IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
Research Article
May 2018 Vol.:9, Issue:3
© All rights are reserved by Messina G et al.

A Study of Prolactin Secretion in Locally Limited and Metastatic Triple Negative Breast Cancer Patients

IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Lissoni P[1], Messina G*[1], Lissoni A[1], Rovelli F[1], Brivio F[2], Fumagalli L[3], Di Fede G[4]

[1]International Institute of PNEI, Milan, Italy;

[2]Surgery Division, Cinisello Hospital, Cinisello Balsamo, Milan, Italy;

[3]Surgery Division, Manzoni Hospital, Lecco, Italy;

[4]Institute of Biological Medicine, Milan, Italy.

Submission: 19 April 2018
Accepted: 25 April 2018
Published: 31 May 2018

HUMAN JOURNALS
www.ijsrm.humanjournals.com

Keywords: Hyperprolactinemia, Prolactin, Prolactin receptors, Triple negative breast cancer.

ABSTRACT

It is a clinical evidence that breast cancer triple negative for ER, PgR and HER-2, the so-called triple negative breast cancer (TNBC), represents the most aggressive form of the mammary tumors. In addition to the stimulatory role of estrogens, EGF, IGF-1 and IGF-2 on breast cancer cell proliferation, PRL has been proven to be another potential important growth factor for breast cancer development either in vitro, or in vivo, since the evidence of hyperprolactinaemia has appeared to be associated with a poor prognosis and with a lack of response to both chemotherapy and endocrine tumor therapies in metastatic breast cancer women. The frequency of hyperprolactinemia in metastatic breast cancer women with tumors other than TNBC is less than 30%, while at present there are no data about PRL behaviour in TNBC. This preliminary study was performed to evaluate PRL secretion in TNBC with locally limited or metastatic disease. The study included 30 patients, 20 of whom showed distant organ metastases. The control group consisted of 50 women with mammary tumors other than TNBC, 26 of whom had distant metastases. No significant difference in the percent of hyperprolactinaemia and in PRL mean levels was seen between non-metastatic TNBC and non-metastatic control patients. On the contrary, both the percentage of hyperprolactinaemia and PRL mean levels observed in metastatic TNBC women were significantly higher than those found in metastatic controls. In addition, both lymphocyte count and lymphocyte-to-monocyte ratio mean values were significantly lower in metastatic TNBC patients with high PRL values than in those with normal PRL levels. The results of this preliminary study, by showing a greater percentage of enhanced PRL secretion, would suggest a possible involvement of PRL, whose stimulatory role on mammary tumor cell proliferation is well known, in TNBC progression and dissemination, as confirmed by the evidence of a more severe status of immunosuppression in metastatic TNBC with hyperprolactinaemia.

INTRODUCTION

The breast cancer represents the human neoplasm, whose prognosis may be better predicted on the basis of well defined biological parameters reflecting the biological malignancy of tumor itself. In particular, it has been established that the expression of estrogen receptor (ER) and progesterone receptor (PgR) is associated with a better prognosis, whereas the positivity for epidermal growth factor (EGF)-receptor-2 (HER-2) may predict a more aggressive malignancy. However, the most aggressive behavior has been found in the presence of mammary tumors, which are negative for the three main prognostic biomarkers of breast cancer, including ER, PgR and HER-2, the so-called triple negative breast cancer (TNBC), because of its preferential early recurrence in visceral organs, including brain, except for the apocrine variant, which is constantly triple negative, but with a better prognosis (1,2). TNBC represents approximately 10-15% of all breast tumors. The more aggressive behaviour of TNBC is also documented by the low efficacy of chemotherapy because of the rapid occurrence of chemo-resistance (1,2). Most TNBC are consisting of basal-like cells rather than luminal ductal cells, as well as the other most typical mammary tumors. Moreover, tumors that are BCRA-1 deficient are most generally basal-like TNBC. Then, because of its lack of ER, PgR and HER-2, the growth of TNBC would depend on the stimulation of receptors for growth factors other than the most commonly investigated, including estrogens and EGF. In fact, the development of both normal and cancer breast cells has appeared to depend on several other growth factors, in particular, prolactin (PRL) (3), insulin-like growth factor (IGF)-1 and -IGF-2 (4), and EGF itself by acting on another EGF receptor, the EGF-R1, also called HER-1 (1,2). IGF-1, IGF-2 and PRL, as well as EGF, exert their activity by acting on their specific receptors. Therefore, it has to be established which relation may exist among the various growth factors for breast cancer. One of the most promising breast cancer growth factor is PRL itself (3), which has appeared to be involved in promoting both onset and progression of mammary tumors. PRL acts on own specific cell surface receptors. PRL receptor (PRL-R) is a transmembrane protein, that belongs to the cytokine receptor superfamily, and at present, at least five isoforms of PRL-R have been identified, which differ only in the length of their intracellular domain (5). PRL-R expression would occur in about 60% of human breast cancers, but its prognostic significance is still controversial since either a better or a poor prognosis have been reported in relation to the positivity for PRL-R in mammary tumors (5). In contrast, most studies agree with the evidence of an over-expression of PRL-R in mammary tumors with respect to the normal breast tissue (5). In any case, PRL would promote breast cancer cell proliferation through several mechanisms (6-9), including the activation of Jak2/Stat 5, PI3K

