



**Sudan University of Science and Technology  
(SUST)**

**College of Graduate studies**

**Assessment of Thyroid Function among Patients with  
Breast Cancer in Khartoum State**

**تقييم وظائف الغدة الدرقية في مرضى سرطان الثدي بولاية الخرطوم**

A dissertation Submitted in Partial Fulfilment for the requirement of  
M.Sc degree in Medical Laboratory Sciences (Clinical Chemistry)

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## **Dedication**

*I would like to dedicate this research to my mother and my father, who prayed for me all the time so that I complete this research, my sister aml and my brothers who spent their life to provide sufficiency, also I dedicate this research for my real best friends, finally I dedicate this research to my colleagues in the master.*

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## List of abbreviations

<b>BSE</b>	Breast self-examination
<b>CBE</b>	Clinical breast examination
<b>FT3</b>	Free Triiodothyronine
<b>FT4</b>	Free Thyroxine
<b>FT4I</b>	free thyroxine index
<b>MRI</b>	Magnetic resonance imaging
<b>SPSS</b>	Statistical Package for Social Sciences
<b>T3</b>	Triiodothyronine
<b>T3U</b>	T3 uptake
<b>T4</b>	Thyroxine
<b>TBG</b>	Thyroxine-Binding Globulin
<b>TPO-AB</b>	thyroperoxidase antibodies
<b>TRH</b>	Thyrotropin Releasing Hormone
<b>TSH</b>	Thyroid Stimulating Hormone
<b>WHO</b>	World Health Organization



## **Abstract:**

**Background:** According to the [world health organization](#) (WHO), breast cancer is the most common cancer among women worldwide, claiming the lives of hundreds of thousands of women each year and affecting countries at all levels of modernization, The incidence of breast cancer in Sub-Saharan African countries is low compared with developed countries, the cancer picture in Sub-Saharan Africa and especially in Sudan is changing. Lately, breast cancer incidence and mortality has been rising. The relationship between breast cancer and thyroid diseases is controversial, recent studies indicate a possible relationship between hypothyroidism and breast cancer in vivo.

**Objective:** This study was performed to assess thyroid function tests among patients with breast cancer in compare with healthy individuals, and to compare means of TSH, T3 and T4 in breast cancer patients with healthy individuals.

**Methods:** The study design was analytical case control done in Khartoum state during the period between 2017 to October 2018. Blood samples were collected from 88 participants who were included in this study, the case group contain 44 patients and 44 healthy individuals as control group, TSH, T3 and T4 was measured by ELISA.

**Results:** The mean of (TSH) level was significantly decreased, the mean of T4 was significantly increased, while the mean of T3 showed no significant deference in patients with breast cancer when compared to control group.

**Conclusion:** Our study revealed that there is an association between breast cancer and thyroid disorders, the incidence of thyroid disease in breast cancer patients is increased when compared to control group.

## المستخلص:

**الخلفية:** وفقاً لمنظمة الصحة العالمية (WHO) ، فإن سرطان الثدي هو أكثر أنواع السرطان شيوعاً بين النساء في جميع أنحاء العالم ، حيث يُزهق أرواح مئات الآلاف من النساء كل عام. ويؤثر على البلدان على جميع المستويات، صورة السرطان في أفريقيا، وخاصة في السودان آخذة في التغير. في الآونة الأخيرة ، معدل الإصابة بسرطان الثدي والوفيات في ارتفاع. العلاقة بين سرطان الثدي وأمراض الغدة الدرقية أمر مثير للجدل ، وتشير الدراسات الحديثة إلى وجود علاقة محتملة بين قصور الغدة الدرقية وسرطان الثدي.

**الهدف:** تم إجراء هذه الدراسة لتقدير اختبارات وظائف الغدة الدرقية بين المرضى الذين يعانون من سرطان الثدي مقارنة مع الأفراد الأصحاء ، ومقارنة مستويات TSH ، T3 و T4 في مرضى سرطان الثدي مع الأفراد الأصحاء.

**الطريقة:** تمت الدراسة في ولاية الخرطوم خلال الفترة ما بين 2017 وحتى أكتوبر 2018. تم جمع عينات الدم من 88 مشاركاً تم تضمينهم في هذه الدراسة ، وتحتوي مجموعة الحالات على 44 مريضاً و 44 شخصاً أصحاء كمجموعة مراقبة ، تم قياس TSH ، T3 و T4 بواسطة ELISA.

