**ABSTRACT**

**BIOCHEMICAL STUDIES OF THYROID HORMONES, ANTI THYROPEROXIDASE, AND SOME PARAMETERS IN BLOOD SERUM OF PATIENTS OF BENIGN AND MALIGNANT BREAST TUMOR PATIENTS**

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This study was conducted at the Oncology Teaching Hospital in Baghdad - Iraq, and the study lasted for 7 months, from March 1, 2021, to September 1, 2021. The study included three groups, each group included 40 patients with benign tumors, 40 patients with malignant tumors, and 40 female control groups. Thyroid disease is a common disease that may lead to other diseases. Breast cancer is also a common and fatal disease, and the causes of the disease must be sought. Thyroid peroxidase (TPO) is required for the thyroid gland's physiological activity. The goal of this study is to see if there's a link between thyroid and lipid problems and benign breast disease and breast cancer. The high incidence of thyroid peroxidase antibodies (TPOAbs) in breast cancer patients, as well as their protective effect, have previously been shown, implying a relationship between breast cancer and thyroid autoimmunity. TPO was recently discovered in breast cancer tissue samples, however, its antigen was not examined. TPO and its antigenic activity may have favorable benefits on TPOAb-positive breast cancer patients by detecting thyroid pathophysiology early. However, further research is needed to establish TPOAbs' positive impact and to better understand the mechanism behind it.

**Keywords:** Thyroid hormones, Antithyroperoxidase, Benign tumors, Malignant tumors.

**1. INTRODUCTION**

Along with lung and colon cancer, breast cancer is the most frequent cancer in women and one of the three most common malignancies globally (Torre *et al.* 2012). With an estimated 12% of women receiving a breast cancer diagnosis and a 3.5% mortality rate, it is a severe public health concern with an increasing frequency. Early detection and screening initiatives, as well as efficient systemic treatments, have decreased mortality in developed nations (Malvezzi *et al.* 2016).

Breast asymmetry, lumps, and discharge are possible symptoms of benign breast tumors, which are illnesses with aberrant cellular development that do not intrude into neighboring tissues. They respond well to conservative or surgical treatment, and the risk of malignant change or recurrence is minimal to nonexistent (Yang *et al.* 2015).

Breast cancer and benign breast illness were linked to endocrine abnormalities because of their well-established relationship with steroid hormones, their hypothesised relationship with thyroid hormone abnormalities, and the fact that they are still a hotly debated topic. Early studies relied on observations of a higher breast cancer occurrence in regions with endemic goiter or on the clinical perception that hypothyroidism was frequently present in breast cancer patients. The primary techniques for assessing thyroid function biochemically in the beginning were the measurement of basal metabolic rate, serum protein bound iodine or 1311 (PBI), or absorption and clearance. Although mild hypothyroidism has been suggested several times, many studies have been unable to conclusively show abnormal thyroid function in breast cancer patients. However, there is conflicting evidence about the incidence of breast cancer in those who use thyroid hormone supplements and the curative and palliative effects of thyroid hormone therapy for the treatment of breast cancer (Kogai *et al.* 2006).

Thyroid hormones are produced in the thyroid gland in response to thyroid stimulating hormone. They are created when active iodine and thyroglobulin molecules combine to form triiodothyronine (T3) or thyroxine (T4), which is thought to be a prohormone for the most active and predominant form (T3). Thyroid hormones affect heat production and have an effect on nearly all body cells. Blood lipid abnormalities are another indicator that is advised since studies have shown that diet may have an impact on the discrepancy in breast cancer incidence among young women from different racial or ethnic groups.

Thus, the Amie of this study is to determine if benign breast disease and breast cancer are related to thyroid and Anti-TPO.

**3. MATERIALS AND METHODS**

**3.1 Materials**

**3.1.1 Design of study**

(120) female cases were selected, their ages ranged between (30-70 years), after obtaining their consent. These cases are divided into three groups, as described in the section below

• 40 cases of malignant tumors (patient group).

• 40 cases of benign tumors (patient group).

• 40 cases are healthy (control group).

