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RESEARCH ARTICLE

ARE THYROID AND SEX HORMONE RATIOS PREDICTIVE OF BREAST CANCER RISK? A PRELIMINARY STUDY AMONG A COHORT OF SRI LANKAN BREAST CANCER PATIENTS

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ABSTRACT

Purpose: The association of thyroid related diseases and sex hormones with breast cancer (BC) is reported with inconclusive results. The study was designed to analyse the thyroid/sex hormone ratios of BC patients.
Material and Methods: TSH, T3, T4, estrogen, progesterone and testosterone concentrations of newly diagnosed breast cancer patients (n=155) aged 30 to 75 years and age-matched normal controls (n=75) were analyzed. Thyroid: sex hormone ratios were calculated. Data on history of thyroid related diseases were collected.
Results: History of thyroid related diseases was significantly higher ($p<0.05$) in breast cancer patients compared to controls. Among the remaining, subclinical hyperthyroidism was found in 14%, but only 7% in healthy women. Significantly higher ($p<0.05$) mean T3 and T4 values and lower TSH levels were observed in patients with breast cancer when compared to healthy. Serum testosterone was significantly low among BC patients. Considering the thyroid to sex hormones ratios among postmenopausal women, T3/testosterone, T4/testosterone, T3/estrogen, T4/ estrogen, ratios were significantly different compared to healthy and the highest significance was found with T3/testosterone. Cutoff values studied from receiver operative characteristic curves indicated that a woman having T3/testosterone above 7.47 showed 12.5 times odds ($p=0.000$) of being diagnosed with BC.
Conclusion: The present study concludes that the incidence of thyroid related diseases is higher among Sri Lankan BC patients and elevation of T3/testosterone ratio is indicative of BC.

Keywords: breast cancer, sex hormones, thyroid hormones, thyroid/sex hormone ratios

INTRODUCTION

The impact of hyper and hypothyroidism on breast cancer (BC) is researched with inconclusive results. Some studies disclose profound effects of hyperthyroidism on BC cell proliferation (1). Some portray association between hypothyroidism and BC (2). The thyroid disease incidence is higher among BC patients when compared to apparently healthy individuals. Significantly high mean T3 and T4 and low TSH values in postmenopausal BC patients when

compared to controls implicate an association of hyperthyroidism and BC (3). Free T3 and T4 concentrations were higher in BC patients when compared to controls and benign breast tumors [4]. A dose-response positive association of T3 with the risk of BC exists with no such association between TSH and BC in postmenopausal women (5). In addition, T3 levels positively associate with invasive BC (6). In contrast, hypothyroidism and low-normal T4 are related with an increased risk of BC in post-menopausal women (2). In contrast to both above observations, some studies report unaltered thyroid profiles in BC women (7). Thyroid disorders such as hypothyroidism, hyperthyroidism or autoimmune thyroiditis did not have a higher incidence in BC patients or patients with benign breast tumors [4]. A negative correlation between TSH and T3 is seen in early BC but not in advanced BC (8). Thus, the exact impact of thyroid hormones in BC development and progression is not recognized (5).

Substantial changes in the expression of thyroid hormone receptors suggest a possible deregulation that could trigger BC development (9). Estrogen like effects of thyroid hormones are suspected to be impacting BC development (10). T3 is believed to promote BC cell proliferation and increase the effect of estrogen on cell proliferation in some BC cell lines indicating the role of T3 in BC development and progression (11). Similarly postmenopausal BC patients have significantly increased thyroid hormone/ estrogen ratios suggesting a possible tumor growth promoting effect due to the misbalance of the hormones (3). However, data on distribution of thyroid to sex hormones of BC patients is not reported.

Thus, this study was designed to analyze the incidence of thyroid related diseases and to analyze the thyroid profiles (TSH, T3 and T4), sex hormones of BC patients and compare with apparently healthy females. Attempts will be made to assess any significant associations with thyroid hormone / sex hormone levels in developing BC among Sri Lankan BC patients.

