ISSN 2348-0319 International Journal of Innovative and Applied Research [2022]
(Volume 10, Issue 06)
06-12

Journal home page: http://www.journalijiar.com

INTERNATIONAL JOURNAL
OF INNOVATIVE AND APPLIED RESEARCH

RESEARCH ARTICLE

ASSOCIATION OF THYROID HORMONES AND ESTROGEN IN PATIENTS WITH BREAST CANCER

Constance N. Nwadike[1], Oluchi Aloy-Amadi[1], Kingsley U. Mbionwu[1] and Emmanuel Ifeanyi Obeagu[2]

1. Department of Medical Laboratory Science,Imo State University, Owerri, Nigeria.
2. Department of Medical Laboratory Science, Kampala International University, Uganda.

Manuscript Info

Manuscript History
Received: 10 April 2022
Final Accepted: 15 June 2022
Published: June 2022

Keywords:
T3, T4, TSH, Estrogen, Breast Cancer, Women

Abstract

Some epidemiological studies have reported a relationship between thyroid dysfunctions and the risk of breast cancer's giving the similarities between estrogen and thyroid function. This study was therefore aimed at evaluating the level of thyroid gland hormones, TSH and estrogen in breast cancer patients. A cross-sectional study was carried out in the month of September to November 2019. A total of 60 subjects attending breast cancer screening clinic of Federal Medical Center and Imo State University, Owerri were recruited for this study. The 60 subjects are between the ages of 20-60 years. The 60 subjects were divided into two groups: Group 1 (Test) consists of 30 females with breast cancer. Group 2 (Control) consists of 30 apparently healthy women with no breast cancer. Blood samples were collected, and the level of thyroid hormone and estrogen was determined. Data generated was then analysed using SPSS version 21. There was no significant difference (p=0.308 and p=0.626 respectively) in the mean value of T_3 and T_4 in breast cancer patients (3.42±0.07 and 1.41±0.15 respectively)µIU/ml when compared to Controls (3.11±0.64 and 1.35±0.23)µIU/ml. The mean value of TSH was significantly increased (p=0.02) in breast cancer patients (2.79±1.05)Pmol/ml when compared to controls (1.40±0.17)Pmol/ml. The mean value of estrogen was significantly increased (p=0.000) in breast cancer patients (540.39±66.70)pg/ml when compared to controls (222.10±58.09)pg/ml. There was a non significant negative correlation of serum estrogen with serum T_3, and T_4, (r= -0.253, p=0.682 and r=-0.107, p=0.864). There was a non significant positive correlation of serum estrogen with serum TSH (r=0.380, p=0.528). There is a strong association between breast cancer and serum concentrations of thyroid stimulating hormone and estrogen, but there is no alteration in TSH hormones in breast cancer.

Corresponding Author:- **Constance N. Nwadike**

Introduction:-

Breast cancer is a cancer that develops from breast tissue. Signs of breast cancer may include a lump in the breast, a change in breast shape, dimpling of the skin, fluid coming from the nipple, or a red scaly patch of skin, (National Cancer Institute, 2014). It is one of the most common neoplasm in women and is a leading cause of cancer-related deaths worldwide (Rajneesh et al., 2018; Obeagu et al., 2021; Obeagu et al., 2017; Obeagu and Obeagu, 2016;; Obeagu et al.,2021). Breast cancer primarily affects women with occasional incidence in men and female to male ratio of breast cancer prevalence is reported to be 100:1 (Jemal et al., 2009). It is the most common malignancy in

women and accounts for 18.4% of all cancers in female patients (WHO, 2013).Risk factors for developing breast cancer include being female, obesity, lack of physical exercise, drinking alcohol, hormone replacement therapy during menopause, ionizing radiation, early age at first menstruation, having children late or not at all, older age, and family history. About 5–10% of cases are due to genes inheritedfrom a person's parents, including BRCA1 and BRCA2 among others (Wernberg et al., 2017). Breast cancer most commonly develops in cells from the lining of milk ducts and the lobules that supply the ducts with milk. Cancers developing from the ducts are known as ductal carcinomas, while those developing from lobules are known as lobular carcinomas. In addition, there are more than 18 other sub-types of breast cancer. Some cancers, such as ductal carcinoma in situ, develop from pre-invasive lesions. The diagnosis of breast cancer is confirmed by taking a biopsy of the concerning lump. Once the diagnosis is made, further tests are done to determine if the cancer has spread beyond the breast and which treatments it may respond to, (National Cancer Institute, 2014).

