RESEARCH ARTICLE

Alteration of Thyroid Function in Indian HER 2-Negative Breast Cancer Patients Undergoing Chemotherapy

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Abstract

Background: Thyroid hormones (TH) are regulated by the hypothalamic-pituitary axis, which plays an important role in cell growth, differentiation, development and other aspects of metabolism. It is believed that an active hypothalamic-pituitary axis increases the susceptibility of thyroid dysfunction during systemic chemotherapy. In order to investigate the relation between thyroid function and chemotherapy the present study was designed to investigate TH in breast cancer patients receiving at least three cycles of chemotherapy. The levels of TH were measured at the baseline and before each cycle of chemotherapy. **Materials and Methods:** Blood samples for estimation of TH levels were collected from 80 (pre-menopausal-40; post-menopausal-40) breast cancer patients just before they were undergoing - 1st, 2nd, 3rd and 4th cycle of chemotherapy. The serum was separated and T_3, T_4 and TSH levels were determined by chemiluminescence method. **Results:** T_3 and T_4 were found significantly decreased and TSH was found significantly increased after 1st ($p<0.001$), 2nd ($p<0.0001$) and 3rd cycle of chemotherapy ($p<0.0001$). The variation of T_3 levels (decreased) and TSH levels (increased) was found more in post-menopausal ($p<0.0001$) women then in pre-menopausal women after 3rd cycle of chemotherapy as compared to baseline ($p<0.001$). **Conclusions:** TH were remarkably altered after each cycle of chemotherapy leading to decline in thyroid function of breast cancer patients. Further, the results also indicated that post-menopausal women were more prone towards decline in thyroid function then pre-menopausal women. The present study proposes the monitoring of TH after each cycle of chemotherapy in breast cancer patients.

Keywords: Thyroid hormone - chemotherapy - breast cancer - subclinical hypothyroidism - menopause

Introduction

Breast cancer is the most common cancer in the urban areas of developing countries. In the last few years due to increase in life expectancy, urbanization and western lifestyles in urban areas of developing countries led to increase incidence of breast cancer in low and middle income countries (Babu et al., 2013; Varughese et al., 2015). Even though advances in the field of cancer therapeutics, chemotherapy remains the mainstay therapeutic modality. Recent progress in chemotherapy has enabled to improve management of various cancers. However, these cytotoxic therapies are generally associated with some immediate or otherwise delayed side effects. Researchers have thoroughly studied the adverse effects of anticancer therapy on breast, gastrointestinal, hepatic, renal and hematological systems. The effect of chemotherapy on endocrine system, however, is comparatively less envisaged (Chapmen et al., 1992; Meistrich et al., 1997; Yeung et al., 1998). Numerous epidemiological studies have shown relationship between plasma thyroid hormones (TH) -triiodothyronine (T_3) and the prohormone thyroxine (T_4) -levels and breast cancer risk, which supports the concept that TH promote tumor growth (Hellevik et al., 2009; Tosvoic et al., 2012-2013; De Groot et al., 2015). TH are regulated by hypothalamic-pituitary axis, which have been found to play a role in cell growth, differentiation, development and other aspects of metabolism. It is believed that active hypothalamic-pituitary axis increases the susceptibility of thyroid dysfunction owing to systemic chemotherapy (Huang et al., 2013). Systemic anticancer treatments include cytotoxic drugs, hormones, immunomodulators and targeted drugs that selectively modulate critical molecules in tumor progression or activate immune response to cancer. These agents might variably disturb thyroid function with impairment leading to modified total but do not affect the free concentration of TH to manifest thyroid disease. A few studies have prospectively evaluated thyroid dysfunction associated with cytotoxic agents (Massarat et al., 1992; Yeung et al., 1998; Hamnvik et al., 2011; Torino et al., 2013). For example, lomustine, vincristine, and cisplatin have shown in vitro effects on thyroid cell lines. Similarly, L-asparaginase had been shown to cause transient central hypothyroidism in cancer patients (Heideman et al., 1981; Torino et al., 2013).