**النتائج:** انخفاض في متوسط مستوى (TSH) بشكل ملحوظ ، زيادة في متوسط T4 بشكل ملحوظ ، بينما لم يُظهر في متوسط T3 أي اختلاف كبير في مرضى سرطان الثدي بالمقارنة مع مجموعة التحكم.

**الخلاصة:** كشفت دراستنا أن هناك علاقة بين سرطان الثدي واضطرابات الغدة الدرقية ، وزيادة الإصابة بمرض الغدة الدرقية في مرضى سرطان الثدي بالمقارنة مع مجموعة المراقبة.

# Chapter One

## Introduction, Rationale and Objectives

### 1.1 Introduction:

Cancer is one of the leading causes of morbidity and mortality worldwide, with approximately 14 million new cases in 2012. The number of new cases is expected to rise by about 70% over the next 2 decades; it is also the second leading cause of death globally and was responsible for 8.8 million deaths in 2015. Globally, nearly 1 in 6 deaths is due to cancer. Approximately 70% of deaths from cancer occur in low- and middle-income countries. (Ferlay *et al.*, 2015)

Breast cancer is a group of diseases that affects breast tissue. Both women and men can get breast cancer, though it is much more common in women (Korde *et al.*, 2010). According to the World Health Organization (WHO), breast cancer is the most common cancer among women worldwide, claiming the lives of hundreds of thousands of women each year and affecting countries at all levels of modernization (Anderson *et al.*, 2008). The incidence of breast cancer in Sub-Saharan African countries is low compared with developed countries; the cancer picture in Sub-Saharan Africa and especially in Sudan is changing. Lately, breast cancer incidence and mortality has been rising, with an incidence rate of 25.1 per 100,000 (Mohammed *et al.*, 2010)

Thyroid gland is butterfly shaped gland located in the front of the neck just above the trachea in the adult human, which is composed of two lobes with the right lobe larger than the left lobe, and the lobes are connected by the isthmus (Burtis *et al.*, 2015). The thyroid gland is responsible for the production of two hormones: calcitonin is secreted by parafollicular C cells and is involved in calcium homeostasis, and thyroid hormone: thyroid gland secretes two hormones, thyroxine (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>). In addition, the thyroid gland secretes very small amounts of biologically inactive T<sub>3</sub> and minute quantities of monoiodotyrosine and diiodotyrosine, which are precursors of T<sub>3</sub> and T<sub>4</sub>, the hypothalamus and the pituitary in the brain communicate to maintain T<sub>3</sub> and T<sub>4</sub> balance. The hypothalamus produces thyroid stimulating hormone (TSH) releasing hormone (TRH) that signals the pituitary to tell the thyroid gland to produce more or less of T<sub>3</sub> and T<sub>4</sub> by either increasing or decreasing the hormone thyroid stimulating hormone (Bishop and Fody., 2010).

Thyroid hormone is critical in regulating body metabolism, neurologic development, promotion of sexual maturation and numerous other body functions (Bishop and Fody., 2010).

As early as the 19th century, thyroid hormones were utilized for therapeutic purposes in breast cancer patients. Since then, many trials have been initiated in order to identify the relationship between thyroid dysfunction and breast cancer disease. Some of these studies showed higher incidence of breast cancer in patients with thyroid dysfunction compared to healthy controls. Furthermore, several studies have provided evidence for the relationship between breast cancer and autoimmune thyroid disorders. In particular, a high prevalence of thyroperoxidase antibodies (TPO-AB) and autoimmune hypothyroidism is present in breast cancer patients and patients with non-malignant breast tumours in comparison to healthy controls(Ditsch, *et al.*, 2010).

The relationship between breast cancer and thyroid diseases is controversial, recent studies indicate a possible relationship between hypothyroidism and breast cancer in vivo. In addition oestrogen-like effects of thyroid hormones on breast cancer cell growth are seen in vitro (Ditsch, *et al.*, 2010).

## **1.2 Rationale:**

Cancer is one of the leading causes of morbidity and mortality worldwide. It is the second leading cause of death globally causing about approximately 70% of deaths in low and middle-income countries (including our country, Sudan).

Breast cancer is the most common cancer among women worldwide as reported by world health organization (WHO), its incidence and mortality has been rising. The relationship between breast cancer and thyroid diseases is controversial; many studies reported that the incidence of thyroid disease in breast cancer patients when compared to control group is increased; and hence this study was performed.

