University of Bahri

College of Applied and Industrial Sciences

Serum Trace Elements and Their Associations with Breast Cancer Subgroups in Sudanese Breast Cancer Patients in Oncology Center of Merowe- Northern State –Sudan - SEP 2026

A proposal of thesis submitted in partial fulfillment of the requirement for the Master Degree in Biotechnology

Prepare by

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SEP 2022

1- Introduction

1-1- Background:

Every year, 1.7 million women are diagnosed with breast cancer, making it the most common cancer in women worldwide and with an estimated 522,000 deaths in 2012, the leading cause of cancer death in women (accounting for 15% of all cancer death).

A comparative analysis of epidemiological data for breast cancer in the USA, Canada, India, China, Taiwan, Japan, South Korea, and Sweden showed notable differences in the median age at diagnosis; the peak age at diagnosis is 40–50 years in Asian.

Countries, and 60–70 years for Western countries -Increasing age, family history of breast cancer, major inheritance susceptibility, breast density, hormone-based therapies, and exposure to ionizing radiation, obesity and alcohol consumption are major associated risks associated with breast tumors

Although many studies have focused on breast cancer etiology, nosogenesis and treatment, aspects of this disease remain poorly defined. A comprehensive understanding of trace element speciation, localization and function under various pathophysiological conditions is becoming increasingly important for understanding disease mechanisms and

discovering novel diagnostic, prognostic, and therapeutic targets for breast cancer . Little is known about beneficial or harmful relationships between serum trace element levels and breast cancer [4]. Trace elements play important roles in biological processes relevant to breast cancer, especially those elements that are essential components of antioxidants [5]. Certain environmental and nutritional factors may elicit estrogenic and/or carcinogenic effects, influencing breast cancer [4].

Although some researchers have worked to identify associations between trace element levels and breast cancer in different populations using samples such as hair, toenails, or blood (including serum and plasma), their results have been inconsistent. Different analytical methods can be used to identify and measure trace elements, such as X-ray fluorescence spectroscopy, atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectrometry (ICP-MS), and inductively coupled plasma atomic emission spectroscopy (ICP-AES). Different assays vary in sensitivity and specificity for trace element determination, which may affect study results. Most studies of trace element status in breast cancer that have been performed in humans included small numbers of subjects, limited numbers of trace elements, or were performed as long ago as the 1990s . Furthermore, no reliable data have been collected regarding multiple serum trace element levels in Koreans, either from breast cancer patients or control females.

2-1- Statement of the problems

Breast cancer is life threating millions women life around the world and barely can be lethal disease in the world.

3-1- Justification

Traces elements are playing an important role in catalyze the enzymes that responsible for metabolic pathways used by tumors. It's known that tumor cells consumed ATP a lot, which means high rate of metabolic pathways such as glycolysis, glycogenesis, lipolysis

4-1- General objective

To study Serum Trace Elements and Their Associations with Breast Cancer Subgroups in Sudanese Breast Cancer Patients in Oncology Center of Merowe- Northern State –Sudan - SEP 2026

5-1- Specific objectives

To determine relationships between serum levels of trace elements (Zinc, Copper, and Magnesium) µg/L and breast cancer patient.

To measure the blood serum elements in breast cancer patients.

To asses risk factors associating with breast cancer.

2- Literature Review

Mawada E. Idriss1 et al, 2015. Assessment of Serum Zinc and Iron among Sudanese Women with Breast Cancer in Khartoum State. Result: The serum zinc in patients and controls respectively were (0.54±0.30, 1.09±0.23 mmol/l) and serum iron patients and controls respectively were (0.55±0.34, 1.04±0.28 mmol/l). Serum zinc and iron were significantly lower in women with breast cancer compare to healthy women (p. value <0.000 and 0.000) respectively. There were statistically significant differences of serum level of zinc and iron between three stages: Zinc (Stage 1: 0.918±0.25) vs (Stage 2: 0.443±0.05) vs (Stage 3: 0.259±0.06) (p. value <0.000 and 0.037) respectively. Iron (Stage 1: 0.954±0.26) vs (Stage 2: 0.433±0.06) vs (Stage 3: 0.239±0.07) (p. value <0.000 and 0.000) respectively. Conclusion: The serum level of zinc and iron were significantly lower in women with breast cancer and significant with different stage of disease. Zinc and iron were lower in stage three, two and one respectively.