and MAPK signaling pathways (5-7), and the stimulation of IGF-1R, IGF-2R and EGF-R expressions (8, 9). Moreover, PRL may promote breast cancer growth by inducing the production of a protein, the so-called PRL-induced protein (PIP), which has appeared to promote tumor cell invasion and to enhance the proportion of TNBC (10). Finally, PRL may act as an angiogenic factor in stimulating breast cancer growth (9). Because of the fundamental role of estrogens in the pathogenesis of mammary tumors, the major question regards the relation between the expression of PRL-R and that of ER (9), which exists in two different forms, ER-alpha and ER-beta. ER-alpha expression has appeared to the higher in mammary tumors, whereas ER-beta expression is predominant in the normal mammary tissue.

PRL-R expression has appeared to be positively correlated with the only ER expression, whereas no correlation was seen with the other main prognostic factors for breast cancer, including PgR expression, grade, tumor size and node involvement (5,6). In contrast, other authors have described a higher PRL-R expression in most cases of ER-negative breast tumors (7). Therefore, at present, it is still unknown whether PRL-R expression may constitute a new independent biological prognostic variable for mammary tumors. However, despite the well demonstrated role of PRL in the pathogenesis of mammary tumors, the clinical studies performed to investigate the importance of the control of PRL secretion in the treatment of advanced breast cancer patients are still at the beginning, and at present there are no data about PRL secretion in TNBC patients and the possible involvement of PRL in the progression of TNBC. The majority of TNBC have been shown to present a high expression of HER-1 (1,2) since an EGF-R1 overexpression has been found in about 55% of TNBC, but its role in TNBC growth is still unclear since controversial results have been reported with EGF-R antagonists in the treatment of TNBC. In addition, TNBC tends to present high intra-5-9).tumoral concentrations of vascular endothelial growth factor (VEGF) (1,2). Then, it is probable that TNBC may be a heterogeneous group of tumors, with several subtypes characterized by different molecular alterations. In particular, it could be possible to identify two different TNBC subtypes in relation to PRL-R expression, with the evidence of a quadruple negative breast cancer in the presence of lack of PRL-R expression and of TNBC only, but positive for PRL-R. Finally, PRL has also been proven to exert immunomodulating effects on the anticancer immunity, whose effects, however, are still controversial, since both immunostimulatory and immunosuppressive effects have been reported (5, 9).