### **1.3 Objectives:**

#### **1.3.1 General objective:**

To assess thyroid functions among patients with breast cancer in Khartoum state.

#### **1.3.2 Specific objectives:**

To estimate TSH, T3 and T4 among patients with breast cancer compared to control group.

## **Chapter Two**

### **Literature review**

#### **2.1 Cancer:**

Cancer is a generic term for a large group of diseases characterized by the growth of abnormal cells beyond their usual boundaries that can then invade adjoining parts of the body and/or spread to other organs. Other common terms used are malignant tumours and neoplasm. Cancer can affect almost any part of the body and has many anatomic and molecular subtypes that each requires specific management strategies(Ferlayet *al.*, 2015).

One defining feature of cancer is the rapid creation of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs, this process is referred to as metastasis. Metastases are the major cause of death from cancer(Ferlayet *al.*, 2015).

Cancer is the second leading cause of death globally and is estimated to account for 9.6 million deaths in 2018. Lung, prostate, colorectal, stomach and liver cancer are the most common types of cancer in men while breast, colorectal, lung, cervix and thyroid cancer are the most common among women. There are over 100 different types of cancers that can affect any organ of the body(Ferlayet *al.*, 2015).

##### **2.1.1 Causes of cancer:**

Cancer arises from one single cell; the transformation from a normal cell into a tumour cell is a multistage process, typically a progression from a pre-cancerous lesion to malignant tumors. These changes are the result of the interaction between a person's genetic factors and three categories of external agents including: physical carcinogens such as ultraviolet and ionizing radiation, chemical carcinogens such as asbestos, components of tobacco smoke, aflatoxin (a food contaminant) and arsenic (a drinking water contaminant), and biological carcinogens such as infections from certain viruses, bacteria, or parasites. Ageing is another fundamental factor for the development of cancer. The incidence of cancer rises dramatically with age, most likely due to a build-up of risks for specific cancers that increase with age(Ferlayet *al.*, 2015).

## **2.2 Breast cancer:**

Breast cancer is a hormone-dependent neoplasm (Turken *et al.*, 2003), it is the most common cancer and also the leading cause of cancer mortality in women worldwide. Approximately 1.38 million new breast cancer cases were diagnosed in 2008 with almost half of all breast cancer cases and nearly 60% of deaths occurring in lower income countries (Shah and Rosso., 2014).

### **2.2.1 Risk factors for breast cancer:**

Several risk factors for breast cancer have been well documented. However, for the majority of women presenting with breast cancer are not possible to identify specific risk factors, a familial history of breast cancer increases the risk by a factor of two or three. Some mutations, particularly in BRCA1, BRCA2 and p53 result in a very high risk for breast cancer. However, these mutations are rare and account for a small portion of the total breast cancer burden (IARC., 2008) (Lacey *et al.*, 2009).

Reproductive factors associated with prolonged exposure to endogenous estrogens such as early menarche, late menopause, late age at first childbirth are among the most important risk factors for breast cancer. Exogenous hormones also exert a higher risk for breast cancer. Oral contraceptive and hormone replacement therapy users are at higher risk than non-users. Breastfeeding has a protective effect (IARC., 2008) (Lacey *et al.*, 2009).

The differences in breast cancer incidence between developed and developing countries can partly be explained by dietary effects combined with later first childbirth, lower parity, and shorter breastfeeding, the increasing adoption of western life-style in low- and middle-income countries is an important determinant in the increase of breast cancer incidence in these countries (Peto., 2001).

### **2.2.2 Diagnosis of breast cancer:**

Early diagnosis remains an important early detection strategy, particularly in low- and middle-income countries where the diseases is diagnosed in late stages and resources are very limited. There is some evidence that this strategy can produce "down staging" (increasing in proportion of breast cancers detected at an early stage) of the disease to stages that are more amenable to curative treatment (Yip *et al.*, 2008).



### **2.2.2.1 Mammography screening:**

Mammography screening is the only screening method that has proven to be effective. Although there is evidence that organized population-based mammography screening programmes can reduce breast cancer mortality by around 20% in the screened group versus the unscreened group across all age groups, in general there appears to be a narrow balance of benefits compared with harms, particularly in younger and older women. There is uncertainty about the magnitude of the harms – particularly overdiagnosis and overtreatment. Mammography screening is very complex and resource intensive and no research of its effectiveness has been conducted in low resource settings (Yip *et al.*, 2008).