**3.1.2 Sample collection**

Based on the reports of a breast oncologist, patients were diagnosed and specimen types identified. After autoclaving, each patient's 5 cm venous blood was collected in vacutainer gel tubes. Blood samples were allowed to coagulate for 15 minutes on a rack at room temperature before being centrifuged for 10 minutes at 3500 rpm. The serum was then isolated from the rest of the cells. The serum was separated into 4 Abendrovites with a tiny pipette and then frozen at 30 °C.

**3.2 Equipment and materials used**

The VIDAS device of French origin was used in this study to measure the levels of thyroid hormones (T3 (REF 3040301), T4 (REF 3040401), TSH (REF 30400, and anti-TPO (ATPO (REF 30461)).

**3.3 Statistical analysis**

Unless otherwise noted, all tests were repeated at least twice. The mean standard deviation (Mean±SD) was used to express all quantitative data. Healthy women's data were compared to samples of diseased women collected from an oncology facility. The SPSS 25 statistical tool was used to analyze the chemistry data. P values of less than 0.05 were used as a statistical significance measure. The Anova one-way test was used to generate P-values and means.

**RESULTS**

 **Biochemical Parameters**

 **Age distribution**

Age levels showed non-significant (P< 0.94) differences between patients with benign tumors and the control group, and age levels showed insignificant differences between patients with malignant tumors and the control group, as shown in Table 4.1

 **Body mass index**

The levels of BMI showed significant (P> 0.03) differences between patients with benign tumors and malignant tumors compared to the control group .

**Table 4.1** The serum Age and body mass index tests in the benign tumor patients group and the malignant tumor group compared with the control group

|  |  |  |
| --- | --- | --- |
| **PARAMETERS** | **MEAN** $\pm $ **SD** | **P-VALUE** |
| **CONTROL****N(40)** | **BENIGN TUMORS****N(40)** | **MALIGNANT TUMORS****N(40)** |
| Age (years) | 49.67 ± 11.35 | 49.70 ± 10.03 | 50.35 ± 10.25 | 0.94 |
| Body mass index (kg/m2) | 25.13 ± 2.55 | 29.35 ± 4.51 | 30.17 ± 6.37 | 0.03 |

 **Triiodothyronine**

Serum T3 level In this study, these results showed a significant increase in patients with non-malignant breast cancer tumor types (P > 0.03) when compared to the control group, as shown in Table 4.2.

 **Thyroxine**

T4 levels in this study showed significantly higher number of patients with benign tumors and malignant breast cancer as compared to the control group (P > 0.04), as shown in Table 4.2.

 **Thyroid-stimulating hormone**

In this study, our findings in TSH levels showed a non-significant elevation in patients with benign and malignant breast tumors when compared to the control group (P > 0.614), as shown in Table 4.2

 **Anti-thyroperoxidase**

In this study ATPO levels were significantly elevated in patients with benign tumors and malignant breast cancer as compared to the control group (P > 0.04), as shown in Table 4.2.

**Table 4.2** The serum thyroid hormones and antithyroid peroxidase tests in the malignant and benign tumor patients group compared with the control group

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| --- | --- | --- |
| **PARAMETERS** | **MEAN** $\pm $ **SD** | **P-VALUE** |
| **CONTROL****N(40)** | **BENIGN TUMORS****N(40)** | **MALIGNANT TUMORS****N(40)** |
| Triiodothyronine (nmol/L) | 1.47 ± 0.46 | 0.78 ± 0.37 | 1.58 ± 0.54 | 0.03 |
| Thyroxine (nmol/L) | 80.38 ± 12.93 | 83.91 ± 11.29 | 100.27 ± 21.26 | 0.04 |
| Thyroid-stimulating hormone (IU/mL) | 1.92 ± 0.94 | 1.91 ± 1.43 | 2.17 ± 1.63 | 0.61 |
| Anti-thyroperoxidase (IU/mL) | 1.09 ± 0.71 | 3.81 ± 7.53 | 102.21 ± 184.14 | 0.01 |

**Discussion**

One of the most recent diseases to spread and affect a lot of people is breast cancer. Many researchers have pushed to look into the connection between the illness and other disorders due to the rapid growth of breast cancer in an effort to stop it from spreading further. In our study, we looked at the relationship between breast cancer and thyroid hormone abnormalities and thyroid peroxidase (TPO), which is important for the physiological function of the thyroid gland. In this investigation, we identified a statistically significant association between breast cancer and BMI, T3, T4, Total Cholesterol, Tg, and LDL. Despite variations in age, TSH, and HDL means as displayed in the tables above, there was no statistically significant difference (Godlewska *et al.* 2017).