Mona Mohamed K. El-Deeb1 et al , 2016. The Role of Serum Trace Elements and Oxidative Stress in Egyptian Breast Cancer Patients. Oxidative stress is considered to be involved in the pathophysiology of all cancers. Studies indicated that the levels of oxidative stress markers increased in breast cancer. Trace metals are essential to normal human homeostasis. When present in an abnormal expression, they contribute in many pathological processes. Some trace metals are claimed to be

carcinogenic and capable of inducing a toxic effect through the formation of free radicles and acting as cofactors in the oxidative damage of biological macromolecules and DNA. Objective: Our aim was to investigate the serum levels of some trace elements (Copper, Zinc and Cadmium), the total oxidative and anti- oxidative capacity (TOC and TAC) in patients with breast cancer in comparison to patients with benign breast tumours. Patients and Methods: The present study included 65 females. The participates were divided into 2 main groups: control group which consisted of 20 apparently healthy female; the patient groups which divided into 3 groups: group B included 15 patients with benign breast tumours, group N consisted of 15 newly diagnosed breast cancer patients and group M included 15 patients with metastatic breast cancer. Results: The mean serum levels of Copper, Zinc, and Cadmium were significantly higher in the three patients groups (B, N and M) than the control group. Similarly, serum uric acid (UA) and (TAC) levels showed significant higher level in both breast cancer groups as compared to the benign group. However TOC levels showed only significantly higher level in metastatic group. Conclusions: The present study suggested elevated TAC, UA and TOC in breast cancer patients. The increased levels of trace elements could lead to formation of free radicals or other reactive oxygen species. The serum profile of these trace metals may be helpful in predicting prognosis of breast cancer.

Rihwa Choi et al , 2018. Serum Trace Elements and Their Associations with Breast Cancer Subgroups in Korean Breast Cancer Patients. The relationships between serum levels of trace element sand breast cancer remain relatively unknown. In this study, we investigate serum levels of seven trace elements in Korean breast cancer patients compared to controls without breast cancer. Serum trace element levels were determined using inductively coupled plasma mass spectrometry in Korean breast cancer patients before initiation of breast cancer treatment. Korean females without breast cancer served as a control group. Trace element levels were measured in the discovery cohort (n = 287) and were validated in an independent cohort (n = 142). We further investigated possible associations between trace element levels and the presence of lymph node metastasis, distant metastasis, or triple-negative breast cancer among breast cancer patients in subgroup analyses. Serum manganese and molybdenum levels were significantly higher ($p < 0.05$) in breast cancer patients than in controls. Serum copper levels were significantly higher in breast cancer patients with distant metastasis, while selenium levels were significantly lower. Other trace elements were neither significantly different between breast cancer patients and controls nor between subgroups of breast cancer patients. Our study provides insights about the potential roles and impacts of trace elements through an assessment of the associations between trace elements and breast cancer.

Borhan Mansouri1etal, 2021. Association between trace elements in cancerous and non-cancerous tissues with the risk of breast cancers in western Iran. This study aimed to assess the tissue content of essential and toxic metals including lead (Pb),cadmium (Cd),arsenic (As),silver (Ag), aluminum (Al), chromium (Cr), copper (Cu), iron (Fe), selenium (Se), and zinc (Zn) in the breast cancerous tissues compared to the non-cancerous tissue. The biopsy specimens of 63 breast cancers along with 63 adjacent healthy tissues in Kurdistan Province, Iran, were collected from 2019 to 2020 and assayed using ICP-MS (Agilent 7900). The results of the Mann-Whitney test illustrated that the concentration of Pb, Cd, As, Cr, Cu, and Se were significantly elevated in cancerous tissue ($p < 0.05$), while Zn was the only trace element with higher levels in healthy subjects ($p < 0.05$). Moreover, weak to moderate correlations between elements were observed in the cancerous group including Al-Cr ($r=0.60$), As-Cu ($r=0.52$), and Cu-Se ($r=0.56$). In contrast, no correlation over 0.50 was found between trace elements in the non-cancerous group. Raw risk differences (RDs) accounted for a significant effect for Pb, Cd, As, Ag, Cr, Se, and Zn on the development of breast cancer. In conclusion, elevated levels of As, Cd, Cu, Pb, and Se may contribute to enhancing the risk of breast cancer.

3. Materials and Methods

3.1 Material:

3.1.1 Study design

This study will be a case control hospital based study.

2.1.2Study area, setting, and duration

This study will be done in Breast Cancer Center – Merowe, it will be conducted in the Center lab in SEP 2022.

3.1.3 Study population

The population consist case and control group.

3.1.3.1 The case:

a- Inclusion criteria

Diagnostic female breast cancer patients aged above 40 years, they are willing or have desire for sharing in the study.

b- Exclusion criteria

The exclusion criteria will be included diagnosis of any type of cancer other than breast cancer.