### **2.2.2.2 Breast self-examination (BSE):**

There is no evidence on the effect of screening through breast self-examination (BSE). However, the practice of BSE has been seen to empower women, taking responsibility for their own health. Therefore, BSE is recommended for raising awareness among women at risk rather than as a screening method (Yip *et al.*, 2008).

### **2.2.2.3 Clinical breast examination (CBE):**

Research is underway to evaluate CBE as a low-cost approach to breast cancer screening that can work in less affluent countries. Promising preliminary results show that the age-standardized incidence rate for advanced-stage breast cancer is lower in the screened group compared to the unscreened group (Sankaranarayanan *et al.*, 2011).

### **2.2.2.4 Magnetic resonance imaging:**

Mammography remains the gold standard for breast imaging but magnetic resonance imaging (MRI) has become an important modality in the detection, assessment, staging, and management of breast cancer in selected patients. Screening MRI is more sensitive but less specific for the detection of cancer in high risk women. MRI is valuable in the screening of select high risk patients, patients in whom breast augmentation prevents effective screening mammography, or in patients with equivocal findings on other imaging modalities. (Shah and Rosso., 2014).

### **2.2.2.5 Ultrasound:**

Whole breast ultrasound may allow the clinician to screen for breast cancers not detected by traditional mammography, especially in dense breasts where mammographic sensitivity is lower. (Shah and Rosso., 2014).

## **2.3 Thyroid:**

The thyroid gland is responsible for the production of two hormones: thyroid hormone and calcitonin. Calcitonin is secreted by parafollicular C cells and is involved in calcium homeostasis. Thyroid hormone is critical in regulating body metabolism, neurologic development, and numerous other body functions. Clinically, conditions affecting thyroid hormone levels are much more common than those affecting calcitonin (Bishop and Fody., 2010).

The thyroid hormones are thyroxine (T4) and triiodothyronine (T3). Four steps are involved in the synthesis of these hormones:

- Inorganic iodide from the circulating blood is trapped (iodide trapping).
- Iodide is oxidized to iodine (oxidation).
- Iodine is added to tyrosine to produce monoiodotyrosine and diiodotyrosine (organification).
- One monoiodotyrosine is coupled with one diiodotyrosine to yield T3 and two diiodotyrosines are coupled to yield T4 (coupling). (DasGupta and Wahed, 2014)

Both T3 and T4 are bound to thyroglobulin and stored in the colloid. Free (unbound) T4 is the primary secretory hormone from the thyroid gland, and T4 is converted in peripheral tissue (liver, kidney, and muscle) to T3 by 5'-monodeiodination. T3 is the physiologically active hormone. T4 can also be converted to reverse T3 by 3'-monodeiodination. This form of T3 is inactive. The majority (99%) of the T3 and T4 in circulation are found to be involved in thyroxine-binding globulin (TBG), albumin, and thyroxine-binding prealbumin. T3 binds to the thyroid hormone nuclear receptor on target cells to cause modified gene transcription. (DasGupta and Wahed., 2014)

### **2.3.1 Thyroid anatomy:**

The thyroid gland is positioned in the lower anterior neck and is shaped like a butterfly. It is made up of two lobes that rest on each side of the trachea, with a band of thyroid tissue called the isthmus running anterior to the trachea and bridging the lobes. Posterior to the thyroid gland are the parathyroid glands that regulate serum calcium levels and the recurrent laryngeal nerves that innervate the vocal cords. These posterior structures become important during thyroid surgery, when care must be exercised to avoid injury that could lead to hypocalcemia or permanent hoarse voice (Bishop and Fody., 2010).

### **2.3.2 Control of thyroid function:**

Understanding of the hypothalamic-pituitary-thyroid axis is essential for correctly interpreting thyroid function testing. This axis is central in the regulation of thyroid hormone production. TRH is synthesized by neurons in the supraoptic and supraventricular nuclei of the hypothalamus and stored in the median eminence of the hypothalamus. When secreted, this hormone stimulates cells in the anterior pituitary gland to manufacture and release thyrotropin (TSH) which in turn, circulates to the thyroid gland and leads to increased production and release of thyroid hormone. When the hypothalamus and pituitary sense that there is an inadequate amount of thyroid hormone in circulation, TRH and TSH secretion increases and will lead to increased thyroid hormone production. If thyroid hormone levels are high, TRH and TSH release will be inhibited leading to lower levels of thyroid hormone production and vice versa if thyroid hormone levels are low. This feedback loop requires a normally functioning hypothalamus, pituitary, and thyroid gland, as well as an absence of any interfering agents or agents that mimic TSH action (Bishop and Fody., 2010).

