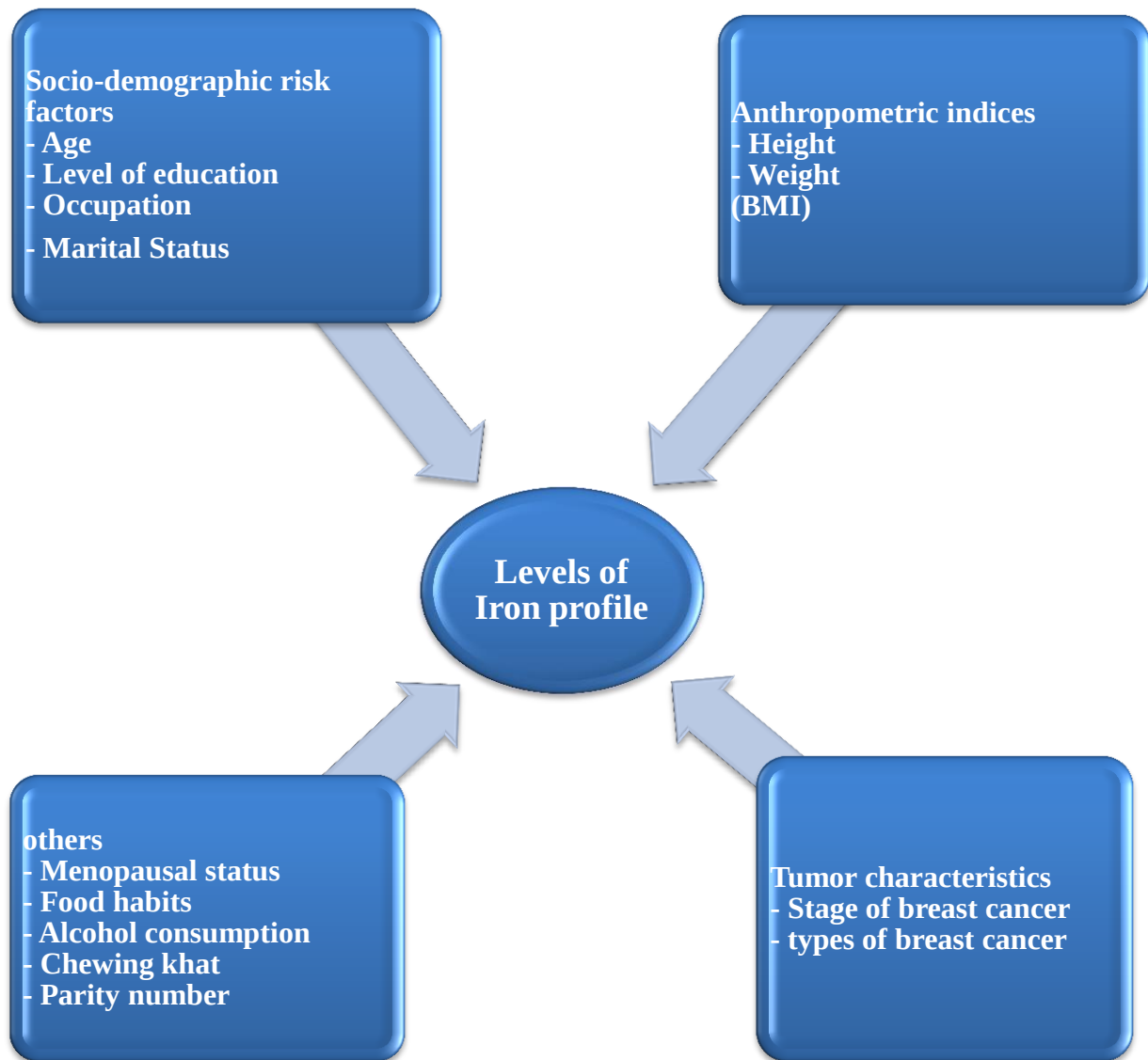


Figure 3:- Conceptual frame work developed to show association of dependent and independent variables.

3. Objectives

3.1 General objective

To assess serum iron profiles and associated factors among newly diagnosed female breast cancer patients attending Tikur Anbessa specialised hospital, Addis Ababa, Ethiopia: A Comparative Cross-Sectional Study

3.2 Specific objective

- To compare serum iron profiles in breast cancer patients and healthy control groups.
- To compare serum iron profile levels at different clinical stages of breast cancer.
- To assess risk factors that affects the serum iron profiles of breast cancer patients.

Hypothesis

Ho: The mean of serum iron, ferritin, TIBC, and transferrin saturation percent between the breast cancer patient and the control group are the same. And the mean of serum iron, ferritin, and TIBC and transferrin saturation percent between different stages of breast cancer patients is the same.

4. Materials and methods

4.1 Study area

Tikur Anbessa Hospital is located in Addis Ababa, Ethiopia. The opening of Tikur Anbessa Specialised Hospital (TASH) was in 1972; it is the largest general public hospital in the country. In 1998, Tikur Anbessa Specialised Hospital, which is also the largest referral hospital in the country, was given to Addis Ababa University (AAU) by the Ministry of Health (MOH) for the faculty as the main teaching hospital. The faculty is the oldest and largest among the health training institutions in the country, staffed with the most senior specialists. The oncology unit of TASH is the cancer referral hospital in the country and has OPD, which provides service to new and follow-up patients, and an inpatients department [47].

4.2 Study design and period

A comparative cross-sectional study was conducted during the period from March to November 2023 at Tikur Anbessa Specialised and Teaching Hospital College of Health Science, Addis Ababa University.

4.3 Populations

4.3.1 Source of population

- The source population was all breast cancer patients at TASH.
- The source population for the control group was all apparently healthy individuals.

4.3.2 Study population

- All breast cancer patients who volunteered and were admitted during the study period and met the inclusion criteria.
- All apparently healthy volunteer adult individuals who met the inclusion criteria.

4.4 Inclusion and Exclusion Criteria

4.4.1 Inclusion criteria

- The test group criteria included females newly diagnosed with breast cancer and pathologically confirmed, regardless of menopausal status.
- The control group comprised apparently healthy females matched to patients based on age group and parity.

4.4.2 Exclusion criteria

- Breast cancer patients with a history of hemochromatosis, infection, inflammatory diseases, like rheumatoid arthritis, viral or alcoholic hepatitis, CVD, pregnancy, taking iron supplements, or undergoing radiation, hormonal, or chemotherapy for breast cancer.

4.5 Study Variables

4.5.1 Dependent Variables

- Serum iron, ferritin, TIBC and transferrin saturation percent

4.5.2 Independent Variables

- Socio-demographic factors (age, marital status, education level, occupation, and BMI)
- Histopathological grade (stage of disease)
- Type of breast cancer
- Menopausal status
- Nutritional status
- Alcohol consumption, Khat chewing, smoking habits

4.6 Sample size determination and sampling technique

4.6.1 Sample size determination

The sample size was calculated using the following formula:

$$n = \frac{2SD^2(Z\beta + Z\alpha/2)}{d^2}$$

Where: SD = Standard deviation for the case (ferritin level for breast cancer is 111.25) [48].

d = Expected mean difference between cases and controls, based on previously published studies, is 53.84 [48].

Z β = the standard normal variate for power is 0.84 for 80% power.

Z $\alpha/2$ = the standard normal variate for significance level

$$\text{Therefore: } n = \frac{2SD^2(Z\beta + Z\alpha/2)}{d^2} = \frac{2*(111.25)^2(0.84 + 1.96)^2}{(53.84)^2} = 2 * \frac{(12,376.56)*(7.84)}{(2,898.75)} = 2*33.5 = 67$$

As per the formula, the sample size is 67 samples and was adjusted to 74 by incorporating a 10% non-response rate. Therefore, 74 samples were taken from each group, giving a total of 148 samples.

4.6.2 Sampling technique/methods

A convenient sampling technique was used for the selection of cases based on inclusion and exclusion criteria.