MATERIAL AND METHODS

Study Sample

The research is a cross-sectional study. Newly diagnosed female BC patients (n=155) who have not had any treatment for breast cancer (surgery, chemotherapy, radiotherapy) were identified from Apeksha Hospital (National Cancer Institute, Maharagama). Age matched apparently healthy females (n=75) were selected for the comparative study. Informed written consent was obtained from all participants before engaging in the study. Data on history of thyroid related diseases, menopausal status, hormonal contraceptive usage and hormone replacement therapies for any clinical condition were collected using an interviewer administered questionnaire.

Thyroid Profile

Venous blood samples from patients who have not undergone treatment for cancer or who have not had hormonal contraceptives/ any hormonal treatments six months before the diagnosis of carcinoma were collected. Blood samples of apparently healthy age matched females those who were not on any hormonal treatment were collected. Thyroid profile (T3, T4 and TSH) was analyzed using an enzyme immunoassay method with final fluorescent detection using MINI VIDAS analyzer (Biomerieux, France) using the separated serum.

Thyroid Stimulating Hormone (TSH)

Serum (200 µL) was introduced to sample well in the strip containing alkaline phosphatase-labeled monoclonal anti-TSH immunoglobulins [mouse], wash buffer [tris, NaCl, tween and sodium azide and substrate [4-methyl-umbelliferyl-phosphate, diethanolamine and sodium azide] and SPR coated with monoclonal anti-TSH immunoglobulins (mouse) were used for the detection.

Free Triiodothyronine (FT3) and Free Tetraiodothyronine (FT4)

Serum (100 µL) was introduced to the sample well. The reagent wells contained alkaline phosphatase labeled T3 derivative or T4 derivative and similar components as for TSH. The SPR had anti-T3 antibodies [rabbit] or anti T4 antibodies [rabbit]. The conjugate enzyme catalyse hydrolysis of substrate in to 4-methyl-umbelliferone of which the fluorescence was measured at 450 nm.

Sex Hormones and Ratios of Hormones

Serum estrogen, progesterone and testosterone levels of the same study sample were measured using MINI VIDAS immune analyzer (Biomerix, France) and thyroid/sex hormone ratios were calculated (12).

Table 1. Thyroid profile of breast cancer patients and apparently healthy females

Test		BC (n= 139) Mean± SD	AHW (n=75) Mean± SD	Reference ranges[1]
TSH (mIU/L)	Premenopausal	2.39 ± 1.87[a]	3.31 ± 1.98[a]	0.4-4.5
	Postmenopausal	2.34 ± 2.30[a]	3.03 ± 2.65[a]	
	All	2.38 ± 1.88[a]	3.19 ± 2.65[a]	
FT3 (pg/mL)	Premenopausal	2.64 ± 0.43[b]	2.47 ± 0.47[c]	2.08-6.74
	Postmenopausal	2.59 ± 0.41[b]	2.32 ± 0.43[c]	
	All	2.61 ± 0.41[b]	2.35 ± 0.33[c]	
FT4 (ng/dL)	Premenopausal	1.18 ± 0.30[d]	1.00 ± 0.37[f]	0.8-2.3
	Postmenopausal	1.13 ± 0.28[d]	0.97 ± 0.41[f]	
	All	1.16 ± 0.25[d]	0.99 ± 0.25[f]	

BC: Patients with breast cancer, AHW: apparently healthy women. Different superscripts in each row indicate significant differences ($p<0.05$) among hormones at each phase among breast cancer and apparently healthy females; TSH- serum thyroid stimulating hormone 3rd generation; FT3- serum free triiodothyronine; FT4- serum free tetraiodothyronine; 1Manual on Standard operation procedure, sample collection and reference range for clinical chemistry, World Health Organization, Ministry of Health and the Department of Biochemistry, Medical Research Institute, Sri Lanka

Statistical Analysis

Statistical data analysis was carried out using SPSS version 16.0 (2007, SPSS for Windows, SPSS Inc., Chicago, IL, USA) package. The quantitative data with skewed distribution were presented as median (Inter quartile range). The qualitative data were expressed by calculating the frequency and percentage. P value of less than 0.05 ($p<0.05$) was considered significant. Non-parametric significances were analysed by Mann-whitney U test. Correlations of parametric and non-parametric data were analysed by Pearson and Spearman test respectively. Receiver operative characteristic (ROC) curve was plotted for determination of cut off values of some selected biochemical parameters.