### **2.3.3 Actions of thyroid hormones:**

Once released from the thyroid gland, thyroid hormone circulates in the bloodstream where free T<sub>4</sub> and T<sub>3</sub> are available to travel across the cell membrane. In the cytoplasm, T<sub>4</sub> is deiodinated into T<sub>3</sub>, the active form of thyroid hormone. T<sub>3</sub> combines with its nuclear receptor on thyroid hormone-responsive genes, leading to production of messenger RNA that, in turn leads to production of proteins that influence metabolism and development. Effects of thyroid hormone include tissue growth, brain maturation, increased heat production, increased oxygen consumption, and an increased number of adrenergic receptors. Clinically, individuals who have excess thyroid hormone (thyrotoxicosis) will have symptoms of

increased metabolism such as tachycardia and tremor, while individuals with hypothyroidism note symptoms of lowered metabolism like edema and constipation.(Hubbard., 2010).

### **2.3.4 Thyroid disorders:**

Thyroid disorders are caused by increased or decreased levels of the circulating hormones T3 and T4. A wide variety of physical diseases can be traced back to a dysfunctional thyroid gland (Hubbard., 2010).

#### **2.3.4.1 Hypothyroidism:**

Is a serum level of thyroid hormone that is insufficient to provide for the metabolic needs of cells. This disorder affects women four times more than men between the ages of 30 and 60 years. Hypothyroidism is usually referred to as primary, secondary, or tertiary, depending on the site of the dysfunction (Hubbard., 2010).

The symptoms of hypothyroidism include an enlarged thyroid gland (goiter), fatigue, impairment of mental processes, and loss of appetite. Myxedema (loss of hair, swelling of the hands and face, course skin) occurs as the disease progresses.(Hubbard., 2010).

The causes of hypothyroidism relate to the area of tissue damage. In addition, hypothyroidism can be caused by lack of dietary iodine.(Hubbard., 2010).

Primary hypothyroidism involves the inadequate secretion of thyroid hormones caused by a damaged or surgically removed thyroid gland. Congenital hypothyroidism is caused by the absence of the thyroid gland. Laboratory results indicate decreased T3, T4, free thyroxine index (FT4I), T3 uptake (T3U), and increased TSH. (Hubbard., 2010).

Secondary hypothyroidism involves decreased production of TSH caused by pituitary disorder leading to low serum levels of the thyroid hormones. Laboratory results indicate all thyroid test values are decreased.(Hubbard., 2010).

Tertiary hypothyroidism is caused by hypothalamic failure leading to a lack of TRH production (Hubbard., 2010).

In the laboratory evaluation of hypothyroidism, the earliest abnormality is increased TSH, followed by decreased serum levels of T4 and T3 (Hubbard., 2010).

Chronic immune thyroiditis (Hashimoto's disease) is caused by a genetic abnormality in the immune system and involves massive infiltration of the thyroid gland by lymphocytes. The symptoms match those of hypothyroidism (Hubbard., 2010).

#### **2.3.4.2 Hyperthyroidism:**

Is caused by excessive thyroid hormone in the circulation, this causes cells to become overactive. The disorder is sometimes referred to as thyrotoxicosis (Hubbard., 2010).

The symptoms of hyperthyroidism include weight loss, loss of muscle mass, hyperactivity yet, quick fatigability, insomnia, increased sweating, nervousness, palpitations, goiter, and bulging eyes. (Hubbard., 2010).

The cause of this disorder includes pituitary tumors that cause excessive TSH secretion, thyroid carcinoma, or toxic multinodular goiter (gland produces excess hormones). (Hubbard., 2010).

The laboratory evaluation of hyperthyroidism in the initial evaluation reveals elevated thyroid hormone serum levels and decreased serum TSH (Hubbard., 2010).

Graves' disease is an autoimmune disorder that occurs six times more frequently in women than in men. In this disorder, immunoglobulins stimulate the thyroid gland by binding to TSH receptors. Symptoms are similar to those of hyperthyroidism. Laboratory results indicate increased T3, T4, FT4I, and T3U, and decreased or normal TSH (Hubbard., 2010).

Thyroiditis is an inflammation of the thyroid gland caused by either bacterial or viral infection (Hubbard., 2010).