3.1.3.2 The control:

a- Inclusion criteria

Healthy female people aged over 40 years will be included.

b- Exclusion criteria

Any female have benign or malignant tumor will be excluded

Any female haven't desire to share in this study.

3.1.4 Sample size

The sample size calculated by the following formula:

n= N/1+N (d) 2

n= sample size

N =Population

d = degree of significant = (0.05)

The estimated number of study population is 100 patients

n= 100/1+100×0.0025= 100.25

The sample size of this study will be about100 Patients.

3.1.5 Sampling technique

A simple random sampling technique will be used for data collection to represent the entire data, where each member of the population has an equal probability of getting selected and included in the sample.

3.2 Method:

3.2.1 Secondary data

Pretested structured data sheet (appendix 1) contains closed questions

3.2.2 Lab investigation

Blood samples from 100 Individuals patients with breast cancer and from 50 individuals healthy people (as control group) will be collected by qualified lab technician in standard conditions. Trace elements will be measured by Buck Scientific Atomic Absorption Spectrophotometer

The biochemical tests such as blood glucose and lipid profile will be carried out according to manufactory's procedures.

3.2.3Buck Scientific Atomic Absorption Spectrophotometer protocol:

Preparation of sample and reagents:

a. Sample:

5ml of blood samples will be collected using EDTA or citrate as an anticoagulant, and be mixed for 10-20 min, then it will centrifuged at the speed of 2000-3000 r.p.m. for 20 min.

Magnesium sample procedure

For the determination of calcium and magnesium dilute the serum or plasma sample 1:50 with a 0.1% (w/v) lanthanum (as chloride) diluent. If calcium is not to be determined, the dilution can be made with deionized water. The dilution ratio can be adjusted to insure that concentrations fall within a suitable absorbance range. NOTE: Refer to

the lanthanum "Standard Conditions" section for details on the preparation of the lanthanum diluent.

Copper and Zinc procedure

For the determination of serum copper, dilute the sample with an equal volume of deionized water. For the determination of serum zinc, dilute the sample 1:5 with deionized water

3.2.5Assay Procedure:

The Buck 210VGP atomic absorption spectrophotometer is designed to measure the concentration of elemental metals in solution. It provides integrated measurements in absorbance or emission intensity, as well as sample concentration in comparison to standard solutions. The readings can be integrated over a period from 0.5 to 10 seconds. For the transient signals obtained using flame-less techniques (cold vapor, hydride, and graphite furnace), absorbance is determined using peak area integration. The Buck 210VGP can be calibrated using up to 8 concentration values, with units of mg/L, mg/L, ppm, ppb, mEq/dL, mM/dL, mg/dL and %. Calculations can be performed using linear regression, 2nd, 3rd, and 4th order curves. Optional accessories allow the user to print absorbance data, background absorbance and concentrations in real time, and to collect absorbance values using a laboratory recorder. The Buck 210VGP interfaces with the Analyze software package for sophisticated data processing, documentation and reporting. An external computer

may also process the data by using a communications program to download the data into a spreadsheet program.

3.2.6 Data analysis

Data will be analyzed by computerized method (SPSS –version 26), Results presented as tables and figures.

3.2.7 Ethical considerations

The proposal was ethically approved by the ethical committee of the University of Bahri. The consent will be taken from each patient, and the permission from the Northern State Ministry of the Health and general manager of the hospital will be obtained.

Research proposes and objectives will be explained to participant in clear simple words.

Participant has right to voluntary informed consent.

Participant has right to withdraw at any time.

Participant has right to benefit from the researcher skills and knowledge.

Participant has right to no harm (privacy and confidentiality by using coded data sheet).

Investigation results will be showed to the participant immediately.

Remaining blood sample will not be reused for other study.

Precautions during sample collection will be taken into a count to protect from Covid 19.

The filled sheets will be burned after completion the study.

Budget

Equipment and Matrial	**Costs / SDG**
Reagents	300.000
Publication	120.000
Other cost	120.00
Total cost	**540.000**

Work time table:

Work	Time
Data collection	3 to 5 months
Data analysis	2 to 3 months
Laboratory investigation	1 to 3 months
Thesis writing	5 months

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Appendix 1

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Serum Trace Elements and Their Associations with

Breast Cancer Subgroups in Sudanese Breast

Cancer Patients in Breast Cancer Center of Oncology Center of Merowe-Northern State –Sudan - SEP 2026

Questionnaire

Personal information:

ID number……
Age……

Mitral status……

Information about breast cancer:

Family history of breast cancer……

Breast cancer diagnostic procedure…….

Other associated diseases and their history…….

Risk factors of breast cancer…….

Other information:

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…….

Thanks for your response