Ethical Approval and Informed Consent

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Ethical clearance for the study was obtained from Ethics Review Committee, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka (Date: 07.11.2012, Number: 651/12/02; Date: 02.08.2014, Number: 28/14). The approval for registering patients and accessing histopathology data were obtained from the Director of National Cancer Institute, Maharagama, Sri Lanka. Informed written consent was obtained prior to enrolling participants.

RESULTS

Incidence of Thyroid-Related Diseases

Among breast cancer patient's majority were (63%) postmenopausal women with an average age of 63±7 years at the diagnosis of the carcinoma. Among the patients 10% (n=16) reported a history of thyroid related diseases and 6 were on medication for different thyroid related disorders. BC patients with known thyroid dysfunctions and the patients who were on hormonal contraceptives within the past six months before the diagnosis of carcinoma were excluded from the study. Serum TSH, T3 and T4 levels of remaining patients and of apparently healthy age matched females were analyzed (Table 1). Subclinical hypothyroidism was observed to be 14%

Table 2. Thyroid hormone/sex hormone ratios of postmenopausal breast cancer and apparently healthy women

Ratio	BC (n=97) Mean ± SD	AHW (n=45) Mean ± SD
T3/ Estrogen	0.20 ± 0.11[a]	0.15 ± 0.05[b]
T4/ Estrogen	0.08 ± 0.04[c]	0.06 ± 0.02[d]
T3/ Testosterone	25.52 ± 48.93[e]	5.14 ± 3.31[f]
T4/Testosterone	6.97 ± 4.38[g]	1.92 ± 1.00[h]
T3/Progesterone	11.38 ± 5.23[i]	10.03 ± 6.73[j]
T4/Progesterone	4.22 ± 2.11[k]	3.82 ± 2.22[k]

BC: Patients with breast cancer, AHW: apparently healthy women. Different superscripts in a row indicate significant differences ($p<0.005$) in hormones among breast cancer and apparently healthy women.

among the remaining BC patients, and only 7% of females categorized as apparently healthy had subclinical hypothyroidism. When compared with apparently healthy females a woman with thyroid disorders had a relative risk of 1.3 (CI 1.04-1.13) of having BC.

Thyroid Profile

The mean serum TSH of apparently healthy individuals was not significantly different when compared with women with BC even though serum TSH of BC patients was noticeably lower ($p>0.05$). Serum TSH was also not significantly different according to the menopausal status. However, serum T3 and T4 concentrations of BC patients were significantly higher ($p<0.05$) when compared with healthy females irrespective of the menopausal status.

Sex Hormone Concentrations of BC Patients

Among the study sample 37% of BC patients (n=57) were premenopausal. Serum estrogen and progesterone concentrations at each phase among premenopausal BC patients were not significantly different ($p> 0.05$) when compared with age matched controls. Serum testosterone concentrations of premenopausal BC patients were significantly lower ($p=0.001$) than apparently healthy females (12). However, since the number of premenopausal BC patients in each menstrual phase is comparatively less, and the hormone concentrations significantly varied according to each phase, the comparative statistical analysis was conducted with the sex hormone levels of postmenopausal BC patients and apparently healthy age matched postmenopausal women.

Serum estrogen and progesterone concentrations of postmenopausal BC patients were not significantly different to that of apparently healthy women. However, serum estrogen of these BC patients was noticeably lower. When compared with premenopausal women, postmenopausal women had significantly lower ($p=0.000$) serum estrogen and progesterone concentrations. Median (Inter quartile range) testosterone concentrations of postmenopausal BC and healthy women were 0.16(0.18) ng/mL and 0.21 (0.22) ng/mL respectively. Serum testosterone concentrations of BC patients were significantly low ($p=0.001$) irrespective of menopausal status when compared with healthy women (12).