BMI 25 was strongly related with greater tumor size in studies by (Biglia *et al.* 2013, Loi *et al.* 2005). According to the researchers, obese premenopausal women also exhibited higher tumor vascular invasion and metastatic axillary nodes. The patients' BMI was shown to be significantly higher than that of the control group. Women with breast cancer in the highest quartile of BMI (25.847 kg/m2) were 2.5 times more likely than women in the lowest quartile of BMI (20.639 kg/m2) to die of their disease in the five years after diagnosis. Each quartile of BMI was associated with a substantial increase in the likelihood of dying (P 0.05) (Daling *et al.*, 2001).

Advanced malignancies have been shown to have lower TPO levels. It was discovered that TPO proteins were expressed in all tissue samples, both benign and malignant. The antigenicity of immunodominant regions (IDRs) in breast TPO is comparable to that of thyroid TPO, according to this data, which is crucial for effective interaction with human TPOAbs. TPO is a significant autoimmune agonist involved in autoimmune thyroid illness and helps make hormones. TPO levels have been discovered to be dramatically changed in autoimmune carcinomas, and it is also known the circumstances in which this enzyme's physiologically normal activity plays a pathogenic function (Ruf *et al.* 2006).

 In the study of Saraiva *et al.* the levels of thyroid function hormones were investigated in women with breast cancer pre- and post-menopause. The researchers discovered that postmenopausal women with breast cancer had much lower TSH levels than the control group. Additionally, compared to the study's control group, postmenopausal women with breast cancer had significantly greater levels of free T3 and free T4. However, they have found a conflicting case involving premenopausal women in which the level of TSH was considerably higher in breast cancer patients compared to controls(Saraiva *et al.* 2005).

 Ditsch *et al.* have reported significant higher levels of T3 and T4 in women with breast cancer compared to the control of their study. Moreover, they have observed lower but non-significant level of TSH in breast cancer patients (Ditsch *et al.* 2010).

 In most recent study, Bach *et al.* have reported a significant link between the increased risk of breast cancer with hyperthyroidism or hypothyroidism. Moreover, they have concluded that the link does not include TSH, but only thyroid hormones (T3 and T4) (Bach *et al.* 2020).

Turken *et al.* have reported high prevalence of thyroid disorders in women with breast cancer (Turken *et al.* 2003). Increased thyroid hormones may be a risk factor for breast cancer, according to several studies that show a considerable incidence of subclinical or overt hyperthyroidism in women with the disease (Yang *et al.* 2020, Cordel *et al.* 2018, Baldini *et al.* 2022). Hall *et al.* have reported a minor effect of T3 hormone (compared to estrogen) on promoting the proliferation of MCF-7 cells. Additionally, they have demonstrated that a high amount of T3 can strengthen the contribution of E2 to the pathogenesis of breast cancer (Hall *et al.* 2008). In another study, Meirovitz *et al.* have concluded that high levels of T3 in women with breast cancer associate with the progression of the disease and poor diagnosis (Meirovitz *et al.* 2022).

 The two hormones of thyroid gland, T3 and T4, have a receptor on the cell membrane that transport non-genomic signals called αβv3. This receptor transmits the signal through the kinases PI3 and ERK, which causes the cells to proliferate (Liu *et al.* 2019). Therefore, targeting the receptor αβv3 on the breast cancer cells is the proposed mechanism by which hyperthyroidism might promote a rise in the proliferation of breast cancer tumors(Voutsadakis *et al.* 2022).

Several studies looked at thyroid function (fT3 and fT4 levels) in breast cancer patients. In preclinical experiments, T3 was able to keep a number of cell types, including breast cancer cells, from proliferating without the need for serum. In contrast to these findings, Kuijpens *et al.* 2005 found that breast cancer patients (n=2775) had considerably lower levels of fT4 than those without breast cancer (Dinda *et al.* 2002 ; Kuijpens *et al.* 2005)

Therapeutically adjusted hypothyroidism was shown to be more common in breast cancer patients than in Tis patients, women with benign breast tumors, and healthy controls in a study by (Ditsch *et al.* 2010).

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