4.7 Measurement and Data collection

4.7.1 Method of data collection and tools

Different data collection tools were used to collect relevant information based on the study objectives. Face-to-face interviews, document reviews, and laboratory measurements were used to collect data from patients. Data was collected by using a questionnaire that contains the

following variables: age, marital status, parity, BMI, nutrition, and patient recorded data that was analyzed for the type of breast cancer, histopathological grade (stages), and menopausal status.

4.7.2 Collection of samples and processing

Laboratory professionals used sterile disposable syringes and adhered to the aseptic standard non-traumatic vein puncture technique to collect 5 ml of venous blood from both patients and controls. The collected sample was then placed into the serum separator tube (SST) that had been appropriately labeled with the participant's ID code. After a 30-minute wait, the blood in the SST was centrifuged at 4000 rpm for 10 minutes. The serum is then transferred to a 1.5-mL Eppendorf tube and stored at the optimal temperature until needed. Chemistry analysis with COBAS 6000 machine was performed at the Ethiopian Public Health Institute (EPHI), the National Reference Laboratory for Clinical Chemistry.

4.7.3 Laboratory analysis

Iron profile tests (serum iron, ferritin, and TIBC) were analyzed in the serum. The measurements of these parameters were carried out by used the electro-chemiluminescence immunoassay “ECLIA” method on a Cobas E immunoassay analyzer. This method uses different test principles, such as sandwich for measurement. All biochemical analysis for this study was according to manufactures protocol.

Spectrophotometry: The Cobas 6000 (c501) uses spectrophotometry to measure the concentration of various analytes in a given sample. This involves passing a beam of light through the sample and measuring the amount of light absorbed by the analyte. The degree of absorption is directly proportional to the concentration of the analyte in the sample.

Immunoassays: The Cobas 6000 (e601) also uses immunoassays to measure the concentration of certain analytes in the sample. This involves the use of antibodies that specifically bind to the analyte of interest, resulting in the formation of an antigen-antibody complex. The concentration of the analyte can then be measured based on the amount of antigen-antibody complex formed.

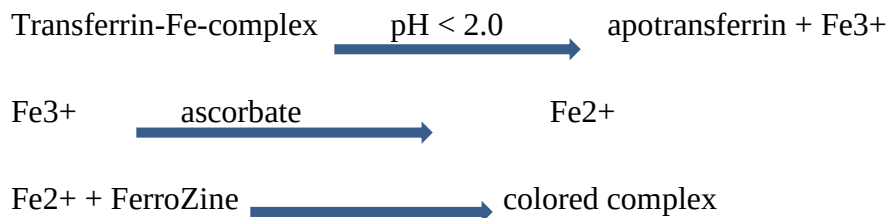
4.7.3.1 Test principles of the laboratory analyte

Ingested iron is primarily absorbed in the form of Fe^{2+} in the small intestine. Dietary iron in the form of Fe^{3+} must be reduced by vitamin C in order to be absorbed. Approximately 1 mg of iron is assimilated daily. In the body, Fe^{2+} is converted to Fe^{3+} by ceruloplasmin, and it is bound to transferrin. Transferrin can bind one or two molecules of iron, and nearly all circulating iron is carried in this manner.

a) Serum iron

Test principle

The Roche method of iron measurement is a three-step process using FerroZine reagents: Fe^{3+} is liberated from transferrin by acid or detergent; Fe^{3+} is reduced to Fe^{2+} by ascorbate; and the reduced iron then reacts with the FerroZine reagent to form a coloured complex. The intensity of this final product is directly proportional to the iron concentration in the sample.



The color intensity is directly proportional to the iron concentration and can be measured photometrically at the Wavelength (sub/main) 700/570 nm.

Reagent

The reagents are standard and ready for use on automated analyzer. Reagents: Citric acid: 200 mmol/L; thiourea: 115 mmol/L; detergent, Sodium ascorbate: 150 mmol/L; FerroZine: 6 mmol/L; preservative

Measuring limits

Lower detection limit of the test: 0.90 $\mu\text{mol/L}$ (5.00 $\mu\text{g/dL}$, 0.05 mg/L)

Measuring range: 0.90-179 $\mu\text{mol/L}$ or 0.33-1.93 mg/l

b) Total Iron-Binding Capacity (TIBC)

Test principle

Total iron binding capacity (T.I.B.C.) is determined by the addition of sufficient Fe^{+3} to saturate iron binding sites on apotransferrin. The excess Fe^{+3} is removed by adsorption with basic magnesium carbonate powder. After centrifugation, the bound iron remaining in the supernatant is measured with the direct method (Ferene) [49].

c) Ferritin

Test principle

Sandwich principle: Total duration of assay: 18 minutes.

- First incubation: by incubating 10 μL of sample, a monoclonal ferritin-specific antibody labeled with a ruthenium and biotinylated monoclonal ferritin-specific antibody form a sandwich complex.
- Second incubation: by adding streptavidin-coated microparticles, sandwich complex bound to solid phase through biotin and streptavidin interaction.
- The mixture of reaction extracted into measuring cell, where microparticles magnetically trapped onto electrode surface.
- Substances which unbound to electrode surface are then eliminated by ProCell/ProCell M.
- Then, voltage application on electrode induce chemiluminescent emission and photomultiplier measure it at 410nm.
- Results are determined with calibration curve, which is generated by 2-point calibration and master curve provided through reagent barcode.

The analyzer automatically calculates the analyte concentration of each sample either in $\mu\text{g/L}$ or ng/ml .

Specimen Type and Requirements: Serum or lithium heparin plasma. The specimen must be free of visible hemolysis. EDTA, citrate, or oxalate are not acceptable anticoagulants as they will bind iron and interfere with the assay. All will reduce the apparent iron concentration in the specimen. The concentration of iron in serum or plasma is dependent upon iron ingestion and is subject to circadian variations. The NHANES Biochem study uses refrigerated or frozen serum. This test is analysed using NHANES Vial 018 or Vial 011.

Measuring range: 0.500-2000 µg/L (ng/ml)

Lower detection limit: 0.50 µg/L (ng/ml).

The Lower Detection Limit represents the lowest measurable analyte level that can be distinguished from zero.

d) Determination of percent transferrin saturation

The transferrin saturation is the ratio of the serum iron concentration and the TIBC expressed as a percentage. Because the serum iron (SI) represents the number of transferrin sites bound with iron and the TIBC represents the total number of transferrin sites for iron binding, the degree to which the available sites are occupied by iron can be calculated. The percent of transferrin saturated with iron is calculated as:

$$\text{SI/TIBC} \times 100\% = \% \text{ transferrin saturation} \dots\dots\dots [50].$$

It is important that both the SI and TIBC be expressed in the same units, but it does not matter which units are used in the calculation.

4.8 Data Quality Assurance

After completing each questionnaire, the principal investigator and data collectors cross-checked to verify the completeness of the information and the data was daily entered into SPSS version 27. Normal control and pathological control were run every morning before the participant's blood sample was run according to manufacturer guidelines. Assure all samples for the presence of interference like hemolysis, lipemia, and icterus.

4.8.1 Pre analytical phases

The collected Blood samples were checked to see if they were labelled properly or not. Phlebotomists were instructed to collect non-hemolyzed blood. Generally, standard operating procedures were applied for each sample collection, transportation, preparation, and storage.

4.8.2 Analytical phases

The reagents and methods were assessed with known control materials.

4.8.3 Post analytical phases

The results of each individual were documented in the registration logbook with the participant's identification number, and the results were checked again before being released for further analysis.

4.9 Data analysis and interpretation

Statistical Package for Social Science (IBM SPSS version 27.0) computer software was used to analyze the data collected. Pearson Chi-Square and the Student t test were used. A P value of 0.05 or less is considered statistically significant.

4.10 Ethical consideration

Ethical clearance and approval were obtained from the department of medical laboratory science in Addis Abeba University College of Health Sciences. The study was classified as human-harm-free since it is a laboratory-based study, and all collected data was analysed anonymously to assure patient confidentiality.

4.11 Dissemination of Result

After the completion of the study, the findings were presented and submitted to the Addis Ababa University department of medical laboratory science as a partial fulfillment of the Master of Science in clinical chemistry. A dissertation based on this study was submitted to Addis Ababa University. The result was sent to different peer-reviewed journals for publication, and it was also shared with the hospital to be used as a guideline for breast cancer patients at TASH.