Thyroid Hormones to/Sex Hormone Ratios

Considering the thyroid profile of the studied BC patients, even though the mean concentrations of thyroid hormones were within the normal reference range, significantly elevated levels of T3 and T4 concentrations were observed among BC patients when compared to apparently healthy. Among the studied sex hormones, serum testosterone was significantly low ($p<0.05$) and a considerable difference in the estrogen concentrations was observed though not significant ($p>0.05$). Thus, to study the possible risk associations with respect to thyroid and sex hormones, the ratio of thyroid hormones to sex hormones were studied and compared with apparently healthy women (Table 2).

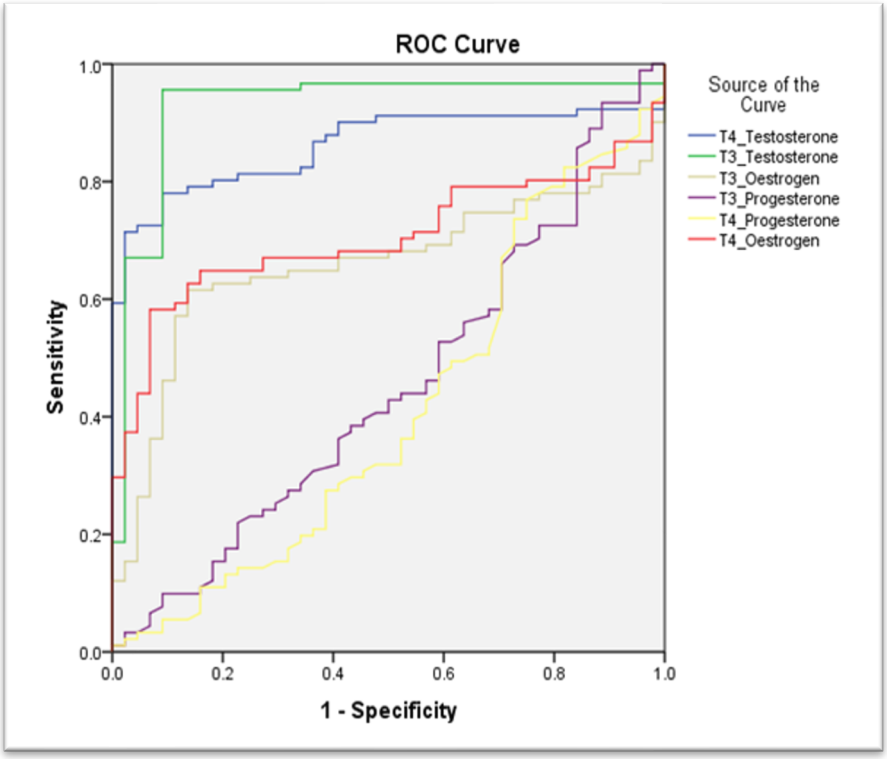

Figure 1. Receiver Operative Characteristic (ROC) Curves to predict the cutoff values

Significant differences in T3/estrogen, T4/ estrogen were found among the two groups and T3/testosterone and T4/ testosterone ratios indicated a high significance (p=0.000). Thus, ROC curves were used (Figure 1) to identify a possible predictor of BC risk and to find a cutoff value with a higher sensitivity and specificity. According to the figure 01, among all studied thyroid/sex hormone ratios, T3/ testosterone ratio showed the highest sensitivity and specificity with highest area under the curve being 93% indicating the possibility of using it as a predictor of risk compared to other studied parameters in BC diagnosis. According to the ROC curve the cutoff value was calculated as 7.47. Thus T3/ testosterone value above 7.47 was identified as a predictive indicator of identifying BC risk. T3/ progesterone or T4/ progesterone ratios were not significant in the study sample.

DISCUSSION

The effect of the changes in the daily lives of female thyroid and sex hormones have been implicated in mammary tumorigenesis and development. Effects of estrogen represent an increase in biological activities and therefore, in conjunction with T3 can act directly on mammary tissue by promoting differentiation (13). Due to these multiple hormonal interactions as well as the ubiquitous role that thyroid hormones play in the body's overall metabolism, the role that thyroid hormones may play in establishing and maintaining BC is exactly not known. Studies have established a direct action of thyroid hormones on the development of the normal mammary gland. But whether an alteration in thyroid status affects mammary tumor risk as well as development and growth are not entirely clear and needs to be studied further.