From a clinical point of view, metastatic breast cancer women may present abnormally high blood levels of PRL in about one third of cases (11,12). Therefore, because of the very high incidence of breast cancer in the world, the metastatic breast cancer would constitute one of the main causes of hyperprolactinaemia in humans. In contrast, despite its frequency, metastatic breast cancer-related abnormally high PRL serum levels are generally not included within the common causes of hyperprolactinaemia by the Endocrinologists, since their clinical studies are still limited to the diseases of the endocrine glands, rather than to involve the endocrine alterations occurring in pathologies other than those of the endocrine glands. Moreover, it has been demonstrated that the evidence of elevated pre-treatment blood levels of PRL may predict not only a poor prognosis in terms survival time, but also in terms of reduced response to chemotherapy and to endocrine therapy, by preventing the apoptosis of breast cancer cells through a stimulation of bcl-2 expression, with a following decrease in the cytotoxic and cytostatic potency of the different anticancer treatments (11-12). The mechanisms responsible for metastatic breast cancer related-hyperprolactinemia are still to be better investigated. Since PRL may be produced not only by the pituitary gland, but also by some other normal cell lines, namely the breast tissue, and breast cancer cells as an autocrine production (13), the high blood concentrations of PRL, which may occur with breast cancer progression, could be the consequence of a possible alteration in the dopaminergic control of PRL release, or be due to a direct autocrine production by breast cancer cells themselves. This finding could explain the controversial results on the effects of a normalization of PRL blood levels by a concomitant anti-prolactinaemic dopaminergic therapy on chemotherapy and endocrine therapy efficacy in hyperprolactinaemic metastatic breast cancer patients, since either no effect (13), or an enhanced efficacy of the various anticancer treatments (14) have been described. These controversial results could simply depend on the fact that the dopaminergic agents may reduce the only pituitary secretion of PRL, whereas they would have no effect on the autocrine tumor production of PRL, whose activity could be blocked only by antagonists of PRL or its receptors (14). Finally, no study has been performed to evaluate which relation may exist between PRL blood levels and immune status of breast cancer patients, at least in part because of the excessive cost of an adequate immune clinical investigation by measuring lymphocyte subsets and cytokine blood concentrations. Recently, however, it has been shown that the simple lymphocyte-to-monocyte ratio (LMR) may reflect the relation existing between the activation of the anticancer immunity, mainly mediated by lymphocytes, and its macrophage- and regulatory T cells (T reg)-mediated immunosuppression, and that the evidence of an abnormally low LMR value less than 2.1 is

associated with a poor prognosis, a diminished response to the different treatments and a lower survival time (15).

This preliminary study was performed to evaluate PRL blood levels in a group of TNBC with locally limited or metastatic disease, by correlating its levels with the immune status of cancer patients.

MATERIALS AND METHODS

The study included 30 consecutive TNBC women (median age: 48 years, range 28-73), 20 of whom had a metastatic disease (visceral lesions: 14; non-visceral lesions: 6), while the remaining 10 patients had a locally limited disease. Dominant metastasis sites were, as follows: soft tissues: 4; bone: 2; lung:3; liver:6; brain:5. The menopausal status was premenopausal in 9 and postmenopausal in the other 21 women. Twelve patients were pretreated by chemotherapy, whereas the remaining 18 patients received no previous chemotherapy. The eligibility criteria were, as follows: histologically and immuno-histochemically proven TNBC, measurable lesions, no double tumor, and no chronic therapy with drugs stimulating PRL secretion, including anti-dopaminergic agents, opioids and neuroleptic drugs. Moreover, none drug was given from at least 24 hours prior to study. The results were compared to those of a historical control group consisting of age-matched 50 patients, 24 of whom were non-metastatic patients, while the other 26 women had a metastatic disease. Serum levels of PRL were measured in duplicate by an immunoradiometric (IRMA) method on peripheral venous blood samples drawn at 8.00 A.M. after an overnight fast. Normal values of PRL observed in our laboratory (95% confidence limits) were below 23 ng/ml. LMR values observed in our laboratory were normal when they were greater than 2.1. Data were reported as mean +/- SE. The results were statistically analyzed by the Student's t-test and the chi-square test, as appropriate.