4.12 Operational definition

Newly diagnosed: - patients with a confirmed breast cancer who does not initiate treatment

Serum iron profiles: - includes serum iron, ferritin, TIBC and transferrin percent saturation

Cases: - Female breast cancer-positive patients (pathology proven) who were not in any treatment service at Tikur Anbessa Specialized Hospital.

Controls: - apparently healthy individuals (have no any history of breast cancer)

Pre menopause: - The respondent is currently menstruating.

Post Menopause: - The respondent has no menstruation for the last 12 months during the interview

5. Results

5.1 Socio-demographic Characteristics of study participants

The study included 148 women in total. The participants in the study were divided into two groups: the case and the control groups. Each group contains 74 study participants. The age range of all breast cancer patients involved in this study was 25–75 years. The average age of women with breast cancer and apparently healthy women was 44.66 + 11.78 years and 46.0 + 12.05 years, respectively. The majority of study participants in both case and control groups were under 50 years old, accounting for 67.6% and 64.9%, respectively.

Out of 148 women who took part in the study, 103 (69.6%) were married, 30 (40.5%) had no formal education, and 55 (74.3%) were private employees as shown below in Table 1.

Table 1:- Socio-demographic Characteristics of breast cancer patients and apparently healthy controls attending Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia, 2023.

Variables		Study participants (n= 148)		P-value
		Breast cancer patients n= 74	Apparently healthy controls n= 74	
Age group	< 50 yrs	50 (67.6)	48 (64.9)	0.496
	≥ 50 yrs	24 (32.4)	26 (35.1)	
	Mean ± SD	44.66 ± 11.78	46.00 ± 12.05	
Marital status	Single	11(14.9)	10(13.5)	0.170
	Married	56(75.7)	47(63.5)	
	Divorced	3(4.1)	9(12.2)	
	Widowed	4(5.4)	8(10.8)	
Educational status	No formal education	30(40.5)	27(36.5)	0.142
	Primary	13(17.6)	20(27)	
	Secondary	13(17.6)	18(24.3)	
	diploma and above	18(24.3)	9(12.2)	
Occupation	House wife	4(5.4)	7(9.5)	0.120
	Government employee	13(17.6)	22(29.7)	
	Private employee	55(74.3)	41(55.4)	
	unemployed	2(2.7)	4(5.4)	

In terms of lifestyle factors, anthropometrics, and food habits of breast cancer patients, the majority 17/74 (23%) were consumed alcohol (consumed on average one or more alcoholic drinks a month for a year), 11/74 (14.9%) were smokers (smoked at least 100 cigarettes in her lifetime), 69/74 (93.2%) never chewed Khat, 22/74 (29.7%) overweighted, 38/74 (51.4%) were multiparous (1-3), 34/74 (45.9%) used contraceptives, 19/74 (25.7%) ate meat at least once per week and from this (27%) had a positive history of breast cancer. Similarly, apparently healthy participants: the majority of 14/74 (18.9%) consumed alcohol (consumed on average one or more alcoholic drinks a month for a year); 69/74 (98.6%) were non-smokers; 7/74 (9.5%) overweighted; 35/74 (47.3%) were multiparous (1-3); 25/74 (33.8%) used contraceptives; and 28/74 (37.8%) ate meat at least once per week (Table 2).

Table 2:- Lifestyle factors, anthropometric, and food habits of breast cancer patients and apparently healthy controls attending Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia: 2023.

Variables		Study participants (n= 148)		P-value
		Breast cancer patients n= 74 (%)	Apparently healthy controls n= 74 (%)	
Alcohol consumption	Yes	17(23.0)	14(18.9)	0.545
	No	57(77.0)	60(81.1)	
Smoking condition	Yes	11(14.9)	5(1.4)	0.112
	No	63(85.1)	69(98.6)	
Khat chewing	Yes	5(6.8)	3(4.1)	0.467
	No	69(93.2)	71(95.9)	
BMI	<18.5 (Underweight)	6(8.1)	0(0)	0.085
	18.5-24.9 (Normal)	42(56.8)	64(86.5)	
	25-29.9 (Overweight)	22(29.7)	7(9.5)	
	≥30 (Obesity)	2(2.7)	0(0)	
	Mean ± SD	23.62 ± 3.54	22.81 ± 1.85	
Parity	None	10(13.5)	11(14.9)	0.118
	1-3	38(51.4)	35(47.3)	
	≥ 4	26(35.1)	28(37.8)	
	Mean ± SD	3.08 ± 2.33	2.99 ± 1.69	
Menopausal status	Yes	39(52.7)	39(52.7)	NS
	No	35(47.3)	35(47.3)	
Oral Contraceptive	Used	34(45.9)	25(33.8)	0.131
	Not used	40(54.1)	49(66.2)	
Eating meat	At least once per week	19(25.7)	28(37.8)	0.112
	Less than once per week	55(74.3)	46(62.2)	
Eating fruit and vegetables	At least once per day	22(29.7)	35(47.3)	0.058
	Less than once per day	52(70.3)	39(52.7)	

5.2 Comparison of laboratory test parameters among study groups

In breast cancer patients, the determined mean $\pm$ SD of serum iron, TIBC, and TS% was (0.66 ± 0.32 , 48.45 ± 11.08 , 24.28 ± 10.43), respectively, while in healthy control groups, the determined mean $\pm$ standard deviation of serum iron, TIBC, and TS% was (0.94 ± 0.31 , 55.19 ± 7.43 , 30.88 ± 9.93), respectively. Serum iron, TIBC, and TS% exhibited significantly lower levels in breast cancer patients compared to the control group, with a P-value of <0.001 . The observed difference was statistically significant ($p < 0.05$) (table 3).

But, in breast cancer patients, the determined mean of serum ferritin was (195.25 ± 136.92), while in healthy control groups, the average serum ferritin level was (115.57 ± 74.72), significantly higher than that of breast cancer patients as reported in Table 3. The observed difference was statistically significant ($p < 0.05$).

Table 3:- Comparison of iron profile in case group and healthy groups attending Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia: 2023.

Test Parameters	Study Groups (n= 148)		t-test for Equality of Means		
	Breast cancer patients (case) n= 74 Mean $\pm$ SD	Apparently healthy (controls) n= 74 Mean $\pm$ SD	p-value	95% CI.	
				Lower	Upper
Serum iron (mg/l)	0.66 ± 0.32	0.94 ± 0.31	<0.001	-0.382143	-0.178695
TIBC (μ mol/l)	48.45 ± 11.08	55.19 ± 7.43	<0.001	-9.80308	-3.66881
Transferrin Saturation percent (%)	24.28 ± 10.43	30.88 ± 9.93	<0.001	-9.90983	-3.29206
Ferritin (ng/ml)	195.25 ± 136.92	115.57 ± 74.72	<0.001	43.758006	115.605777

Standard deviation (SD) and Student's t-test were used to compare the means.

5.3 comparisons of iron profile in some independent variables among breast cancer study participants

The disease-stage-wise division of cases included 14.9%, 36.5%, 29.7%, and 18.9% of cases found in stages I, II, III, and IV, respectively. The rise in serum ferritin and TS% levels was noted in all stages of the disease [i.e., I, II, III, and IV] subgroups; an ascending course of means from stage I to stage IV can be noted. Inversely, TIBC levels decreased in the ascending course of the means from stage I to stage IV [Table 4].

Table 4:- Comparing of iron profiles in breast cancer clinical stages attending Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia: 2023.

