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ORIGINAL ARTICLE

Interplay of Thyroid hormones, Nitric oxide and Nitric oxide synthase in Potentiating the Breast Cancer metastasis

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ABSTRACT

Background: Thyroid hormones are able to induce proliferation of breast cancer(BC) cells and these growth promoting effects were similar to estrogen suggesting a significant small talk between the two hormones.

Aims and Objectives: A cross sectional study is designed to see the Interplay of thyroid hormones (fT3 and fT4), nitric oxide and nitric oxide synthase in potentiating BC metastasis.

Material and Methods: 288 consented breast cancer patients within the age range of 30-60 years and 100 age matched healthy controls were included in the study. Estimation of thyroid hormones including fT3 and fT4, vascular endothelial growth factor (VEGF), Matrix Metalloprotienase 9 (MMP-9) and inducible nitric oxide synthase (iNOS) was carried out by ELISA. Data was analyzed by SPSS 20.0. Variables of subjects and patients was compared by student 't' test. The Correlation analysis was carried out by Pearson Correlation Coefficient. .

Results: Increased levels of T3, T4, VEGF,NO, NOS AND MMP 9 were observed in subjects as compared to controls. Correlation coefficient showed positive correlation between fT3,NO and NOS. On the other hand, negative correlation was observed between fT4, NO and NOS.

Conclusion: This study indicates a probable relationship between hyperthyroidism, NO,NOS,VEGF in the progression and metastasis of BC.

Key Words: Thyroid hormones, breast cancer, nitric oxide, nitric oxide synthase, MMP 9 and VEGF.

INTRODUCTION

Breast cancer (BC) is the most commonly occurring malignancy in women and comprises of almost 1/3rd of all malignancies in women. Thyroid hormones(TH) T3 and T4 play an important role in proliferation of cancer cells and invasiveness via many non-genomic pathways including motivation of the membrane receptor integrin, which is over expressed in tumor cells and vasculature. In addition metastasis of cancer cells are also affected by dysregulation of TH[1].

Nitric oxide (NO) is a short-lived, signaling molecule that takes part in many important functions in human body. However the bimodal functions of NO in carcinogenesis and tumor progression is controversial. It is suggested that pro- and anti-tumorigenic role of NO depend on its generation in tissue, its level of production via oxidative and reductive based environment and the tumor microenvironment [2]. It is thought that AKT and P53 are the pathways for signaling of NO to stimulate growth of cancer cell and promote metastasis. In breast cancer, the working of pathways of NO is depends on its concentration. It is found that concentration of NO <100 nmol activates cGMP-dependent pathways, while concentrations of NO in a range of 200–600 nmol stimulate cAMP independent pathways. The concentrations of NO > 600nM phosphorylate p53 and block the DNA repair enzymes. However, NO-mediated nitrosylation process of metabolic enzymes, and initiation of vascular endothelial Growth factor (VEGF) are major pathways of BC growth and metastasis[3,4].

BC cells, express inducible nitric oxide synthase (iNOS) and have the ability to produce NO. Cancer cells with stromal cells and iNOS-positive tumor suggesting that iNOS take part in the stimulation of apoptosis and angiogenesis and having a good chance for invasion of tumor cells and metastasis[5].

It is proposed that interaction of TH with NO may effect on the development of tumor and angiogenesis[6]. The hormone T3 activates chains of signal transduction proteins (CGMP-dependent protein kinase, extracellular signal-regulated kinase) and iNOS[7]. It is demonstrated that cellular uptake of arginine and NOS is reliant on the transporter of arginine. TH stimulates this transporter through the activation of its receptor integrin and the signaling pathways of ERK1/2, and intracellular Ca2+, resulting in the stimulation of metabolism of arginine and production of NO[8]. Besides the expression of some genes (which encode VEFG, MMP-9 and iNOS) have been linked to the process of angiogenesis via the modulation of thyroid hormones through their receptor integrin[9].

Several studies have conflicting results concerning the association between TH,NO and BC metastasis. A cross sectional study was therefore designed to see the interplay of TH,NO and NOS in potentiating the BC metastasis.

MATERIAL AND METHODS

288 consented breast cancer patients within the age range of 30-60 years and 100 age matched healthy controls were included in the study. Women with history of any other systemic disease, any other malignancy other than BC were also excluded from the study. Study duration was 2018-2019. All of the protocols were approved from the research ethical committee of Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore, Pakistan. For the assessment of biochemical variables, 5ml of blood was drawn and stored for the future analysis. Estimation of fT3, fT4, VEGF, MMP-9 and NOS was carried by technique of ELISA (Abcam). NO was estimated with the help of specialized colorimetric method known as Griess assay.