RESULTS

High PRL serum levels were seen in 12/30 (40%) TNBC patients. The percentage of hyper-prolactinaemia observed in non-metastatic breast cancer patients of the control group was not statistically significantly different with respect to that found in those with non-metastatic TNBC women (1/24 (4%) vs 1/10 (10%)). On the contrary, metastatic TNBC patients showed a percentage of abnormally elevated PRL serum levels statistically significantly higher with respect to that seen in metastatic breast cancer patients with tumors other than the TNBC (11/20 (55%) vs 6/26 (27%),

P < 0.05). PRL mean serum levels observed in TNBC patients and in breast cancer controls are shown in Table 1. In the non-metastatic group, no significant difference in PRL mean levels was found between TNBC and breast cancer women. On the contrary, metastatic TNBC showed PRL mean levels statistically significantly higher than those observed in metastatic breast cancer women other than TNBC (P < 0.05). Moreover, within the TNBC group, metastatic patients showed PRL mean levels statistically significantly higher than those found in non-metastatic patients (P < 0.001). No statistically significant differences in PRL mean levels were seen between metastatic TNBC patients with visceral lesions and those with non-visceral lesions. On the same way, significant differences were seen neither between untreated and pre-treated patients by chemotherapy nor between premenopausal and postmenopausal TNBC patients. LMR mean values and lymphocyte mean count in relation to PRL levels are reported in Table 2. Lymphocyte mean number observed in TNBC patients with normal PRL levels were significantly higher than that found in the normo-prolactinaemic ones (P < 0.025). On the same way, within the metastatic TNBC group, hyperprolactinaemic patients showed lymphocyte mean count significantly lower than those with normal PRL levels (P <0.01). In addition, an abnormally low LMR was seen in 3/20 (15%) TNBC metastatic patients, and in none of those with locally limited tumor. TNBC patients with high PRL levels showed a statistically significantly lower LMR mean values than the normo-prolactinaemic ones P <0.025). Finally, within the metastatic group, LMR mean values observed in patients with abnormally high PRL levels were significantly lower than in the non-prolactinemic metastatic ones (P < 0.01).

Table 1. PRL mean serum levels in triple negative breast cancer (TNBC) and in mammary tumors other than TNBC as a control group

SUBJECTS	n	PRL mean +/- SE
CONTROL BREAST CANCER	50	
- Non-metastatic patients	24	11.2 +/- 1.6
- Metatstatic patients	26	18.6 +/- 1.4 *
TNBC PATIENTS	30	
Disease extension		
- Non-metastatic patients	10	10.5 +/- 1.6
- Metastatic patients	20	33.3 +/- 6.6**
- Non-visceral lesions	6	35.6 +/- 6.8
- Visceral lesions	14	31.4 +/- 7.1
Menopausal status		
- Premenopausal	9	19.8 +/- 5.3
- Postmenopausal	21	29.2 +/- 9.7
Therapies		
- Previous chemotherapy	12	36.6 +/- 7.8
- No previous chemotherapy	18	20.1 +/- 3.9

* P < 0.05 vs non-metastatic controls

** P < 0.05 vs metastatic controls; P < 0.001 vs non-metastatic TNBC

Table 2. Lymphocyte mean count and lymphocyte-to-monocyte ratio (LMR) mean values in metastatic and in non-metastatic TNBC patients.

PATIENTS	n	Lymphocytes (n/mm3) X +/- SE	LMR X +/- SE
Metastatic patients	20	1,604 +/- 211	2.6 +/- 0.3
-Normal PRL values	9	1,988 +/- 148*	3.2 +/- 0.2**
-High PRL levels	11	1,125 +/- 202	1.8 +/- 0.3
Non-metastatic patients	10	2,052 +/- 93	4.6 +/- 0.3***

*P < 0.05 vs metastatic patients with high PRL levels;**P < 0.01 vs metastatic patients with high PRL levels; ***p < 0.01 vs metastatic patients with high PRL levels.