Parameters	Clinical stage of breast cancer	N	Mean $\pm$ SD	P. value
Serum iron (mg/l)	I	11(14.9)	0.57 $\pm$ 0.29	0.326
	II	27(36.5)	0.61 $\pm$ 0.28	
	III	22(29.7)	0.71 $\pm$ 0.36	
	IV	14(18.9)	0.76 $\pm$ 0.34	
TIBC (μ mol/l)	I	11(14.9)	56.55 $\pm$ 10.88	0.015
	II	27(36.5)	48.34 $\pm$ 10.47	
	III	22(29.7)	48.42 $\pm$ 8.98	
	IV	14(18.9)	42.36 $\pm$ 12.36	
Transferrin Saturation percent (%)	I	11(14.9)	18.33 $\pm$ 9.25	0.010
	II	27(36.5)	22.20 $\pm$ 9.10	
	III	22(29.7)	25.45 $\pm$ 10.69	
	IV	14(18.9)	31.37 $\pm$ 11.26	
Ferritin (ng/ml)	I	11(14.9)	54.72 $\pm$ 52.09	<0.001
	II	27(36.5)	103.01 $\pm$ 48.28	
	III	22(29.7)	277.13 $\pm$ 100.74	
	IV	14(18.9)	354.91 $\pm$ 94.58	

Standard deviation (SD) and one-way ANOVA test were used for comparing the means.

Serum iron profile levels were compared across stages I, II, III, and IV of BRCA using a one-way ANOVA. The results showed significant findings in all three markers, with a p-value of

For TIBC, stage I compared to stage IV, the p-value was 0.007.

For TS%, Stage I compared to stage IV, the p-value was 0.010,

Stage II compared to stage IV; the p-value was 0.035

For serum ferritin, Stage I with stage III, the p-value was <0.001,

Stage I with stage IV, the p-value was <0.001

Stage II with stage III, the p-value was <0.001,

Stage II with stage IV, the p-value was <0.001

Clustered Bar Mean of total serum iron in micro gram per deci litre(mcg/dL), Mean of total iron binding capacity in micro mole per litre(μ mol/l), Mean of percent transferrin saturation(%), Mean of serum ferritin in nano gram per deci litre(ng/dl)...

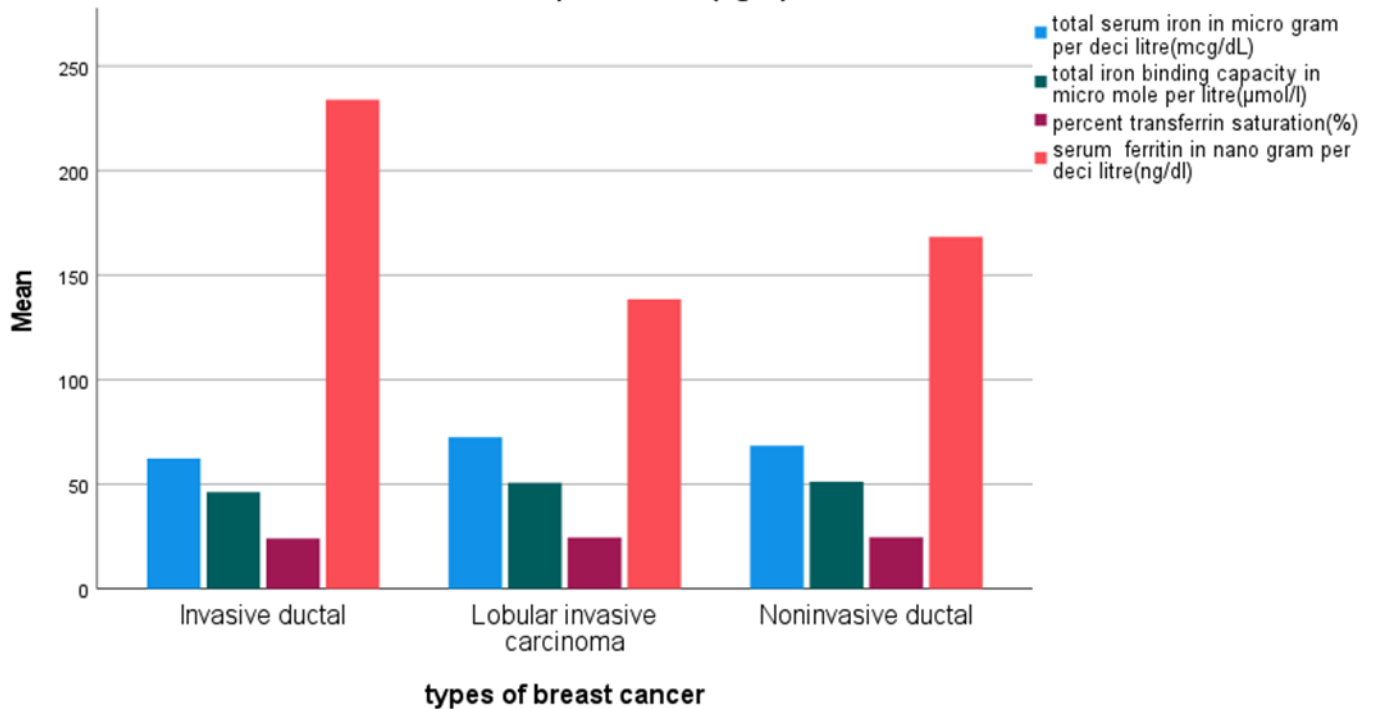


Figure 4:- Bar plot showing comparison of mean serum iron profile levels (serum iron, ferritin, TIBC, and %TS) between different types of breast cancer

Various types of breast cancer were represented on a bar chart, with the y-axis indicating the average levels of various iron profiles, while the x-axis displayed the types of breast cancer using different colors.

As we observed from the above figure regarding breast cancer types, No significant correlation with serum iron, TIBC, or TS% has been found., while only serum ferritin shows a significant association between invasive ductal (233.95 ± 138.29) and lobular invasive carcinoma (138.50 ± 87.29) among the three types of breast cancer with a significance level of 0.028.

Table 5:- Comparisons of menopausal status with serum iron profiles in breast cancer patients and apparently healthy controls attending Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia: 2023.

Parameters	Menopausal status	Breast cancer patients		Healthy controls	
		Mean $\pm$ SD	p-value	Mean $\pm$ SD	p-value
Serum iron (mg/l)	Yes(39)	0.72 $\pm$ 0.35	0.087	0.96 $\pm$ 0.28	0.634
	No(35)	0.60 $\pm$ 0.27		0.93 $\pm$ 0.34	
TIBC (μ mol/l)	Yes(39)	47.20 $\pm$ 10.39	0.306	54.45 $\pm$ 7.30	0.369
	No(35)	49.98 $\pm$ 11.85		56.01 $\pm$ 7.58	
Transferrin Saturation percent (%)	Yes(39)	27.11 $\pm$ 11.96	0.015	31.75 $\pm$ 8.83	0.427
	No(35)	21.22 $\pm$ 8.08		29.90 $\pm$ 11.09	
Ferritin (ng/ml)	Yes(39)	274.01 $\pm$ 127.72	<0.001	147.89 $\pm$ 80.47	<0.001
	No(35)	107.49 $\pm$ 83.52		79.55 $\pm$ 47.08	

Standard deviation (SD) and Student's t-test were used to compare the means.

The iron levels were compared between pre-menopausal and post-menopausal statuses in both breast cancer patients and healthy control groups. In all parameters, Compared to premenopausal women, the levels were higher in postmenopausal women. In this study, serum ferritin levels were significantly elevated in postmenopausal patients (mean $\pm$ SD = 274.01 $\pm$ 127.72) and controls (mean $\pm$ SD = 147.89 $\pm$ 80.47) as compared to the respective pre-menopausal groups (mean $\pm$ SD = 107.49 $\pm$ 83.52 and mean $\pm$ SD = 79.55 $\pm$ 47.08), and the p-value is considered highly significant (p<0.05). Beside this, the TS% in postmenopausal patients was significantly higher compared to premenopausal patients, with a p-value of 0.015.

5.4 Risk factors associated with iron profile changes among breast cancer patients

Alcohol consumption, BMI, and eating meat had a significant association with the iron levels in the serum of breast cancer patients.

Ages were significantly associated with serum ferritin. Elderly breast cancer patients were 4.29 times more likely to have hyperferritinemia than younger age groups (COR = 2.72; AOR (95% CI) = 4.39 (1.24–15.51) with a significance level of 0.02) (Table-6).