#### **2.3.5 Assays for thyroid function:**

Include testing for serum level of total (both bound and free) or free T3, total or free T4, TSH, and TBG. These tests are typically immunoassays. Other thyroid tests include the following:

A. T3 resin uptake analyzes the capacity of TBG to bind thyroid hormones. It is an indirect measurement of the number of free binding sites on the TBG molecule.

B. Free thyroxine index (FT4I) indirectly assesses the concentration of circulating free T4. It is calculated by multiplying the value of the total T4 by the percentage value of the T3 resin uptake.

C. Thyroid antibody screens assay for the presence of thyroid-stimulating immunoglobulins, such as those in Graves' disease and Hashimoto's thyroiditis.

D. TRH stimulation test measures pituitary TSH stores and is considered conclusive for hyperthyroidism, although it is not needed in most hyperthyroid patients. In patients with slightly elevated hormone levels (but other symptoms of hyperthyroidism), TRH is injected, and blood samples are assayed for TSH, it is level rise rapidly in a normal person but will not rise in a hyperthyroid patient (Hubbard., 2010).

#### **2.4 Background studies:**

In Libya, 2018 Abdalla Mohammed Jarari, *et al* conducted a research titled serum thyroid hormone profile in breast cancer patient. There was no significant difference in thyroxine (T4) measurement between breast cancer groups and normal healthy control group. ( $P > 0.05$ ). The triiodothyronine (T3) measurements showed no significant difference between breast cancer groups, fibrocystic group and normal healthy control group ( $p > 0.05$ ). The thyrotropin (TSH) measurements showed significant difference comparing both breast cancer patients, pre-treatment ( $p = 0.016$ ) and post-treatment ( $p = 0.01$ ) respectively to the control group (Mohammed Jarari *et al.*, 2018).

Also in Mexico, 2018 Carolina Ortega-Olvera, *et al* conducted a study about thyroid hormones and breast cancer association according to menopausal status and body mass index. The study concluded that lower TT3 concentrations were associated with breast cancer in both premenopausal and postmenopausal women, and there is a strong association between breast cancer and serum concentrations of TT3 and TT4 (Ortega-Olvera *et al.*, 2018).

Also another study was done in Iran, 2017 by Mohammad Reza Motie, *et al* about evaluation of thyroid dysfunction in breast cancer before surgery. The results revealed that mean serum TSH level was not significantly different between the two groups, but the mean serum levels of T4 was higher in patients ( $p < 0.05$ ) (Reza *et al.*, 2017).

In 2017 Shi Y, *et al* performed a research in China titled study on the status of thyroid function and thyroid nodules in Chinese breast cancer patients. The results revealed that the thyroxine (T4) level in initially diagnosed breast cancer patients were significantly higher than those in benign breast diseases patients ( $7.68 \pm 1.51$  vs  $7.29 \pm 1.52$  ug/dl,  $p < 0.001$ ), while the TSH levels were slightly lower than in benign breast diseases patients ( $3.23 \pm 4.59$  vs.  $3.60 \pm 6.74$  uIU/ml,  $p = 0.302$ ) (Shi *et al.*, 2017).

Furthermore in 2016 study was conducted by Renija V, *et al* about thyroid hormone profile in early breast cancer patients. It showed that there was statistically significant low T4 and high TSH in breast cancer patients and concluded that compared to hyperthyroidism, hypothyroidism was found to be clinically significant in breast cancer patients (Renija, Purayil and Purayil., 2016).

In Iran, 2015 study under the title of: is there any association between thyroid autoimmunity and breast cancer? was done by Valizadeh N, *et al*. There was no statistically significant difference between the mean values of T3 in patients with breast cancer and the control group, the mean TSH level in patients with breast cancer was significantly higher than healthy women. It concluded that measurement of T3 in all women with breast cancer is not recommended but measurement of TSH seems reasonable (Valizadeh., 2015).

Mahmood Rasoo, *et al* did a research in Saudi Arabia, 2014 titled comparative study of alterations in tri-iodothyronine (T3) and thyroxine (T4) hormone levels in breast and ovarian cancer. The results revealed that statistically significant difference ( $P=0.000^*$  and  $P=0.017^*$ ) was obtained among all groups. A significant increase in T3 ( $P=0.000^*$ ) and T4 ( $0.005^*$ ) levels was observed among breast cancer patients as compared to healthy controls (Mahmood *et al.*, 2014).