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However, 5-fluorouracil and L-asparaginase modified the levels of thyroid hormone-binding proteins without any clinical significance in cancer patients (Beex et al., 1977; Garnick et al., 1979; Ferster et al., 1992; Mamby et al., 1995; Surks et al., 1995; Dong et al., 2000; Torino et al., 2012).

Thyroid dysfunction is emerging as a variably common endocrine toxicity of several anticancer drugs. A small number of studies assessed the effects produced by poly-chemotherapy on thyroid function in cancer patients (Torino et al., 2012). Due to this scarcity of data on the functioning of thyroid gland with respect to different cycles of chemotherapy, the present study was aimed to investigate effects of different regimen of chemotherapy on thyroid functions in breast cancer patients undergoing at least three cycles of chemotherapy.

Materials and Methods

Patients

The sample consisted of 80 newly diagnosed breast cancer patients undergoing chemotherapy. All patients were HER2-negative cases of breast cancer, diagnosed with solid tumors, treated with chemotherapy. It was a longitudinal prospective cohort study. Patients were enrolled by the informed consent process. Patients were excluded for the following reasons: patient undergone radiotherapy, any kind of previously diagnosed thyroid disease, recent elevation of serum creatinine or chronic kidney failure to values greater than normally expected for that particular age, abnormal hepatic function; patient having autoimmune disorder; use of iodine contrasts for a 6-month period before and during the study; and patients having brain tumor. All patients were between 18 and 55 years of age. The patients were receiving CAF (cyclophosphamide, adriamycin and 5-fluorouracil), CMF (cyclophosphamide, methotrexate and 5-fluorouracil) and CEF (cyclophosphamide, epirubicin and 5-fluorouracil) regimens during the study. The study was conducted in agreement with the Declaration of Helsinki and approved by Institutional Review Board (IRB) of Rajiv Gandhi Cancer Institute and Research Centre, Rohini, New Delhi, India.

Sample collection

3 ml blood samples were taken from each enrolled subjects at four time points. First sample was collected one day before the start of chemotherapy (baseline) and then other samples were collected before the start of 2nd, 3rd and 4th chemotherapy cycle. Blood samples were collected in plain vials (without anticoagulant) and vials were kept in ice for one hour in standing position. Samples were then centrifuged at 4,000 rpm for 15 minutes to separate serum. Supernatant serum was separated using pipettes and processed to analyze T3, T4 and TSH levels.

Method

T_3, T_4 and TSH were determined using chemiluminescence methods (CLIA). Following reference ranges were used; T_3 60-181 ng/dl, T_4 3.20-12.6 μg/dl and TSH 0.35-5.50 μIU/ml

Percentage change (%)

A percentage change is a method to communicate a change in a variable. It represents the relative change between the old value and the new one. In the present study variables were T_3, T_4 and TSH concentrations calculated at four time points longitudinally. Initial mean value was the mean values of T_3, T_4 and TSH at pre-chemotherapy and final mean values were taken at 3rd cycle of chemotherapy in this study.

% change = Initial value-final value x 100/Initial value

Statistical analysis

The study variables contain both categorical and continuous variables. Frequencies with proportions were represented the categorical variables and scale variables as mean ± SD. All scale parameters were tested for normality using the Kolmogorov-Smirnov test. Data of various thyroid function variables over time for Breast cancer was analyzed using repeated measures analysis of variance (ANOVA) by adjusting age and other parameters. The Bonferroni correction was applied for within group comparisons. The two-sided critical region with $p \leq 0.05$ was considered as statistical significance. IBM SPSS 22.0 statistical software (IBM Corp., Armonk, NY, USA) was used for all statistical analyses in the present study.

Results

Patient demographics

80 newly diagnosed breast cancer patients were enrolled in the study. The median was 43 years (range, 18-55). 53 patients were found estrogen receptor-positive (ER+) or progesterone receptor-positive (PR+) and 27 patients were estrogen receptor-negative (ER-) or progesterone receptor-negative (PR-). 40 patients were pre-menopausal and 40 patients were post-menopausal women. Patients were at different clinical stages of breast cancer before chemotherapy. 14, 27, 27 and 12 patients were at clinical stage I, II, III, IV at the time of enrollment, respectively. FEC, CMF and FAC were used