BMI was significantly associated with serum iron. Breast cancer patients had normal weights (0.25) times less likely to have low serum iron levels than overweight patients (COR = 1.18; AOR (95% CI) = 0.25 (0.09–0.72) at P-values of 0.01 (Table-6).

Alcohol consumption and eating meat were significantly associated with serum iron. Breast cancer patients who consumed alcohol (0.16) and eat meat at least once per week (0.18) times less likely to have low serum iron levels than non-alcohol consumers and eat meat less than once per week (COR = 0.30; AOR (95% CI) = 0.16 (0.03-0.92); COR = 0.24; AOR (95% CI) = 0.81 (0.04-0.78) with P-values 0.04 and 0.02, respectively. (Table-6)

Table 6:- Shows binary logistic regression analysis with factors associated with abnormal ferritin, and lower serum iron among breast cancer patients attending Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia: 2023.

Variables		Serum Ferritin ≥ 200 ng/ml			Serum iron ≤ 0.6 mg/l		
		COR	AOR (95% CI)	P-value	COR	AOR (95% CI)	P-value
Alcohol drinking	Yes	3.392	3.658(0.86-15.59)	0.080	0.303	0.155(0.03-0.92)	0.040*
	No	1			1		
Smoking status	Yes	2.844	2.85(0.54-15.07)	0.218	0.487	1.152(0.21-6.24)	0.870
	No	1			1		
Khat chewing	Yes	0.920	0.083(0.011-2.20)	0.169	4.118	18.06(0.9-359.1)	0.058
	No	1			1		
Age	≥ 50	2.718	4.388(1.24-15.51)	0.022*	0.721	0.418(0.12-1.52)	0.185
	< 50	1			1		
BMI	$< 25\text{kg/m}^2$	1.046	1.086(0.48-2.46)	0.843	1.177	0.248(0.09-0.72)	0.010*
	$\geq 25\text{kg/m}^2$	1			1		
Contraceptive use	Yes	1.184	1.267(0.37-4.32)	0.706	0.467	0.816(0.23-2.86)	0.751
	No	1			1		
Meat consumption	At least once per week	1.799	1.78(0.49-6.54)	0.385	0.238	0.181(0.04-0.78)	0.022*
	Less than once per week	1			1		
fruit and vegetables consumption	At least once per day	2.728	2.805(0.78-10.08)	0.114	0.549	0.489(0.14-1.76)	0.274
	Less than once per day	1			1		

There is no significant association between independent variables, total iron binding capacity, and transferrin saturation (%) among breast cancer patients, as shown in Table 7 below.

Table 7:- Shows binary logistic regression analysis with factors associated with lower TIBC and TS% among breast cancer patients attending Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia: 2023.

Variables		TIBC ≤ 42.5 μmol/l			Transferrin saturation ≤ 15%		
		COR	AOR (95% CI)	P-value	COR	AOR (95% CI)	P-value
Alcohol drinking	Yes	1.280	1.316(0.33-5.30)	0.699	0.725	0.976(0.20-4.76)	0.976
	No	1			1		
Smoking status	Yes	1.102	1.431(0.28-7.34)	0.667	0.320	0.460(0.05-4.46)	0.503
	No	1			1		
Khat chewing	Yes	0.708	0.577(0.04-7.62)	0.676		NA	NA
	No	1			1		
Age	≥50	1.304	1.161(0.35-3.88)	0.809	0.407	0.462(0.11-1.99)	0.299
	<50	1			1		
BMI	<25kg/m2	0.631	0.642(0.28-1.45)	0.286	0.848	0.896(0.37-2.20)	0.811
	≥25kg/m2	1			1		
Contraceptive use	Yes	0.811	0.892(0.26-3.02)	0.855	0.893	1.197(0.32-4.46)	0.789
	No	1			1		
Meat consumption	At least once per week	0.711	0.700(0.18-2.75)	0.610	0.606	0.671(0.15-3.10)	0.609
	Less than once per week	1			1		
fruit and vegetables consumption	At least once per day	1.103	1.140(0.33-4.00)	0.838	1.575	1.244(0.33-4.74)	0.749
	Less than once per day	1			1		

6. Discussion

The current study assessed serum iron, ferritin, total iron binding capacity (TIBC), and percent transferrin saturation (TS %) among breast cancer patients and apparently healthy participants. Serum ferritin levels were significantly elevated in breast cancer patients in comparison to the healthy control group. Serum iron, total iron binding capacity (TIBC), and percent transferrin saturation (TS %) were significantly lower in breast cancer patients compared to the apparently healthy study group.

In this study, Serum ferritin levels showed a significant rise in breast cancer patients (195.25 ± 136.92) compared to apparently healthy group (115.57 ± 74.72) at $P < 0.001$. The data reveals a significant increase in serum ferritin levels among breast cancer patients. compared to controls, which is in line with previously published studies by Pavithra et al., Rakesh Dhankhar et al., Basima Sadiq Ahmed et al., Harshal P. Manohar et al., Kher et al., Moore et al., Ulbrich et al., Maira Mahmoud et al., and Harshal Narkhede et al. [37, 38, 43, 51-56]. These findings indicated that elevated serum ferritin levels may suggest the presence of malignant disease. In terms of cancer mechanisms, a study discovered that increased serum ferritin levels are associated with inflammation and oxidative stress, rather than iron overload.

This study reveals the significantly decreased serum iron levels of patients with breast cancer (0.66 ± 0.32 mg/l) compared to the apparently healthy group (0.94 ± 0.31 mg/l) ($P < 0.001$). The present finding has similar findings to previous studies by BasimaSadiq *et al.* and Nigerian researcher Kayode S. Adedapo, in which the mean of patients with breast cancer was significantly lower than that of healthy controls (P -value < 0.0001) [43, 45]. This finding aligned with another study conducted by Mawada *et al.* [57]. and Skrajnowska *et al.* [58]., an observation was made that patients with breast cancer showed a significant variation in iron levels compared to the control group. However, it has a different finding than a study conducted by Rufaida *et al.* [46]., who observed that the serum iron level increased across the study groups. The differences between our results and these results may be because of the differences in method used for investigation and sample size.

In the present study, the results revealed that the average serum TIBC among breast cancer patients was $48.45 \pm 11.08 \mu\text{mol/l}$; compared to the control group (mean $\pm$ SD $55.19 \pm 7.43 \mu\text{mol/l}$), these values were significantly lower ($P < 0.001$). The results of the current research are in line with a prior study conducted in Iraq by Basima Sadiq A.; significantly ($P < 0.0001$) lower serum TIBC levels were observed in breast cancer patients than normal healthy controls [43]. And also in other studies, similar findings were observed in reducing TIBC levels in infection, inflammation, and neoplasia [59]. Three potential factors may contribute to the reduction in TIBC: increased iron demands due to heightened erythropoiesis, inadequate iron release from the body's iron reserves, inadequate iron absorption [60].

According to the present finding, the percentage of transferrin saturation in the serum was found to be significantly lowered in patients diagnosed with breast cancer ($24.28 \pm 10.43\%$) compared to healthy controls ($30.88 \pm 9.93\%$). This result was consistent with the study conducted by Basima Sadiq *et al.* and Mustafa *et al.*, who observed that a significantly lower serum percent transferrin saturation level was observed in breast cancer patients than normal healthy controls [43, 46]. This observation may be due to the fact that when the amount of iron in the blood decreases below the recommended range or due to chronic inflammation of the cancer itself.

According to the study's findings, a significant rise in serum ferritin levels and percent transferrin saturation was observed with the progression of the disease's clinical stages ($p < 0.001$ and $p = 0.015$, respectively). These values were significantly elevated in patients with advanced stages of breast cancer (stage 4 and stage 3) in comparison to early stages (stage 1 and stage 2) ($p < 0.05$). This study finding aligns with the observations previously mentioned by Rakesh Dhankar and Coombes RC *et al.* Elevated ferritin levels were found to be markedly higher in advanced-stage carcinoma compared to early-stage carcinoma ($p < 0.001$). Moreover, higher levels of ferritin were linked with more advanced malignant characteristics and were significantly elevated when compared to less advanced stages ($p < 0.001$) [38, 61].