In Brasil, 2005 a research under the title of profile of thyroid hormones in breast cancer patients was done by Saraiva, *et al*. The results showed that hyperthyroidism was associated with postmenopausal patients as shown by significantly higher mean T3 and T4 values and lower TSH levels in this group of breast cancer patients than in controls, also postmenopausal breast cancer patients have a significantly increased thyroid hormone (Saraiva *et al.*, 2005).

## **Chapter three**

### **Materials and methods**

#### **3.1 Materials:**

##### **3.1.1 Study area:**

This study was carried out in Khartoum centre for radiotherapy and nuclear medicine, Khartoum state.

##### **3.1.2 Study design:**

Analytical case control study design was conducted.

##### **3.1.3 Study duration:**

The study was conducted in the period from January 2017 to October 2018.

##### **3.1.4 Study population and sample size:**

Eighty eight subjects were involved in the study, 44 had breast cancer and 44 healthy individuals.

##### **3.1.5 Inclusion criteria:**

Study includes female patients with breast cancer.

##### **3.1.6 Execution criteria:**

Study excludes patients with thyroid disorders, pregnant women, medications i.e.(estrogen), acute hepatitis and sever ill patients.

#### **3.2 Data collection method, technique and analyzed plan:**

##### **3.2.1 Data collection:**

The data collected by using questionnaire.

##### **3.2.2 Technique:**



### **3.2.2.1 Sampling technique:**

Under a septic condition, 2.5 ml venous blood was collected in plain containers. Venous blood was collected from all participants by using disposable syringe after cleaning the skin by 70% alcohol, and the puncture site was tied with a light bandage. Then drawn specimens were poured slowly into plain container. After that the specimens were stand for half an hour and placed in centrifuge to collect the serum.

### **3.2.2.2 Estimation technique:**

#### **Thyroid stimulating hormone (TSH):**

TSH is estimated by immunoenzymometric assay which includes high affinity and specificity antibodies (enzyme conjugated and immobilized) with different and distinct epitope recognition, in excess, and native antigen.

In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-TSH antibody.

Upon mixing the biotinylated monoclonal antibody, the enzyme labelled antibody and serum containing the native antigen, reaction result between the native antigen and antibodies without competition or steric hindrance to form soluble sandwich complex.(Appendix I)

#### **Triiodothyronine (T3):**

**Competitive enzyme immunoassay:** the essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme antigen conjugate and native antigen.

Upon mixing immobilize antibody, enzyme-antigen conjugate, and serum containing the native antigen a competitive reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of insolubulized binding sites.

After equilibrium is attained, the antibody bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration. (Appendix II)

#### **Thyroxin (T4):**

**Competitive enzyme immunoassay:** the essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme antigen conjugate and native antigen.

Upon mixing immobilize antibody, enzyme-antigen conjugate, and serum containing the native antigen a competitive reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of insolubulized binding sites.

After equilibrium is attained, the antibody bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration. (Appendix III)

### **3.2.3 Quality control:**

The precision and accuracy of all tests used in this study was checked by commercially prepared control samples before its application for the measurement of test.

### **3.2.4 Ethical consideration:**

Permission of this study has been obtained from the ministry of health which insures all ethical considerations for conducting the research in a way that protects patient's confidentiality and privacy.

The objectives of the study were explained to all individuals participating in this study and the informed consent was obtained from all participants.

### **3.2.5 Statistical analysis:**

Presented in form of tables, a statistical analysis is then made depending on computerized programs mainly excel and SPSS (version 20) to utilize the results which were obtained at the end of the study.

## **Chapter four**

### **Results**

This study include eighty eight subjects, 44 had breast cancer and 44 healthy individuals. results of TSH, T3 and T4 in patients with breast cancer and control group are given in table

#### **4.1 Describe the statistical analysis for the variables between the study groups:**

Table (4.1) Describe the statistical analysis for the variables between the study groups as the following:

The results showed that the mean of thyroid stimulating hormones (TSH) level was significant decreased and the mean of thyroxin (T4) was significant increase in patients with breast cancer in compare with healthy individuals, there was no significant deference in the mean of triiodothyronine (T3) among patients with breast cancer in compare with healthy individuals.

**Table (4.1):** the statistical analysis for the variables between the study gropes

<b>Parameter (unit)</b>	<b>Case (mean± SD)</b>	<b>Control (mean± SD)</b>	<b>P vale</b>
<b>TSH (<math>\mu</math>IU/ml)</b>	1.57± 0.87	2.59±0.76	0.00*
<b>T3 (ng/ml)</b>	1.31±0.31	1.29±.43	0.781
<b>T4 (<math>\mu</math>g/dl)</b>	8.70±.81	7.7±1.2	0.00*

- P value less than 0.05 that's considered as statistically significant.
- \*\*Significant.