Table 1. Patient Demographics

Breast cancer patients (n=80)	
Median Age (range), years	43 (18-55)
Hormone receptor status	
ER+ and or PR+	53 (66%)
ER- and PR-	27 (34%)
Treatment	
FEC	27 (34%)
CMF	27 (34%)
FAC	26 (32%)
Menopause	
Pre	40 (50%)
Post	40 (50%)
Stage	
I	14 (17%)
II	27 (34%)
III	27 (34%)
IV	12 (15%)

CEF Cyclophosphamide Epirubicin 5-Fluorouracil; CMF Cyclophosphamide; Methotrexate 5-Fluorouracil; FAC 5-Fluorouracil Adriamycin Cyclophosphamide

as a chemotherapy regimen in 27, 27 and 26 patients respectively (Table No. 1).

Effect of different cycles of chemotherapy on T_3 levels

T_3 levels after the 1st cycle of chemotherapy were found to be significantly decreased as compared to the baseline (prechemotherapy) (p<0.001). After 1st cycle changes in T_3 levels were evident and keep on changing till 3rd cycle of chemotherapy. The levels of T_3 were further decreased when the patients underwent 2nd cycle (p<0.0001) and 3rd cycle of chemotherapy as compared to baseline (p<0.0001) (as shown in Figure 1). Differences in mean T_3 levels after the 1st cycle compared with 2nd cycle and 3rd cycle were found statistically significant (P<0.001). T3 levels were also found to be decreased from 2nd cycle to 3rd cycle (p<0.0001) (Table No. 2). Percentage change of T_3 from pre-chemotherapy to 3rd cycle of chemotherapy was found to be 28.50% decreased (Table No. 2).

Effect of different cycles of chemotherapy on T_4 levels

T_4 levels after the 1st cycle of chemotherapy were found to be significantly decreased as compared to the baseline (p<0.001). After the 1st cycle changes in T_4 levels were evident and keep on changing till 3rd cycle of chemotherapy (as shown in figure 1). The levels of T4 were further decreased when the patients underwent 2nd cycle (p<0.0001) and 3rd cycle of chemotherapy as compared to baseline (p<0.0001). Differences in mean T_4 levels after 1st cycle compared with 2nd cycle (p<0.01) and 3rd cycle were found to be significantly decreased (P<0.0001). T_4 levels were also found to be decreased from 2nd cycle to 3rd cycle (p<0.0001). Percentage change of T_4 from pre-chemotherapy to 3rd cycle of chemotherapy was found to be 34.40% decreased (Table No. 2).

Effect of different cycles of chemotherapy on TSH levels

TSH levels after the 1st cycle of chemotherapy were found to be significantly increased as compared to the baseline (p<0.05). After 1st cycle changes in TSH levels were evident and keep on changing till 3rd cycle of chemotherapy (as shown in figure 1). The levels of TSH were further increased when the patients underwent 2nd cycle (p<0.0001) and 3rd cycle of chemotherapy as compared to baseline (p<0.0001). Differences in mean TSH levels after the 1st cycle compared with 2nd cycle (p<0.005) and 3rd cycle were found statistically significant (P<0.0001). TSH levels were found to be significantly increased from the 2nd cycle to 3rd cycle (p<0.0001). Percentage change of TSH from pre-chemotherapy to 3rd cycle of chemotherapy was found to be 94% increased (Table No. 2).

Effects of different cycle of chemotherapy on T3, T4 and TSH levels in post-menopausal women as compared to pre-menopausal women

Differences in mean T_3 and T_4 levels significantly decreased in both post-menopausal and pre-menopausal patients from baseline to 3rd cycle of chemotherapy. T_3 levels were found more decreased in post-menopausal women compared to pre-menopausal women with respect to chemotherapy treatment. T_3 levels were found to be more significantly decreased after the 3rd cycle in post-menopausal women (p<0.0001) than pre-menopausal women when compared with baseline (p<0.001) (as shown in Figure 2). Percentage change of T3 in post-menopausal women was higher 38.1% than pre-menopausal women 18.1% (Table 3).

Changes in T_4 levels for both pre & post-menopausal women were almost same (as shown in Figure 2). Percentage change of T_4 in post-menopausal women was almost same 35.6% than pre-menopausal women 36.2% (Table 3).