Furthermore, Maira Mahmoud and co-authors indicated a significant increase in mean serum ferritin levels among breast cancer patients as opposed to the control group. Additionally, a substantial variance in mean serum ferritin levels was noted across the various stages of breast cancer (i.e., stage I, II, III, and IV) [55]. This is due to tumor-associated macrophages (TAMs)

have been suggested as the primary origin of elevated serum ferritin levels in individuals diagnosed with cancer. TAMs are responsible for the production and secretion of ferritin within the microenvironment. This process leads to the metabolic reprogramming of cancer cells, thereby facilitating their proliferation, promoting angiogenesis, and inducing immunosuppression through paracrine signaling.

This study observed higher serum iron profile levels in post-menopausal women, comparing them to pre-menopausal women, both in breast cancer patients and healthy groups. But only serum ferritin and TS% levels were significant, with P-values of <0.001 and 0.015, respectively. This study finding is supported by Panag *et al.*, Yan Li *et al.*, Cho SH *et al.*, and Cho GJ *et al.*, who have observed elevated serum ferritin levels in postmenopausal women. The observed elevation in iron levels is likely a result of the discontinuation of menstrual bleeding. As a consequence, the loss of iron through menstruation is no longer occurring, it accumulates in the body and iron requirement goes down considerably [62-65].

Ferritin synthesis is mostly regulated by the iron level [66]. The premenopausal, healthy women in this study had the lowest ferritin concentrations. This result is not surprising, as low ferritin levels are caused by physiological loss that is not adequately compensated by consumption, which results in a relatively small iron pool in females within the reproductive age range. Reduced iron loss in postmenopausal women results in an increase in stored iron levels, and rise in ferritin levels that eventually reach levels comparable to those seen in males. Continuously building up iron during the postmenopausal period may be connected to an age-related elevated risk for breast cancer [67].

Logistic regression analysis revealed a significant positive association between alcohol consumption, age, BMI, and meat consumption with serum iron and ferritin levels. In this study, patients who were older had a 4.388-fold higher risk of developing hyperferritinemia than younger patients (AOR (95% CI) = 4.388 (1.24–15.51); P = 0.022). In addition, patients with breast cancer who consumed alcohol (0.155) and eat meats at least once per week (0.181) times less likely to have low serum iron levels than non-alcohol consumers and eat meats less than once per week (AOR (95% CI) = 0.155 (0.03-0.92); AOR (95% CI) = 0.183 (0.04-0.78); P = 0.040 and P = 0.022, respectively. And breast cancer patients had normal weights (0.248) times

less likely to have low serum iron levels than overweight weight patients (AOR (95% CI) = 0.248 (0.09–0.72), $P = 0.010$). Further investigations have indicated a favorable association between age and hyper-ferritinemia [68]., alcohol consumption and high serum iron levels [69]., and overweight and low serum iron levels [70].

The findings of this study indicate that the neoplastic process causes a significant decrease in iron levels in the serum, possibly as a result of iron being redirected towards tumor development. Since iron is essential for proper catalase function, the observed decline in enzyme activity may be linked to the marked reduction in iron content in the blood [15].

The role of iron in the tumor microenvironment is increasingly evident, with a substantial amount of supporting evidence. which suggests that iron absorption, efflux, and regulatory pathways are all disturbed in cancer [71]. Increased angiogenesis has also been associated with iron deficiency. Haung reported that iron plays a crucial role as a cofactor for the enzyme hypoxia inducible factor (HIF)-1 α prolyl-4-hydroxylase, which is responsible for hydroxylating HIF-1 α . Hence, low iron levels inhibit proline hydroxylation and maintain the stability of the HIF-1 α protein, resulting in a rise in angiogenesis [72]. Breast cancer patients exhibited a notable reduction in iron levels compared to the control group, potentially leading to an increased susceptibility to breast cancer onset. The findings align with the Jian et al. findings, which found that human breast cancer growth and metastasis are facilitated by a Fe deficiency [71].

7. Strength and limitation of the study

7.1. Strength of the Study

- ✓ This study is the first to be conducted in breast cancer patients in our country, serving as a reference for future research ahead.
- ✓ Data and sample collection were conducted by experienced nurses and laboratory technologists in strict adherence to SOPs.
- ✓ The information was obtained from a demographic sample that was more diverse, reflecting a wider distribution of ages.
- ✓ Laboratory tests were analyzed using fully automated clinical chemistry instruments.

7.2 Limitation of the Study

- ✓ Restricted resource.
- ✓ Research focusing on the analysis of serum iron profiles in breast cancer patients is limited, especially in the Ethiopian setting.
- ✓ This study is restricted by a small sample size and the exclusion of tissue studies.
- ✓ The cutoff values for serum iron, TIBC, and TS% were not used; only ferritin was used during risk factor assessments.
- ✓ Lack of comparison according to treatment received to observe the effect of different modalities of treatment.

8. Conclusion and recommendation

8.1 Conclusions

The objective of this study was to assess the levels of serum iron, ferritin, total iron binding capacity, and percent transferrin saturation in breast cancer patients and compare them with those of healthy individuals. According to the findings of the study, it can be inferred that breast cancer patients consistently exhibit elevated levels of serum ferritin activity. It can be inferred from the data that individuals suffering from breast cancer experience a marked decline in serum iron, transferrin saturation percentage, and total iron binding capacity, indicating a substantial decrease in these iron-related parameters.

The results indicate a strong association between serum ferritin levels and the development of breast cancer, suggesting that they may serve as useful and affordable biomarkers for the timely identification and prediction of the disease.

Therefore, it is possible to draw the conclusion that iron metabolism plays a significant role in the pathophysiology of breast cancer and may be useful in determining the disease's severity and progress. The assessment of these serum levels of iron may play an essential role in breast cancer detection and monitoring.

Iron metabolism is strongly involved in the pathogenesis of breast cancer and can serve to assess the severity of the disease. Exploring the impact of iron dysregulation in breast cancer may pave the way for novel therapeutic interventions, offering potential advantages to patients through reduced recurrence rates, lower incidence rates, and improved survival outcomes.

8.2 Recommendations

- Further studies involving a substantial sample size are necessary to verify and validate the utility of estimating the iron profile in conjunction with other tumor markers.
- Breast cancer patients should check their iron profile levels in collaboration with RBC and Hgb counts.
- Combining ferritin with other biomarkers for breast cancer could potentially enhance its utility.
- Utilizing the iron profile as a regular screening tool is essential for breast cancer patients.
- Serum ferritin might be used with other cancer markers for staging breast cancer.
- Tissue analysis with immunohistochemistry is needed to support the present finding.

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Annexes

Annex I: Information sheet (English)

CODE _____

This informed consent form is for newly diagnosed breast cancer patients admitted in TASH.

Introduction: Hello! I'm from Addis Abeba University and I'm here with other colleagues to research on Iron profiles in breast cancer patients. I'm going to inform you and ask you to participate in this research project. You can decide whether or not to take part in the study.

Aim of the study: In order to assess serum iron levels and its associated factors among females breast cancer patients at TASH.

Type of Research Intervention: This research will involve your participation for anthropometry measurements, giving blood specimen (for this research). We will make 5 minutes interview with you to collect little clinical information.

Voluntary Participation: This study just asks for your voluntary involvement. Whether or not you participate is entirely up to you. Nothing will change in your daily life if you decide not to take part in this study. Any medical care you receive is unaffected by your choice not to volunteer.

Risks and discomforts: When collecting blood, there may occasionally be minor bleeding and minor discomforts or pains, which can be managed by trained health professionals collecting the data.

Benefits: Although there will be no direct benefit to you, your involvement will help us to assess serum iron level among breast cancer patients.

Incentive: There won't be any additional compensation for your participation in this study.

Confidentiality: The data acquired about your health and the lab results will be kept completely confidential. Individual information will remain private and findings will only be released in aggregate form. **Right to Refuse or Withdraw:** If you want to do so, you are always free to revoke your consent to the study for any reason.

Annex II: Consent Form (English)

Code of study subject _____

I have been informed about a study plan that is entitled with “Assessment of Iron profiles and its associated factors among females with Breast Cancer in TASH, Ethiopia” and for this purpose some information and blood sample will be taken from me. I was informed about the objectives of this study. The sample would be collected using the standard method for laboratory investigation; however drawing blood can be painful.

