Thyroid and Sex Hormones Ratios in Predicting Breast Cancer Risk

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Abstract

Purpose: The study intended to analyze the incidence of thyroid related diseases and to assess thyroid to sex hormones ratios of breast cancer (BC) patients

Methods: TSH, T_3, T_4, Estrogen, progesterone and testosterone levels of newly diagnosed BC patients (n=155) aged 30 to 75 years and age-matched normal controls (n=75) were analyzed. Data on history of thyroid related diseases were collected from an interviewer administered questionnaire. Thyroid/sex hormone ratios were analyzed and compared against healthy women.

Results: History of thyroid related diseases was significantly higher (p<0.05) in BC patients compared to controls. Patients (10%) with history of thyroid related diseases were excluded from the study. Subclinical hyperthyroidism was identified among 14% of the remaining BC patients and was the only dysfunction (7%) among healthy women. Significantly higher (p<0.05) mean T_3 and T_4 values, lower TSH levels and non-significant levels of estrogen and progesterone were observed in patients with BC when compared to healthy. Serum Testosterone of BC patients were significantly low (p<0.05). Considering the thyroid to sex hormones ratios among postmenopausal women, T_3/testosterone, T_4/testosterone, T_3/estrogen, T_4/estrogen, ratios were significantly different in the two groups and the highest significance was found with T_3/testosterone. Cutoff values studied from receiver operative characteristic curves indicated that a woman having T_3/testosterone above 7.47 showed 12.5 times odds (p=0.000) of being diagnosed with BC.

Conclusion*:* Incidence of thyroid related diseases are higher among BC patients and elevation of T_3/testosterone ratio indicated a significant risk of BC. However, a study involving a larger number of participants could confirm the above.

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 T_3 and T_4 and low TSH values in postmenopausal BC patients when compared to controls implicate an association of hyperthyroidism and BC [3]. Free T_3 and T_4 concentrations were higher in BC patients when compared to controls and benign breast tumors [4]. A dose-response positive association of T_3 with the risk of BC exists no such association between TSH and BC in postmenopausal women [5]. In addition, T_3 levels positively associate with invasive BC [6]. In contrast, hypothyroidism and low-normal T_4 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 T_3 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 is suspected to be impacting BC development [10]. T_3 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 T_3 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.

Previous study conducted on the same study sample reported significant low levels of serum testosterone concentrations among BC patients irrespective of the menopausal status. However, serum estrogen or progesterone concentrations of post-menopausal BC patients were not significantly different when compared with postmenopausal healthy women [12].

Thus this study was designed to analyze the incidence of thyroid related diseases and to analyze the thyroid profiles (TSH, T_3 and T_4) 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.

Methodology

Study sample

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.

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) [12] and thyroid/sex hormone ratios were calculated.

Statistical Analyses

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 to be 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 (652/12, 13/48). The approval for registering patients and accessing histopathology data were obtained from the Director, National Cancer Institute, Maharagama, Sri Lanka. Informed written consent was obtained prior to enrolling participants.

Results

Incidence of Thyroid related diseases

Among breast cancer patients 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 past history of thyroid related diseases and 6 were on medication for different thyroid related disorders. BC patients with known thyroid dysfunctions before diagnosis of BC were excluded from the study. Serum TSH, T_3 and T_4 levels of remaining patients and of apparently healthy age matched females were analyzed (Table 1). Subclinical hypothyroidism was observed to be 14% 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.

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]	
FT_3 (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]	
FT_4 (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; TSH3- serum thyroid stimulating hormone 3rd generation; FT_3- serum free triiodothyronine; FT_4- serum free tetraiodothyronine; [1]Manual 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.

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 were 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 T_3 and T_4 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 differences in the estrogen concentrations were was observed though not significant ($p > 0.05$). Thus in order 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).

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

Ratio	BC (n = 97) Mean ± SD	AHW (n = 45)
T_3/ Estrogen	0.20 ± 0.11^a	0.15 ± 0.05^b
T_4/ Estrogen	0.08 ± 0.04^c	0.06 ± 0.02^d
T_3/ Testosterone	25.52 ± 48.93^e	5.14 ± 3.31^f
T_4/Testosterone	6.97 ± 4.38^g	1.92 ± 1.00^h
T_3/Progesterone	11.38 ± 5.23^i	10.03 ± 6.73^j
T_4/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.

Significant differences in T_3/estrogen, T_4/ estrogen were found among the two groups and T_3/testosterone and T_4/ testosterone ratios indicated a high significance ($p = 0.000$). Thus ROC curves were used (Fig. 1) to identify a possible predictor of BC risk and to find a cutoff value with a higher sensitivity and specificity. According to the Fig. 01, among all studied thyroid/sex hormone ratios, T_3/ 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 T_3/ testosterone

value above 7.47 was identified as a predictive indicator of identifying BC risk. T_3/ progesterone or T_4/ progesterone ratios were not significant in the study sample.