Most types of breast cancer are easy to diagnose by microscopic analysis of a sample—or biopsy—of the affected area of the breast. Also, there are types of breast cancer that require specialized lab exams. The two most commonly used screening methods, physical examination of the breasts by a healthcare provider and mammography, can offer an approximate likelihood that a lump is cancer, and may also detect some other lesions, such as a simple cyst, (Saslow et al., 2014). When these examinations are inconclusive, a healthcare provider can remove a sample of the fluid in the lump for microscopic analysis (a procedure known as fine needle aspiration, or fine needle aspiration and cytology—FNAC) to help establish the diagnosis. The needle aspiration may be performed in a healthcare provider's office or clinic using local anaesthetic if required. A finding of clear fluid makes the lump highly unlikely to be cancerous, but bloody fluid may be sent off for inspection under a microscope for cancerous cells. Together, physical examination of the breasts, mammography, and FNAC can be used to diagnose breast cancer with a good degree of accuracy. Other options for biopsy include a core biopsy or vacuum-assisted breast biopsy, which are procedures in which a section of the breast lump is removed; or an excisional biopsy, in which the entire lump is removed. Very often the results of physical examination by a healthcare provider, mammography, and additional tests that may be performed in special circumstances (such as imaging by ultrasound or MRI) are sufficient to warrant excisional biopsy as the definitive diagnostic and primary treatment method, (Yu et al., 2010). The management of breast cancer depends on various factors, including the stage of the cancer and the age of the patient. Increasingly aggressive treatments are employed in accordance with the poorer the patient's prognosis and the higher the risk of recurrence of the cancer following treatment. Breast cancer is usually treated with surgery, which may be followed by chemotherapy or radiation therapy, or both. A multidisciplinary approach is preferable. Hormone receptor-positive cancers are often treated with hormone-blocking therapy over courses of several years. Monoclonal antibodies, or other immune-modulating treatments, may be administered in certain cases of metastatic and other advanced stages of breast cancer, (Saini et al., 2011).

The conventional prognostic markers are: dimension and histopathology type of the tumour, number of afflicted lymph nodes, grading, hormone receptor status and Her/2-neu-status. A high percentage of positivity of antibodies to thyroid peroxidase has been proven in women with breast cancer in comparison to healthy controls (Reinehr, 2013). Some studies have indicated thyroid autoimmune changes as prognostic factors in breast cancer.

The association between thyroid hormones and the risk of breast cancer (BC) has been reported in epidemiological studies (Tosovic et al., 2012). A positive association has been reported between thyroxine (T4) and risk of breast cancer, which is more pronounced in overweight and obese women. Negative associations have been reported between triiodothyronine (T3) and breast among premenopausal women; in contrast, positive associations have been observed among postmenopausal women (Tosovic et al., 2012). It has been shown that in vitro studies, thyroid hormones affect the growth of Breast Cancer-derived cell lines, lung cancer, and glioblastoma (Lin et al., 2009). T4 has been shown to increase cell proliferation through the αvβ3 integrin receptor found on the plasma membrane of cells (Tosovic et al., 2010). In contrast, the effect of T3 on cell proliferation has not been well-established because it differs by the type of cell line used (Hall et al., 2008). These effects are important, since abnormalities in thyroid function tests have been observed in a variety of non-thyroidal illnesses, without preexisting thyroid or hypothalamic-pituitary disease. Furthermore, these abnormalities might change with body mass index (BMI) because thyroid hormones are involved in the regulation of various metabolic pathways (e.g., adaptive thermogenesis and glucose metabolism) that are relevant for resting energy expenditure and changes in body weight (Reinehr, 2013).Recent studies showed increased serum levels of thyroid-stimulating hormone (TSH), with subclinical or manifest hypothyroidism in 10.0-19.7% of breast cancer patients (Davis et al., 2016). As early as the early 19th century, thyroid hormones were utilised 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.