Among the BC patients in the present study sample, a considerable number of BC patients (n=16) reported a history of thyroid related diseases and among the remaining BC patients, the incidence of subclinical hypothyroidism was twice as high as among apparently healthy individuals. Studies reveal increased risk of BC in women with hyperthyroidism (14). Indicating an association between level of thyroid function and BC risk and the present study confirms the same for the first time in Sri Lanka.

Among the BC patients even though serum TSH levels were noticeably lower, serum T3 and T4 levels were significantly elevated indicating a possible impact of these on tumor development or progression. Cell line studies reveal that T3 can promote BC cell proliferation and increase the effect

of estrogen on cell proliferation. Thus, T3 may play a role in BC development and progression (11).

Circulating estrogens and androgens are found to be positively associated with the risk for BC in premenopausal women (15). However, previous findings indicate non-significant difference in serum estrogen and progesterone levels in BC patients and significant low levels of testosterone (12). The higher bioavailability of testosterone counteracts the proliferative effects of estrogen on mammary tissue and thereby exert a protective role to the breast, inhibiting cancer development and/or tumour growth (16) which might be a considerable stakeholder in the study group. Also, majority (75%) of the BC patients in the present study were either obese or overweight (17) and thus the impact of adiposity related secretions of androgens on BC cannot be undermined (18). A study reveals a synergistic response between T3 and high carbohydrate meals (19) whereas the BC patients in the present study were not regularly consuming balanced meals but were on frequent carbohydrate rich meals (unpublished observations). Thus the diet, the sedentary lifestyle and being either overweight or obese might have contributed to the present observations.

Lipid-soluble hormones in the blood are bound to hormone-specific transport proteins, while a smaller portion is bound to serum albumin. Testosterone-estrogen-binding globulin (SHBG) is a sex hormone-binding globulin that binds to testosterone and estradiol in the blood. Other known steroid-binding globulins are transcortin, primarily associated with progesterone and thyroxine-binding globulin (TBG), for transporting T4. Increased estrogen concentrations increase TBG concentrations. The rise in TBG is paralleled by a T4 increase to maintain a physiological concentration of free T4. Besides the effects on TBG concentrations, sex hormones also affect deiodinase activity which might together contribute to BC development [20].

In vitro studies reveal direct stimulatory effects of T3 on basal production of testosterone and estradiol (21) and according to the present study T3/testosterone above 7.47 indicated the highest risk. In other words, while elevated T3 contribute to BC cell proliferation, lower testosterone concentrations might have reduced the anti-proliferative and pro-apoptotic effect of testosterone on BC. Thus, the present study identifies that T3/ testosterone ratio can predict BC with higher odds when compared with other studied thyroid hormone/sex hormone ratios in identifying BC risk. The imbalance of thyroid hormones causes the dysfunction of the reproductive system (22) which might also impact on the concentrations of sex hormones.

Interestingly testosterone sometimes functions via conversion to estradiol [23] and lower testosterone in females might impact on obesity and poor glucose control. Considering the HbA1c levels 20% of BC patients showed values above 7% after excluding 13% of BC patients who were already on glycemic control drugs at the time of enrolment to the study. However further studies are needed to confirm the exact impact of lower serum testosterone and elevated T3 on developing BC as research on molecular mechanisms involving androgenic pathways in BC is still in their infancy.

CONCLUSION

Thyroid related diseases are significantly higher among BC patients and BC patients showed significantly elevated serum T3 and T4 levels than controls indicating the possible impact of hyperthyroidism in BC. Considering the thyroid hormone/sex hormone levels, significantly increased serum T3/ estrogen, T3/testosterone ratios among postmenopausal BC women implies the impact on hormone imbalance on BC development. Considering the Thyroid hormone/sex hormone ratios, serum T3/testosterone above 7 was identified as a potent marker in identifying BC risk among the study sample.

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Author contributions: H.M.K.A conducted the study and drafted the manuscript; S.E. supervised the study and reviewed the manuscript; K.S. supervised the study.

Conflict of interests: Authors declare no conflict of interests.

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