Statistical Analysis: Data was analyzed by SPSS v20.0. Variables were expressed as mean ± SD. Student 't' test was used to compare the levels of fT3,fT4, NO, NOS, MMP-9 and VEGF of subjects was compared to the values of mentioned parameter of controls. P value <0.05 was taken as significant. Correlation of thyroid hormones with nitric oxide and nitric oxide synthase was estimation by Pearson correlation Coefficient. r- Values from 0.4 to 0.5 was considered as significant.

RESULTS

Demographic profile showed that mean age of controls and subjects was 67.59±1.59 and 70.59±10.59 years respectively. Body weight and both systolic as well as diastolic BP of subjects was more than controls. Majority of the subjects were educated ,married belong to lower and middle socioeconomic class. Majority of the patients were diagnosed with TNM staging 2 (Table 1). Increased levels of fT3, fT4, NO, iNOS, VEGF and MMP-9 were observed in subjects as compared to controls, however a

statistically significant difference was only observed in the levels of NO, iNOS, VEGF and MMP 9. Correlation coefficient showed a non-significant positive correlation between fT3, NO (r=0.12) and iNOS (r=0.02). On the other hand a non-significant negative correlation was observed between fT4, NO (r=-0.04) and iNOS (r=0.-0.17).

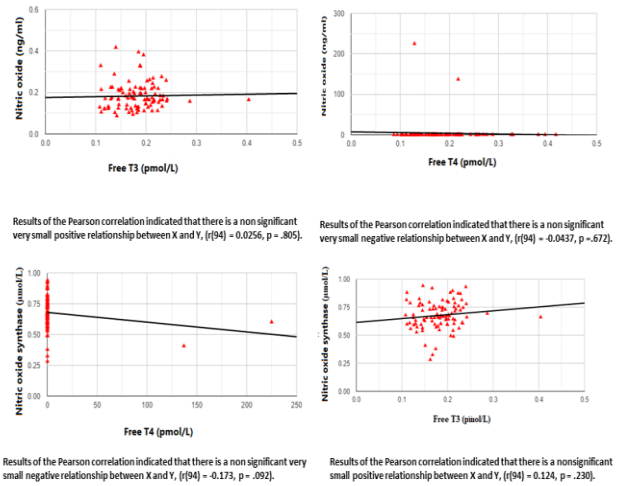
Table 1: Demographic profile of breast cancer patients and controls

VARIABLES	CONTROL (n=100)	SUBJECT (n=288)	p-VALUE
Body weight (Kg)	67.59±12.59	70.59±10.59	0.525
Age (years)	41.59±8.33	40.11±9.58	0.235
Marital status	Married = 95%	Married = 97%	-
Education (Atleast 12 years of education)	Educated= 70%	Educated =66 %	-
Systolic BP (mmHg)	121.59±10.59	135.29±15.29	0.015
Diastolc BP (mmHg)	80.15±3.29	88.59±6.59	0.041
Socioeconomic status	Middle/lower class	Middle/lower class	-
Occupation	Professional 70 women	Professional 188 women	-
TNM Staging	-	Stage 2	-

Table 2: Variation in thyroid hormones and parameters associated with breast cancer in patients and controls

VARIABLES	CONTROL (n=100)	SUBJECT (n=288)	p-VALUE
FT4 (pmol/L)	12.59±3.25	18.59±4.59	0.015
FT3 (pmol/L)	4.33±1.08	5.03±0.95	0.027
NO (ng/ml)	21.25±4.59	35.29±6.35	0.05
Nitric oxide synthase (iNOS) (µmol/L)	9.58±2.48	29.65±3.28	0.05
Vascular endothelial growth factor (VEGF) (pg/ml)	7.88±1.99	13.58±1.48	0.037
MMP-9 (ng/ml)	35.29±7.48	201.58±9.58	0.001

Figure 1: Pearson Correlation between fT3,fT4 with NO and iNOS



DISCUSSION

According to the current study the mean age of females with BC was 40.11 ± 9.58 years with TNM stage 2. Overall, their body weight and BP was also more than controls. Majority of the subjects had achieved secondary education, were married and belonged to middle and lower socio economic class. However, a cross-sectional study carried out on 520 breast cancer women in a Malaysian study found the average age of the patients was 44.64 years old, majority of them women married, had tertiary level of education and belonging to middle class [10].

We observed increased levels of fT3 and fT4 in subjects as compared to controls. We agreed with a German study including 65 BC women conducted to find the role of TH in BC. Apart from familial predisposition, occupational, reproductive and hormonal factors, no clinically important risk factors for breast cancer were found. The study found higher levels of both fT3 and fT4 in patients as compared to controls[11]. According to another study conducted in Netherlands including 80343 diagnosed hyperthyroid females, it was found that hyperthyroid females are more likely to develop BC as compared to hypothyroid females. This also indicates an association between thyroid function and BC[12].