Estrogen is the primary female sex hormone as well as a medication. It is responsible for the development and regulation of the female reproductive system and secondary sex characteristics. Oestrogen may also refer to any substance, natural or synthetic, that mimics the effects of the natural hormone (Trichopoulos et al., 2012). The oestrane steroid estradiol is the most potent and prevalent endogenous oestrogen, although several metabolites of estradiol also have estrogenic hormonal activity. Estrogens are used as medications as part of some oral contraceptives, in hormone replacement therapy for postmenopausal, hypogonadal, and transgender women, and in the treatment of certain hormone-sensitive cancers like prostate cancer and breast cancer**.** Estrogens play a pivotal role in breast cancer development and sustained tumor growth. Observational studies conducted before the era of estrogen replacement therapy revealed that early loss of ovarian function reduced breast cancer risk by 60% to 70% (Trichopoulos et al., 2012).In premenopausal women, the main estrogen source for breast tissue is circulating E_2, which is secreted by the ovaries. At menopause, ovarian production of estrogen ceases. In postmenopausal women, circulating E_1, which is produced in different tissues (e.g., skin, soft tissue, muscle, and liver) by aromatization of androstenedione taken up from the circulation, is the main unconjugated plasma estrogen (Russo and Russo, 2014). However, due to conflicting evidence, the relative contribution from circulating versus locally synthesized estrogens to intratumor E_2 levels in postmenopausal women has been controversial for 2 decades.

Due to the paucity of information of hormonal level in breast cancer patients, this study is aimed at evaluating the association of thyroid hormone, thyroid stimulating hormone and estrogen level in breast cancer patients.

Materials and Methods:-

Subjects:

A cross-sectional study was carried out in the month of September to November 2019. A total of 60 subjects attending breast cancer screening clinic of Federal Medical Center and Imo State University, Owerri were recruited for this study. The 60 subjects between the ages of 20-60 years were divided into two groups of 30 females with breast cancer which served as test subjects and 30 apparently healthy women with normal functioning breast which served as control subjects.

Inclusion

The participants include those that met the enrollment criteria. The criteria were as follows: Subjects who were diagnosed of breast cancer, Breast cancer patients within the age 20 years to 60 years and breast cancer subjects who gave us their consent

Sample collection and preparation

Five Milliliters (5mls) of venous blood samples were collected aseptically by venipuncture from each of the subjects using Five Milliliters (5mls) sterile disposable syringe and needle. The whole blood samples were dispensed into a pre labeled plain dry specimen container and allowed to clot. The clotted samples were centrifuged at 3000 rpm for 5 minutes to separate and obtain the serum. The serum was extracted using a pipette and was dispensed into another container and stored at 20^0c prior to use.

Laboratory procedures

Serum Oestrogen (estradiol) Estimation (Russo and Russo, 2014)

This was determined using the bixactestradiol EIA test kit, catalog number ED102.

Principle

The estradiol E2 enzyme immuno-assay test is based on the principle of competitive binding between E2 in the test specimen and E2-horse radish peroxidase conjugate for a constant amount of rabbit anti-estradiol. In the incubation, goat anti-rabbit IgG-coated wells are incubated with 25ul E2 standards, controls, patient samples,100ul estradiol-HRP conjugate reagent and 50ul rabbit anti-estradiol reagent at room temperature (18-25^0c) for 90 minutes. During the incubation, a fixed amount of HRP-labeled E2 competes with the endogenous E2 in the standard, sample and quality control serum for a fixed number of binding sites of the specific E2 antibody. Thus, the amount of E2 peroxidase conjugates immunologically bound to the well progressively decreases as the concentration of E2 in the same specimen increases. Unbound E2 peroxidase conjugate is then removed and the wells washed. Next, a solution of TMB reagent is added and incubated at room temperature for 20 minutes, resulting in the development of blue

color. The color development is stopped with the addition of 1N HCl and the absorbance is measured spectrophotometrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled E2 in the sample.

Calculations
The Mean absorbance value (A_{450}) for each duplicate set of standard, control and patient sample was calculated.

A standard curve was constructed by plotting the mean absorbance obtained for each standard against its concentration in pg/ml on a linear-linear graph paper, with absorbance values on the vertical Y-axis and concentrations on the horizontal X-axis. The mean absorbance values for each specimen was used to determine the corresponding concentration of oestrogen (oestradiol) in pico gram per milliliter (pg/ml).

Reference Range;
<30pg/ml in males

Serum Thyroid Stimulating Hormone Estimation (Davis et al., 2016)
This was determined using the Abnova ELISA kit, catalog number SA120135 based on the modified enzyme immunoassay technique.

Principle:
The Thyroid Stimulating Hormone (TSH) ELISA is used for the quantitative measurement of TSH in human serum. It is a solid phase sandwich ELISA method. The samples, and anti-TSH-HRP/Biotin conjugate are added to the wells coated with Streptavidin. TSH in the sample forms a sandwich between two specific antibodies to TSH. Unbound protein and HRP conjugate are washed off. Upon the addition of the substrate, the intensity of color is proportional to the concentration of TSH in the samples. A standard curve is prepared relating color intensity to the concentration of the TSH.