I've also been told that the questionnaire's data will be kept private. In addition, I am fully aware of my rights to refuse to participate in the study, hold onto information, and withhold my consent. I have been told that my laboratory results will be shared with me as soon as they are available, and if they contain any abnormal indicators, I will be sent to the proper facility for additional evaluation and care.

I freely gave the researcher my informed agreement to utilize the sample I provided for the investigation since I was fully aware of the circumstances. I've had the chance to inquire about it as well, and I've gotten answers that satisfy me.

Participant's signature: _____ Investigator's signature: _____

Witness name: _____ Signature: _____

Annex III: Questionnaire (English Version)

Questionnaire No ☐ date.....

Part I: Socio Demographic characteristics

1. Age: _____
2. Age at menarche: _____
3. Marital status: (a) Single (b) Married (c) widowed (d) divorced
4. Educational Status: (a) illiterate (b) elementary (c) Secondary (d) diploma and above
5. Occupation: (a) Farmer (b) Government employee (c) Private Sector employee
(e) Unemployed
6. Parity number _____
7. BMI: _____
8. Menopausal status: Yes ☐ No ☐
9. Family history with breast cancer Yes ☐ No ☐
10. Consumed on average one or more alcoholic drinks a month for a year
Yes ☐ No ☐
11. Smoked at least 100 cigarettes in her lifetime yes ☐ No ☐
12. Chewing chat yes ☐ No ☐
13. Do you use contraceptive? yes ☐ No ☐
14. How many times do you eat meat per week?
1. At least once per week 2. Less than once per week
15. How many times do you eat fruit and vegetables per a day?
1. At least once per day 2. Less than once per day

Part II: Clinical characteristics

1. Stage of breast cancer (a) Stage I (b) Stage II (c) Stage III (d) Stage IV
2. Types of breast cancer (a) Invasive ductal (b) lobular invasive carcinoma
(c) Noninvasive ductal

Laboratory test result

Iron profile:

Serum iron..... mg/l

Ferritin ng/dl

Total iron binding capacity..... $\mu\text{mol/l}$

Transferrin Saturation percent.....%

Annex IV: Information sheet to participants (Amharic version)

አባሪ 1፣ የተሳታፊዎች መረጃ ቅጽ

ሰላም ጤና ይስጥልኝ መላኩ ፀጋዬ እባላለሁ። በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ በክልካል ኬሚስትሪ ትምህርት ክፍል የሁለተኛ ዲግሪ ተመራቂ ተማር ስሆን የዚህ ጥናት ራዕስ “Assessment of iron profiles and associated factors in female breast cancer patients attended at TASH, AA, Ethiopia” የሚል ነው። እባክዎ በዚህ ጥናት ውስጥ ለመሳተፍ ከመስማማትዎ በፍት ቀጥለው የምገኘውን ሀሳብ በጥሞና ያንብቡና ግልፅ ያልሆነ ካለ ይጠይቁ። የእርስዎ በዚህ ጥናት ላይ የሚኖርዎት ተሳትፎ ሙሉ በሙሉ በግል ፈቃደኝነት ላይ የተመሰረተ ሲሆን ለመሳተፍ አልያም ላለመሳተፍ ቢወስኑ እንኳ በዚህ ሆስፒታል ውስጥ የሚያገኙት ማንኛውም አገልግሎት አይቋረጥም። የጥናቱ ተሳታፊ ከሆኑ የሚጠበቅበዎት በዚህ ጥናት ለመሳተፍ ፈቃድዎ ከሆኑ አንዳንድ ቃለ መጠይቆችን ለመመለስና በስተመጨረሻ ሁለት የሻይ ማንክያ ያክል የደም ናሙና ለመስጠት መስማማት ይጠበቅቦታል። በዚህ ጥናት መሳተፍ የሚያስገኘው ጥቅም ይህ ጥናት የሁለተኛ ዲግሪ መመረቂያ ፅሁፍ እንደመሆኑ መጠን የምከፈልዎት ገንዘብ ባይኖርም በዉጤቱ ተጠቃም ነዎት። ምክንያቱም የእርስዎ ተሳትፎ በእርስዎና በወገነዎ ደም ውስጥ የሚገኝ የአይረን መጠን ከጡት ካንሰር ጋር ያለውን ተያያዥነት እና ስጋት ለጤና ባለሙያዎች ስለሚያሳይ እንደ እርሶ ያሉ ታካሚዎችን በአማራጭ መንገድ ይበልጥ እንዲረዱት ያግዛል። በዚህ ጥናት መሳተፍ የሚያስከትለው ጉዳት መጠነኛ የደም ናሙና ከእጆዎ በምወሰድበት ጊዜ አነስተኛ የህመም ስሜት ሊኖር ይችላል። ነገር ግን ናሙናዉ የሚወሰደዉ በከፍተኛ ጥንቃቄ ልምድ ባለዉ ባለሙያ ስለሆኑ ህመሙ በጥቂት ደቂቃዎች ውስጥ ይተዋል።

የተሳታፍዎችን ምስጥር ስለመጠበቅ

ይህ የሚሰጡን ግለሰባዊ መረጃ ምስጢራዊነቱ የተጠበቀ ነው። ይህም መረጃ በኮምፒውተር በምስጢር ከተመዘገበ በኋላ ስምዎት አይጠቀስም። እናም በምንም ዓይነት መንገድ ሊታወቅ አይችልም። በኮምፒውተር ውስጥ ያለው መረጃም በምስጢር ኮድ ታስሮ ይቀመጣል። ወረቀቶችም በምስጢር ይቀመጣሉ። ይህን መረጃ የሚሰጡት ያለምንም ግዴታ በሙሉ ፈቃደኝነት ነው። ጥናቱ ከተጀመረ በኋላ በማንኛውም ጊዜ ተሳትፎዎን የማቋረጥ ሙሉ መብት ይኖርዎታል። በጥናቱ ላይ መሳተፍ በሆስፒታሉ ካለዎት ግንኙነት ጋር ተፅእኖ የለውም። በጥናቱ ላይ ለመሳተፍ የሚፈልጉ ከሆነ ወይ የተያያዘውን መረጃ ስምዎን መፈረም ወይም ደግሞ መስማማትዎን ጥናቱን ለሚሰበስበው ሠው ይገናኙ እነሱም በርስዎ ስም ይፈርማሉ።

ከምስጋና ጋር

መላኩ ፀጋዬ

አባሪ 2 ፈቃደኝነትን የሚያረጋግጥ ቅጽ

የጥናቱ ተሳታፊ መለያ ቁጥር _____

"ስለ አይረን መጠን ከጡት ካንሰር ጋር ያለውን ተያያዥነት፣ በኢትዮጵያ" የአይረን ሁኔታ በጡት ካንሰር ህመምተኞች ላይ ለመለየት ስለሚረዳው የማጥኛ ዕቅድ ተነግሮኛል። ለዚህም ጥቂት መረጃ እና የደም ናሙና ይወሰዳል። የዚህ ጥናት ዓላማ ለእነ ተብራርቷል። የናሙና ማሰባሰብ የተለመደውን የምርመራ ሂደት ይከተላል። ነገር ግን ከደም መሰብሰብ ጋር የተያያዘ ህመም ሊኖር ይችላል።

በመጠይቁ ውስጥ የተካተቱ ሁሉም መረጃዎች በምሥጢር እንደሚጠበቁ ተብራርቶታል። ከዚህም በላይ የመረጃ መብቴን የመጠበቅ፣ የመተባበር መብቴን እና ከምርመራው ራሴን የማውጣት መብቴ እንደሚጠበቅ ተነግሮኛል። ውጤቱ ዝግጁ በሆነ ጊዜ የላቦራቶሪ ውጤቶች እንደሚገለጹኝ ተነግሮኛል። በምርመራውም የህመም ምልክት ልሆን የሚችል ነገር ከተገኘብኝ ለተጨማሪ ምርመራ እና ሕክምና እንዳገኝ ወደ ተገቢ ቦታ እንደሚገናኝ ተነግሮኛል። ከዚህም በላይ ስለ ጉዳዩ ጥያቄዎች የመጠየቅ እድል አግኝቻለሁ እና በተሰጡኝ መልስ ረክቻለሁ።