TSH levels were found more increased in post-menopausal women compared to pre-menopausal women

Table 2. Effect of different cycles of chemotherapy on T3, T4 and TSH levels (n=80)

Thyroid Hormone	Baseline Mean±SD	1st Cycle Mean±SD	2nd Cycle Mean±SD	3rd Cycle Mean±SD	% change
T3 (ng/dl)	137.6±22.0	122.0±22.0[a]	114.2±19.3[b,1]	98.3±18.8[b,1,2]	28.50%
T4 (μg/dl)	8.93±1.6	7.97±1.5[a]	6.84±1.6[b,3]	5.85±1.4[b,1,2]	34.40%
TSH(μIU/ml)	1.84±0.7	2.25±0.7[c]	2.76±0.8[b,4]	3.57±0.8[b,1,2]	-94.00%

*P<0.05 is statistically significant; Different letters show statistical significance; Minus sign indicate increased percentage change; [a]p<0.001 *vs* Baseline, [b]p<0.0001 *vs* Baseline; [c]p<0.01 *vs* Baseline, 1p<0.0001 *vs* 1st cycle, 2p<0.0001 vs 2nd cycle; 3p<0.01 vs 1st cycle, 4p<0.005 *vs* 1st cycle,

Table 3. Effects of Different Cycle of Chemotherapy on T3, T4 and TSH Levels in Post-menopausal women (n=40) as Compared to Pre-menopausal women (n=40)

Menopause	Baseline Mean±SD	1st Cycle Mean±SD	2nd Cycle Mean±SD	3rd Cycle Mean±SD	change%
			T3 (ng/dl)		
Pre	137.4±22.2	132.7±20.3	127.9±18.5	112.5±16.6a	18.10%
Post	137.6±22.6	110.9±23.9	100.1±19.9	85.1±14.4b	38.10%
			T4 (μg/dl)		
Pre	8.83±1.60	7.61±1.18	6.80±1.27	5.63±1.25[a]	36.20%
Post	9.14±1.56	8.11±1.71	6.85±1.92	5.88±1.63[a]	35.60%
			TSH (μIU/ml)		
Pre	1.85±0.75	2.01±0.70	2.49±0.82	2.78±0.73[a]	-50.20%
Post	1.72±0.59	2.41±0.79	2.92±0.71	4.23±0.83[b]	-145.90%

Minus sign indicate increased percentage change; [a]p<0.001 *vs* baseline, [b]p<0.0001 *vs* baseline

with respect to chemotherapy. TSH levels were found to be more significantly increased after the 3rd cycle in post-menopausal women (p<0.0001) than pre-menopausal women when compared to baseline (p<0.001) (Table No. 3) (as shown in Figure 2). Percentage change of T_3 in post-menopausal women was higher -145.90 % than pre-menopausal women -50.20% (Table 3).

Discussion

The present study shows that thyroid function attenuates during treatment in HER2-negative breast cancer patients treated with chemotherapy. T_3 and T_4 levels were found to be decreased and TSH levels were found to be increased after 2nd cycle and 3rd cycle of chemotherapy. Chemotherapy is the most effective treatment modality for cancers. Thyroid gland disorders are usually associated with cancer and chemotherapy (Yeung et al., 1998). These disorders cover a broad variety of pathophysiological mechanisms which have been hypothesized as i) alteration due to involvement of active hypothalamic pituitary axis (Huang et al., 2013); ii) altered synthesis or clearance of thyroid hormone-binding proteins observed in certain cancers, or caused by cancer treatment that modifies total but not free concentration of TH; iii) alteration of TH metabolism, more commonly known as euthyroid sick syndrome which may occur in chronically ill cancer patients (Yeung et al., 1998).