Calculations
The Mean absorbance value (A_{450}) for each duplicate set of standard, control and patient sample was calculated. A standard curve was constructed by plotting the mean absorbance obtained for each standard against its concentration in pg/ml on a linear-linear graph paper, with absorbance values on the vertical Y-axis and concentrations on the horizontal X-axis. The mean absorbance values for each specimen were used to determine the corresponding concentration of thyroid hormones.

Reference Range
Adults 0.4–4.2
Serum $T_{3\ and}$ T_4 Hormone Estimation (Larson, 1972)
Principle:
The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in standards, controls and patient samples) and an enzyme-labeled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed is inversely proportional to the concentration of T3 in the sample. A set of standards is used to plot a standard curve from which the amount of T3 in patient samples and controls can be directly read.

Calculations:
Conc. of T3 and T4= Absorbance of test X Concentration of Std
Absorbance of standard

Statistical Analysis
The results of the present study were expressed as mean ± standard deviation. The student t-test was calculated using SPSS version sixteen (16) and it was used to compare the parameters (at levels of significance 0.05). $P<0.05$ was considered as statistically significant and $P>0.05$ was considered not statistically significant and results presented in tables.

Results:-

Table 1:- Mean Value of T_3, T_4, TSH and Estrogen in Breast Cancer Patient (Test) Vs Apparently Healthy Subjects (Control).

Parameter	Test	Control	p-value
T_3 (µIU/ml)	3.42±0.07	3.11±0.64	0.308
T_4 (µIU/ml)	1.41±0.15	1.35±0.23	0.626
TSH (Pmol/ml)	2.79±1.05	1.40±0.17	0.02
Estrogen (pg/ml)	540.39±66.70	222.10±58.09	0.000

Table 1 shows that there was no significant difference (p=0.308 and p=0.626 respectively) in the mean value of T_3, and T_4 in breast cancer patients (3.42±0.07 and 1.41±0.15 respectively)µIU/ml when compared to Controls (3.11±0.64 and 1.35±0.23)µIU/ml.

The mean value of TSH was significantly increased (p=0.02) in breast cancer patients (2.79±1.05)Pmol/ml when compared to controls (1.40±0.17)Pmol/ml.

The mean value of estrogen was significantly increased (p=0.000) in breast cancer patients (540.39±66.70)pg/ml when compared to controls (222.10±58.09)pg/ml.

Table 2:- Correlation of Serum Estrogen with T_3, T_4, and TSH in Patients with Breast Cancer.

Dependent Variable	N	R	p-value
T_3	20	-0.253	0.682
T_4	20	-0.107	0.864
TSH	20	0.380	0.528

There was a non significant negative correlation of serum estrogen with serum **T_3**,and **T_4**, (r= -0.253, p=0.682 and r=-0.107, p=0.864).

There was a non significant positive correlation of serum estrogen with serum TSH (r=0.380, p=0.528).

Discussion:-

Breast cancer is the most frequent malignant tumour in women worldwide with about 1 million women being affected (Tosovic et al., 2012). In Nigeria, almost one in ten women is diagnosed with breast cancer (WHO, 2013).

In this study, there was no significant difference (p>0.05) in the mean value of T_3 in breast cancer patients when compared to Controls. The result of this study is in consistent with previously published studies. For example, in studies of breast cancer cell lines that were transfected with estrogen, T3 inhibited cell proliferation (Cestariet al., 2009). Also, Tosovic et al., (2012) reported a non-statistically significant association between serum T3 and breast cancer, independently of menopausal status.

The present study revealed that there was no significant difference (p>0.05) in the mean value of T_4 in breast cancer patients when compared to Controls. Although there was an increase in thyroxine level in breast cancer, but the increase was not significant. The result of this study contradicts with the findings of previous studies. In contrast to these findings, Kuijpens et al. (2015) showed significantly lower levels of T4 in breast cancer patients compared to women without breast cancer. Tang et al., (2014) observed that T4 hormone promoted breast cancer cell proliferation through the stimulation of the mitogen-activated protein kinase (MAPK) pathway by estrogen receptor and such it level is significantly elevated in breast cancer. In several study models, it has been observed that at physiological concentrations, T4 is more active than T3 at stimulating breast cancer proliferation (Li et al., 2017). Thus, the higher levels of T4 in breast cancer cases may be due to the effect of breast cancer on thyroid function rather than T4 acting as risk factors for breast cancer.