ስለዚህ በፈቃደኝነቴ ስለ ጥናቱ ሙሉ በሙሉ በመረዳት ለምርመራውም አሰፈላጊ መረጃ እና የደም ናሙና እንዲወሰድ ተስማምቻለሁ። ይህንን ስምምነቴን ለማረጋገጥ ከስር በፍረማዬ አረጋግጣለሁ።

የተሳታፊ ፊርማ _____ የመርማሪ ፊርማ _____

የምስክር ስም _____ ፊርማ _____

Annex V: Questionnaire (Amharic Version)

አባሪ 3፣ የመጠይቅ ቅጽ

ክፍል 1፣ ግለሰባዊ መረጃዎ

1. ዕድሜ-----

2. የጋብቻ ሁኔታ ሀ. ያላገባ (ች) ለ. ያገባ (ች) ሐ. በሞት የተለያየች መ. በፍች የተለያየች

3. የትምህርት ሁኔታ ሀ. አልተማርኩም ለ. ሁለተኛ ደረጃ ሐ. ድፒሎምና ከዛ በላይ

4. የሥራ ሁኔታ ሀ. ገበረ ለ. የመንግስት ሰራተኛ ሐ. የግል ድርጅት መ. የቤት እመቤት ሠ. ስራ አጥ

5. የእርግዝና ቁጥር _____

6. BMI (Kg/m² _____

7. ከዚህ በፍት ከቤተሰብ/ከቤተ ዘመድ መካከል ካንሰር የታመመ አለ? ሀ. አዎ ለ. አይ

8. አልኮል ጠጥተው ያውቃሉ (ለአንድ አመት በአማካይ በወር አንድ ወይም ከዚያ በላይ የአልኮል መጠጦችን መጠቀም)? ሀ. አዎን ለ. አልጠጣም

9. ሲጋራ አጭሰው ያውቃሉ (በህይወት ዘመኗ ቢያንስ 100 ሲጋራዎችን አጭስ ነበር? ሀ. አዎን
ለ. አላጤስኩም

10. ጫት ይቅማሉ? ሀ. አዎን ለ. አልቅምም

11. የቤተሰብ እቅድ ይጠቀማሉ ሀ. አዎን ለ. አይ

12. በሳምንት ውስጥ ለምን ያህል ጊዜ ስጋ ይመገባሉ?

ሀ. ቢያንስ በሳምንት ለአንድ ጊዜ ለ. በሳምንት ከአንድ ጊዜ በታች

13. በቀን ለምን ያህል ጊዜ አትክልትና ፍራፍሬ ይመገባሉ?

ሀ. በየቀኑ ቢያንስ በቀን አንድ ጊዜ ለ. በቀን ከአንድ ጊዜ በታች

Annex VI: Test principles of the laboratory analyte

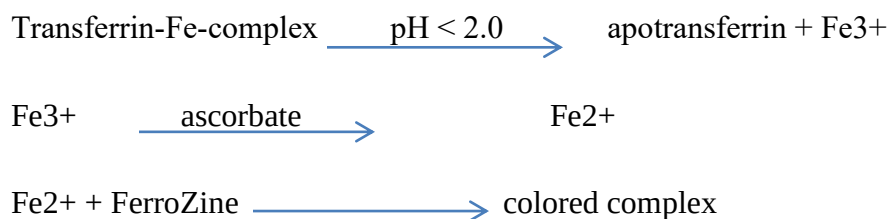
Ingested iron is primarily absorbed in the form of Fe^{2+} in the small intestine. Dietary iron in the form of Fe^{3+} must be reduced by vitamin C in order to be absorbed. Approximately 1 mg of iron is assimilated daily. In the body Fe^{2+} is converted to Fe^{3+} by ceruloplasmin, and it is bound to transferrin. Transferrin can bind one or two molecules of iron, and nearly all circulating iron is carried in this manner.

Current study used electrochemiluminescence immunoassay “ECLIA” method on cobas e immunoassay analyzer. This method uses different test principles such as sandwich, competitive and bridging for measurement.

e) Serum iron

Test principle

The Roche method of iron measurement is a three step process using FerroZine reagent: Fe^{3+} is liberated from transferrin by acid/detergent, Fe^{3+} is reduced to Fe^{2+} by ascorbate, and the reduced iron then reacts with the FerroZine reagent to form a colored complex. The intensity of this final product is directly proportional to the iron concentration in the sample.



The color intensity is directly proportional to the iron concentration and can be measured photometrically.

Reagent

The reagents are standard and ready for use on automated analyzer. Reagents: Citric acid: 200 mmol/L; thiourea: 115 mmol/L; detergent, Sodium ascorbate: 150 mmol/L; FerroZine: 6 mmol/L; preservative

Total Iron-Binding Capacity (TIBC)

Test principle

Total iron binding capacity (T.I.B.C) is determined by addition of sufficient Fe^{+3} to saturate iron binding sites on apotransferrin. The excess Fe^{+3} are removed by adsorption with basic magnesium carbonate powder. After centrifugation, bound iron remaining in supernatant is measured with direct method (Ferene) [49].

f) Transferrin

Test Principle

The transferrin is a quantitative turbidimetric test for the measurement of transferrin in human serum or plasma. Anti-transferrin antibodies when mixed with samples containing transferrin, form insoluble complexes. These complexes cause an absorbance change, dependent upon the transferrin concentration of the patient sample that can be quantified by comparison from a calibrator of known transferrin concentration [50].

g) Ferritin

Test principle

Sandwich principle: Total duration of assay: 18 minutes.

First incubation: by incubating 10 μL of sample, a monoclonal ferritin-specific antibody labeled with a ruthenium and biotinylated monoclonal ferritin-specific antibody form a sandwich complex. Second incubation: by adding streptavidin-coated microparticles, sandwich complex bound to solid phase through biotin and streptavidin interaction. The mixture of reaction extracted into measuring cell, where microparticles magnetically trapped onto electrode surface. Substances which unbound to electrode surface are then eliminated by ProCell/ProCell M. Then, voltage application on electrode induce chemiluminescent emission and photomultiplier measure it at 410nm. Results are determined with calibration curve, which is generated by 2-point calibration and master curve provided through reagent barcode.

Reagents

Reagents for the assay are ready-to-use and predispensed in the sealed reagent strips.

Procedure

All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times.

Specimen Type and Requirements: Serum, or lithium heparin plasma. The specimen must be free of visible hemolysis. EDTA, citrate, or oxalate are not acceptable anticoagulants as they will bind iron and interfere with the assay. All will reduce the apparent iron concentration in the specimen. The concentration of iron in serum/plasma is dependent upon ingestion of iron and is subject to circadian variations. The NHANES Biochem study uses refrigerated or frozen serum. This test is analyzed from NHANES Vial 018 or Vial 011.

Specimen Volume: Optimum/Minimum volume: 100 μ L in a sample cup or 2 mL microtube (8.5 μ L serum or plasma; remainder for dead volume).

Acceptable Specimens/Unacceptable Specimens: Serum, or lithium heparin plasma. EDTA, citrate, or oxalate are not acceptable anticoagulants as they will bind iron and interfere with the assay. All will reduce the apparent iron concentration in the specimen. Other anticoagulants are not acceptable. The specimen must be free of hemolysis.

Specimen Stability and Storage: Separated serum or plasma should be removed from the cells within one hour of collection. Serum or plasma is stable for 3 weeks at 4^o8 °C, seven days at 20-25°C, several years at -20°C, and longer at -70°C. Specimens must be at room temperature prior to assay. Specimens containing precipitate must be centrifuged prior to analysis.

Quality Control

Serum controls with known normal and abnormal values should be run routinely to monitor the validity of the reaction.

Declaration

I, the undersigned declare that this thesis complies with the regulations of the University and meets the accepted standards with respect to originality and quality. I also agree to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports.

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