Another study investigated the levels of TH in BC and verified the role of T3 on genes controlled by estrogen and by T3 itself in breast cancerous tissues. The study found increased level of fT4 in BC patients. They also observed that T3 increased the expression of progesterone receptors (PGR). This showed the significance of TH status and their ability to interfere with the expression of gene target estrogen in tissue samples of BC[13]. In contrast to these findings, another study showed significantly lower levels of fT4 in 2775 BC patients as compared to controls. The overall incidence of hypothyroidism in initially diagnosed BC and benign breast diseases was 28.65% and 32.74%[14,15].

Many biological processes and signalling mechanisms may be found in the association of thyroid dysfunction and risk of developing BC. The TH were able to sustain serum free proliferation of several cell lines, including the breast carcinoma cells[11]. It is experimentally proved that T3 and estrogen synergistically stimulate certain breast cancer cell lines[16,17]. Some studies have indicated thyroid autoimmune changes as key prognostic factors in the development of BC[1,18].

Multiple therapies (chemotherapy, radiation and anti-hormonal treatment) may influence the development of thyroid dysfunction via disturbances of the autoimmune system[19]. Irradiation causes direct damage of the cell along with decreased flow of blood resulting in dysfunction and death of cell[20]. Moreover, ionizing radiation induces DNA damage, leading to genomic instability, chromosomal rearrangements, and cellular transformation either directly or by generating reactive oxygen species(ROS)[21].

We also observed increased levels of NO and iNOS in patients as compared to controls. Many studies have experimentally proven the association of hyperthyroidism with NO and iNOS. Thyroid dysfunction impairs the capability for synthesis and responding to NO[22]. Another study conducted on rats showed that TH affects the level and activity of iNOS and therefore NO levels[23]. cGMP dependent pathways were noted to be dysregulated within BC suggesting that lower concentration of NO-mediated cGMP signaling may actually provide protection against BC[3].

The present study also observed increased levels of VEGF and MMP 9 in diseased subjects as compared to controls. It is proposed that impaired balance of angiogenic process or an imbalance between the schemes of proangiogenic (VEGF; FGF & PDGF) and anti-angiogenic factors (TGF-α; EGF& IGF-1).VEGF, is a key angiogenic factor that induces angiogenesis, vascular permeability, and tortuosity. VEGF plays a main role in vascularization of tumors including mammary and thyroid tumors[24]. It is reported that vascular tumors are sensitive to the blockage of VEGF receptors[25] and with the switching of growth factor receptors, estrogen can trigger estrogen receptor (ER) on the nuclear membrane or cell membrane. Membrane-related ER binds to PI3K and stimulates RAS and AKT molecular pathway resulting in an increased tumor growth and metastasis of cancer cells[26]. The proteins related with angiogenesis are desmin, smooth muscle actin and VEGF, and these also have a role in dysregualtion of tumor stroma[27] .

We also observed increased levels of TH and MMP 9 in patients as compared to controls. A study stated that estradiol

increased the proliferation of thyroid cancer cells and in vitro increases the events related with metastasis including adhesion of cells, migration of cell and cellular invasion. Study also found that MMP-2 and MMP-9 play an important role in thr promotion and propagation of metastasis[28]. Another study reported that TH can upregulate the expression of MMPs non-genomically through singalling pathways of PI3K and MAPK, thereby increasing its invasiveness[9].

Correlation coefficient in the current study have showed a non-significant positive correlation between fT3, NO and iNOS. On the other hand a non-significant negative correlation was also observed between fT4, NO and iNOS. A positive correlation of NO was observed with fT3 and fT4. No studies have previously found the correlations of fT4 with NO and iNOS. However, a study demonstrated that the expression of both NO and iNOS were modulated in both hyper as well as hypothyroidism[30]. Another study reported that gene encoding for iNOS, FGF2, MMP 9 and VEGF are related with angiogenesis and their expression is nongenomicaly modulated by TH via the hormone receptors on integrin[9].

In summary, we found a close association of TH with NO and iNOS in potentiating of BC metastasis. As NO-mediated nitrosylation process of metabolic enzymes, and initiation of VEGF and MMP 9 play an important role in the growth of neoplastic tissue. Besides iNOS also takes part in the stimulation of apoptosis and angiogenesis and having a good chance for invasion of tumor cells

CONCLUSION

Our study indicates a probable relationship between hyperthyroidism, NO, iNOS and VEGF in the development and metastasis of BC. However, data must be confirmed in larger multi centred setups with more follow-up. Further studies are also needed to identify the role of TH in females with BC cancer women and to emphasize the possible effects on cancer progression.

Conflict of interest: Authors declares no conflict of interest.

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