The mean value of TSH was significantly increased (p<0.05) in breast cancer patients when compared to controls. Thyroid dysfunction (especially hypothyroidism) seems to appear during long-term follow-up of breast cancer patients. The mechanism behind the increased level of TSH in breast cancer might be as a result of the low level of thyroid hormones which by negative feedback mechanism is increased. Previous studies has it that T3 and T4 is heavily utilized in breast cancer proliferation and is therefore reduced, due to negative feedback mechanism, the

body senses the low level of thyroid hormone and send signals to the pituitary gland which then releases thyroid stimulating hormone, hence the reason behind the increased level of TSH in breast cancer. Jiskra et al., (2013) showed increased TSH serum levels and hypothyroidism in almost 20% of breast cancer patients. Hence the increased TSH observed in breast cancer patients may be an effect of breast cancer on the thyroid function rather an effect of the hormone on the disease development. Kuijpens et al., (2015) did not demonstrate differences in TSH values between breast cancer patients (at the time of diagnosis) and controls.

The mean value of estrogen was significantly increased ($p<0.05$) in breast cancer patients when compared to controls. Estrogens play a pivotal role in breast cancer development and sustained tumor growth, however, the mechanism behind this phenomenon remains obscure. Several explanations have been proposed, including active uptake from the circulation, local estrogen synthesis, and enhanced tissue binding all results to increase in estrogen level in breast cancer patients. The result of this study is in agreement with the study carried out by (Russo and Russo, 2014).

There was a non significant negative correlation of serum estrogen with serum $T_{3,}$ and $T_{4,}$ and a non significant positive correlation of serum estrogen with serum TSH. There are no studies backing this claim, the non significant difference clearly indicates that there is no association between estrogen, thyroid hormone and thyroid stimulating hormone.

Conclusion:-

There is a strong association between breast cancer and serum concentrations of thyroid stimulating hormone and estrogen.The increase in the individual thyroid hormones though not statistically significant suggests a possible link between hyperthyroidism and breast cancer.

Recommendation:-

Further studies with large sample size are needed to specify the role of thyroid hormones in breast cancer patients and to highlight possible influences on cancer outgrowth and progression.

References:-

1. Cestari, S.H., Figueiredo, N.B., Conde, S.J., Clara, S., Katayama, M.L. and Padovani, C.R. (2009). Influence of estradiol and triiodothyronine on breast cancer cell lines proliferation and expression of estrogen and thyroid hormone receptors. Journal Endocrinology and Metabolism. 53:859–864.
2. Davis, P.J., Goglia, F. and Leonard, J.L. (2016). Nongenomic actions of thyroid hormone. National Review on Endocrinology. 12:111–121.
3. Hall, L.C., Salazar, E.P., Kane, S.R. and Liu, N. (2008). Effects of thyroid hormones on human breast cancer cell proliferation. Journal of Steroid Biochemical and Molecular Biology. 9:57–66.
4. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. (2009). Cancer statistics.CA Cancer. Journal of Clinical Chemistry. 59:225-249.
5. Jiskra, J., Limanova, Z., Barkmanova, J. and Friedmanova, Z. (2013). Prevalence of autoimmune thyroid diseases in women with breast cancer in comparison with colorectal cancer. Journal of Oncology. 16: 149-153.
6. Kaiser, J. (2013). Cancer. Cholesterol forges link between obesity and breast cancer". Science, 342 (6162): 1028-1030.
7. Kuijpens, J., Nyklictek, I., Louwman, M., Weetman, T., Pop, V. and Coebergh, J. (2015). Hypothyroidism might be related to breast cancer in post-menopausal women. Thyroid. 12: 1253- 1259.
8. Larson, P.R. (1972). Thyroid Hormone: Review of Recent Studies of its Physiology and Pathophysiology in Man. Metabolism. 21:1073-1092.
9. Li, H., Sun, X., Miller, E., Wang, Q. and Tao, P. (2017). BMI, reproductive factors, and breast cancer molecular subtypes: a case-control study and metaanalysis. Journal of Epidemiology. 27:143–151.
10. Lin, H.Y., Sun, M., Tang, H.Y., Lin, C., Luidens, M.K. and Mousa, S.A. (2009). L-thyroxine vs. 3,5,3′-triiodo-L-thyronine and cell proliferation: activation of mitogenactivated protein kinase and phosphatidylinositol 3-kinase. AmericaJournal of Physiological Cell Physiology. 296: 980–981
11. National Cancer Institute. (2014). Breast Cancer Treatment (PDQ) .N. Archived from the original on 5 July 2014.