Discussion

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 T_3 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 is 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 past 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 T_3 and T_4 levels were significantly elevated indicating a possible impact of these on tumor development or progression. Cell line studies reveal that T_3 can promote BC cell proliferation and increase the effect of estrogen on cell proliferation. Thus T_3 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 counteract 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 T_3 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 T_4. Increased estrogen concentrations increase TBG concentrations. The rise in TBG is paralleled by a T_4 increase to maintain a physiological concentration of free T_4. Besides the effects on TBG concentrations, sex hormones also affect deiodinase activity which might together contribute in BC development [20].

In vitro studies reveal direct stimulatory effects of T_3 on basal production of testosterone and estradiol [21] and according to the present study T_3/testosterone above 7.47 indicated the highest risk. In other words, while elevated T_3 contribute in 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 T_3/ testosterone ratio can predict BC with higher odds when compared with other studied thyroid hormones/ 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 T_3 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 T_3 and T_4 levels than controls indicating the possible impact of hyperthyroidism in BC. Considering the thyroid hormones/sex hormone levels significantly increased serum T_3/ estrogen, T_3/testosterone ratios among postmenopausal BC women implies the impact on hormone imbalance on BC development. Considering the Thyroid hormones/ sex hormone ratios, serum T_3/testosterone above 7 was identified as a potent marker in identifying BC risk among the study sample.

Declarations

Conflict of Interest Statement

Authors have not conflicts of interest to declare.

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Figures

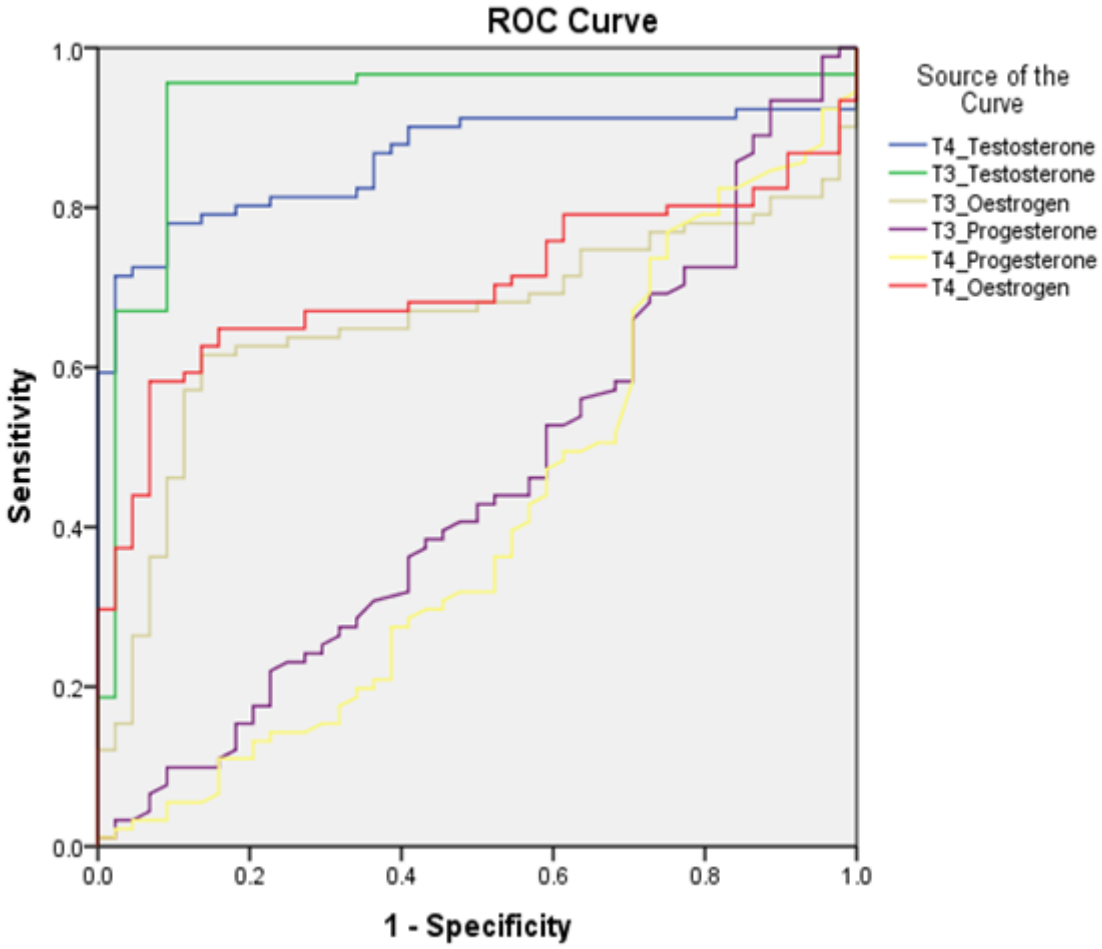

Figure 1

ROC curves to predict the cutoff values