## Chapter five

### Discussion, conclusion and recommendation

#### 5.1 Discussion:

This study was carried out in Khartoum state, from January 2017 to October 2018 in order to estimate thyroid functions among patients with breast cancer in compare with healthy individuals.

This study revealed that there is an association between breast cancer and thyroid disorders; the incidence of thyroid diseases in breast cancer patients is increased as compared to controls. This is in agreement with the study done by Carolina Ortega-Olvera, *et al* in Mexico, 2018 this is also agreed with Saraiva, *et al* findings in Brazil, 2005. (Saraiva *et al.*, 2005)(Ortega-Olvera *et al.*, 2018)

The results of the current study showed that the mean of thyroid stimulating hormones (TSH) level was significantly decreased in breast cancer patients when compared with healthy individuals. This is agreed with the study done in Brazil by Saraiva, *et al.*, and the study done in Libya by Abdalla Mohammed Jarari, *et al.* (Saraiva *et al.*, 2005)(Mohammed Jarari *et al.*, 2018)

Renija V, *et al* in 2016, and Valizadeh N, *et al* in Iran reported a significant high TSH while the studies conducted by Mohamed Riza, *et al* in Iran and Shi Y, *et al* in China reported insignificant difference in TSH as compared to control group; which are not agreed with our findings. (Renija, Purayil and Purayil., 2016)(Valizadeh., 2015)(Reza, *et al.*, 2017)(Shi *et al.*, 2017)

Regarding thyroxin (T4), the mean of T4 was significantly increased in patients with breast cancer when compared with healthy individuals. This is agreed with many previous studies; including the study conducted by Mohamed Riza, *et al* , the study done by Shi Y, *et al*, the study done by Mahmood Rasool, *et al*, and the study done by Saraiva, *et al* who reported the same result. (Reza, *et al.*, 2017) (Shi *et al.*, 2017)(Mahmood *et al.*, 2014) (Saraiva *et al.*, 2005)

This is not in agreement with Renija, *et al* finding which reported a significant decreased T4, and Abdallah Mohammed, *et al* finding which reported insignificant difference T4 in patients as compared to control groups. This may be due to that T4 is involved in the proliferation of the cells. (Renija, Purayil and Purayil., 2016)(Mohammed Jarari *et al.*, 2018)

Regarding T3, there was no significant difference in the mean of triiodothyronine (T3) among patients with breast cancer in compare with healthy individuals.

In Libya, 2018 Abdalla Mohammed Jarari, *et al* and in Iran, 2015 Valizadeh N, *et al* also found that there is insignificant difference in T3 when compared to control group. (Mohammed Jarari *et al.*, 2018) (Valizadeh., 2015)

While the studies performed by Carolina Ortega-Olvera, *et al*, Mahmood Rasool, *et al*, and Saraiva, *et al* reported significant difference in T3 in patients when compared with control group which are disagreed with our findings; this may be due to the short observation time in the present study population. (Ortega-Olvera *et al.*, 2018) (Mahmood *et al.*, 2014) (Saraiva *et al.*, 2005)

Most of these studies were retrospective and used different diagnostic criteria for detection of thyroid diseases. However, examination of thyroid hormones was always performed after treatment (breast surgery, chemotherapy). This is particularly important since the autoimmune system can be influenced by surgery or chemotherapy, triggering or worsening autoimmune diseases (Ditsch, *et al.*, 2010).

Thyroid dysfunction (especially hypothyroidism) seems to appear during long-term follow-up of breast cancer patients. The cumulative incidence of (subclinical) hypothyroidism was about 50% after 20 years' follow-up (Ditsch, *et al.*, 2010). All this may be influence in the differences in our study results with other previous studies.

## **5.2 Conclusion:**

This study revealed that:

- There is an association between breast cancer and thyroid disorders.
- The incidence of thyroid disease in breast cancer patients is increased when compared to control group.

## **5.3 Recommendations:**

- Thyroid function test should be regularly checked in patients with breast cancer.
- Larger sample size should be tested in wider study area.
- Assessment of thyroid functions before surgery and treatment (chemotherapy, radiotherapy and hormonal therapy).

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