Clinical studies on cancer patients indicated that various combination of chemotherapy blunt thyroid function (De Groot et al., 2015). A combination regimen of etoposide, bleomycin, vinblastine, cisplatin and dactinomycin given in testicular cancer patients has been found to induce primary hypothyroidism in 15% of patients (Stuart et al., 1990; Torino et al., 2013). Sutcliffe et al (1981) found that patients with Hodgkin disease receiving mechlorethamine, procarbazine, vinblastine, and prednisolone (MOPP regimen) had increased serum TSH levels in 44% of cases (Sutcliffe et al., 1981; Torino et al., 2013). Regimens which were used in the present study included drugs 5-fluorouracil (5-FU), cyclophophamide, methotrexate and adriamycin. T_3 and T_4 levels decreased after 3rd cycle of chemotherapy may be due to the common drug 5-fluorouracil in every regimen. It can be hypothesized that thyroid dysfunction caused by fluoropyrimidines (5-fluorouracil) may possibly be due to alteration in TH metabolism. This effect may be ascribable to the structural resemblance between 5-fluorouracil and propylthiouracil. Propylthiouracil is a thioamide drug commonly used in the treatment of hyperthyroidism which acts by inhibiting the thyroperoxidase activity that releases iodine for addition onto tyrosine residues on thyroglobulin for the production of T_4 or T_3, as well as TH and also block the conversion of T_4 to the active form T_3 by inhibiting the enzyme 5'-deiodinase (Fujiwara et al., 2013).

The decrease of T_3, T_4 concentrations and increase of TSH concentrations observed in the present study indicated that the possible damage to the thyroid gland could be due to chemotherapy. In observance with this inference, throughout the long term follow-up of breast cancer survivors have high cumulative prevalence of overt hypothyroidism (Khan et al., 2011; Groot et al., 2015). The increase of TSH observed in the study could be explained with respect to recovery of ''*non thyroidal illness*'' (NTI), an adaptive response to (chemotherapy-induced) cellular damage. It has been documented that in seriously ill patients, down-regulation of the hypothalamus-pituitary-thyroid axis due to an adaptation to adverse physical conditions (Warner et al., 2010; Groot et al., 2015). Similarly, in another study on breast cancer patients treated with FEC or TEC, NTI-like plasma markers were observed one to three days after chemotherapy administration (Huang et al.,2013; Groot et al., 2015). It was hypothesized that NTI could be a primary adaptive response to chemotherapy-induced cellular damage. The similar results were also been reported recently where thyroid functions were found altered after 6th cycle of neoadjuvant chemotherapy in breast cancer patients (NEOZOTAC trial) (Groot et al., 2015).

In the present study, in post-menopausal women T_3 levels were found decreased and TSH levels were found increased more than pre-menopausal women after the 3rd cycle of chemotherapy. However, there was not much difference in T_4 levels. The clinical reports suggest that the diminished thyroid function is more common among women of advancing age (Hollowell et al., 2002; Aoki et al., 2007; LeGrys et al., 2013). A cohort study conducted for 20 years found that 7.5% of adult women were diagnosed with subclinical hypothyroidism and the risk of developing hypothyroidism in women increased with age, reaching a value of 13.7/1000 per year between 75 and 80 years of age (Ghianda et al., 2014). Diagnosis of overt hypothyroidism is based on decreased T3, T4 levels and increased TSH. The levels of T3 and T4 were within the population reference range in these individuals, but elevated TSH indicates that T_3 and T_4 concentration is not normal for them (Andersen et al., 2002; LeGrys et al., 2013). The mechanism can be explained by the fact that the levels of serum TBG changed immediately before and quickly after menopause; this could be due to increased levels of TBG and the lack of estrogen during the ageing process. Ageing related changes in thyroid physiology included: decline in thyroid iodine uptake, synthesis of free thyroxine (FT_4) and free triiodothyronine (FT_3) and catabolism of FT_4 while reverse triiodothyronine (rT_3) increases; the level of TSH remains normal, sometimes with a propensity to higher limits (DelGhianda et al., 2014).

In conclusion, TH levels were found to be altered in breast cancer patients at different cycles of chemotherapy. Alteration of TH leads to decline in thyroid function as chemotherapy progresses. Post-menopausal women were found more susceptible to decline in thyroid function then pre-menopausal women. Thus, the present research warrants routine testing for thyroid hormones in breast cancer patients receiving chemotherapy. Additionally, the underlying mechanism of thyroid toxicity induced by anticancer drugs needed further investigation.

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