12. Obeagu, E. I., Adepoju, O. J., Okafor, C. J., Obeagu, G. U., Ibekwe, A. M., Okpala, P. U., & Agu, C. C. (2021). Assessment of Haematological Changes in Pregnant Women of Ido, Ondo State, Nigeria. J Res Med Dent Sci, 9(4), 145-148.
13. Obeagu, E. I., Azuonwu, O., Didia, B. C., & Obeagu, G. U. (2017). Evaluation of Impact of Graves Disease on some Selected Haematological Markers among Subjects in Enugu, Nigeria. Open Access Blood Research & Transfusion Journal, 1(4), 74-78.
14. Obeagu, E. I., & Obeagu, G. U. (2016). A review on haematological profile in menstruating, premenopausal and menopausal women. International Journal of Advanced Research in Biological Sciences, 3(11), 92-108.
15. Obeagu, E. I., Babar, Q., Vincent, C. C. N., Udenze, C. L., Eze, R., Okafor, C. J., ... & Amaeze, F. N. (2021). Therapeutic targets in breast cancer signaling: A review. Journal of Pharmaceutical Research International, 33(56A), 82-99.
16. Rajneesh, C..P., Manimaran, A., Sasikala, K.R. and Adaikappan, P. (2018). Lipid peroxidation and antioxidant status in patients with breast cancer. Singapore Medical Journal. 49: 640-643.
17. Reinehr, T. (2013). Obesity and thyroid function. Molecular and CellEndocrinology. 316:165–171.
18. Russo, J. and Russo, I.H. (2014). Genotoxicity of steroidal estrogens. Trends Endocrinology and Metabolism. 15:211–214.
19. Saini, K., Taylor, C., Ramirez, A., Palmieri, C., Gunnarsson, U., Schmoll, H., Dolci, S., Ghenne, C., Metzger-Filho, O., Skrzypski, M., Paesmans, M., Ameye, L., Piccart-Gebhart, M. and de Azambuja, E. (2011). Role of the multidisciplinary team in breast cancer management: results from a large international survey involving 39 countries. Annals of Oncology. 23 (4): 853–859.
20. Saslow, D., Hannan, J., Osuch, J., Alciati, M., Baines, C., Barton, M., Bobo, J., Coleman, C., Dolan, M., Gaumer, G., Kopans, D., Kutner, S., Lane, D., Lawson, H., Meissner, H., Moorman, C., Pennypacker, H., Pierce, P., Sciandra, E., Smith, R. and Coates, R. (2014). "Clinical breast examination: practical recommendations for optimizing performance and reporting. CA:A Cancer Journal for Clinicians. 54(6): 327–344.
21. Tang, H.Y., Lin, H.Y., Zhang, S., Davis, F.B. and Davis, P.J. (2014). Thyroid hormone causes mitogen-activated protein kinase-dependent phosphorylation of the nuclear estrogen receptor. Endocrinology. 145:3265–272.
22. Tosovic, A., Becker, C., Bondeson, A.G., Bondeson, L., Ericsson, U.B. (2012). Prospectively measured thyroid hormones and thyroid peroxidase antibodies in relation to breast cancer risk. International Journal of Cancer. 31:2126–2233.
23. Tosovic, A., Bondeson, A.G., Bondeson, L., Ericsson, U.B., Malm, J. and Manjer, J. (2010). Prospectively measured triiodothyronine levels are positively associated with breast cancer risk in postmenopausal women. Breast Cancer Research. 12: 33-35.
24. Trichopoulos, D., MacMahon, B. and Cole, P. (2012). Menopause and breast cancer risk. Journal of National Cancer Institute. 48:605–613.
25. Wernberg, J.A, Yap, J., Murekeyisoni, C., Mashtare, T. and Wilding, G.E. (2017). Multiple primary tumors in men with breast cancer review. Journal of Surgical Oncology. 99:16-19.
26. WHO, (2013). World Health Organization Classification of Tumours. Pathology and Genetics of Tumours ofthe Breast and Female Genital Organs. In: Tavassoli FA, Devilee P (editions.). Lyon: pp. 9-112.
27. Yu, Y., Liang, C. and Yuan, X. (2010). Diagnostic value of vacuum-assisted breast biopsy for breast carcinoma: a meta-analysis and systematic review. Breast cancer research and treatment. 120(2): 469–479.