DISCUSSION

In agreement with previous results reported in the literature (12-17), the study confirms the possible occurrence of an enhanced PRL production in the metastatic breast cancer. Moreover, the study would suggest that the metastatic TNBC is associated with hyperprolactinemia in a percentage superior to that occurring in metastatic breast tumors other than TNBC. Since PRL may be a growth factor for breast cancer, the more aggressive behaviour of the TNBC could be due at least in part to the enhanced endogenous PRL production, which may depend on a pituitary endocrine alteration and/or a direct autocrine production by cancer cells themselves (13). Obviously, the potential stimulatory role of PRL on breast cancer growth would require the expression of PRL-R. Therefore, further studies will be required to identify a possible TNBC subtype, negative for ER, PgR, HER-2, but positive for PRL-R. If successive studies will confirm the pro-tumoral role of PRL in TNBC and most in general in mammary tumors, the control of PRL endogenous production and activity by long-term dopaminergic agents, such cabergoline and bromocriptine (20, 21), and/or PRL and PRL-R antagonists, could constitute a new hormonal strategy in the endocrine approach to cancer

therapy. At present, it is not possible to establish the prognostic significance of an enhanced PRL secretion in TNBC, and further longitudinal studies will be required to establish whether the enhanced PRL production may allow a different survival. However, according to these preliminary results, the occurrence of hyper-prolactinaemia in metastatic TNBC seems to be associated with a more severe status of immunosuppresion, as shown by the lower lymphocyte count and LMR in hyperprolactinaemic TNBC, which have both seen to predict a worse prognosis and to be associated with a reduced survival.

REFERENCES

1. Chavez K, Garimella SV, Lipkowitz S. Triple negative breast cancer cell lines: one tool in the search for better treatment of triple negative breast cancer. Breast Dis 32: 35-489, 2010.
2. Arnedos M, Bihan C, Delaloge S, Andre F. Triple- negative breast cancer: are we making head way at least? Ther Adv Oncol 4:195-210, 2012.
3. Welsch C, Nagasawa H. Prolactin and murine tumorigenesis: a review. Cancer Res 17: 951-963, 1997.
4. The Endogenous Hormones and Breast Cancer Collaborative Group. Insulin-like growth factor 1 (IGF-1), IGF binding protein 3 (IGFBP 3) and breast cancer risk: pooled individual data analysis of 17 prospective studies. Lancet Oncol 11: 530-542, 2010.
5. Gills S, Peston D, Vonderhaar B, Shousha S. Expression of prolactin receptors in normal benign and malignant breast tissue: an immunohistological study. J Clin Pathol 54: 9056-960, 2001.
6. Carver KC, Schuler LA. Prolactin does not require insulin-like growth factor intermediates, but synergize with insulin-like growth factor 1 in human breast cancer cells. Mol Cancer Res 6: 634-643, 2008.
7. Baqai T, Peston D, Shousha S. An immunohistochemical characterisation of oestrogen receptor negative ductal carcinoma in situ of the breast. J Pathol 192 (Suppl): 207, 2000.
8. Maus MV, Reilly LM, Schuler LA. Complex prolactin crosstalk in breast cancer: new therapeutic implications. Mol Cell Endocrinol 307: 1-7, 2009.
9. La Pensee EW, Ben-Jonathan N. Novel roles of prolactin and estrogens in breast cancer: resistance to chemotherapy. Endocr Relat Cancer 17: R91-R107, 2010.
10. Naderi A, Meyer M. Prolactin-induced protein mediates cell invasion and regulates integrin signaling in estrogen-receptor negative breast cancer. Breast Cancer Res 14: R111-R116, 2012.
11. Holtkamp W, Nagel GA, Wander HE, Rauschecker HF, von Heyden D. Hyperprolactinemia is an indicator of progressive disease and poor prognosis in advanced breast cancer. Int J Cancer 34:323-328, 1984.
12. Bhatavdekar JM, Shah NG, Balar DB, Patel DD, Bhaduri A, Trivedi SN, Karelia NH, Ghosh N, Shukla MK, Giri DD. Plasma prolactin as an indicator of disease progression in advanced breast cancer. Cancer 65:2028,2032, 1990.
13. Ginsburg E, Vonderhaar BK. Prolactin synthesis and secretion by human breast cancer cells. Cancer Res 55:2591-2595, 1995.
14. Lissoni P, Bucovec R, Malugani F, Ardizzoia A, Villa S, Gardani GS, Vaghi M, Tancini G. A clinical study of taxotere versus taxotere plus the antiprolactinemic agent bromocriptine in metastatic breast cancer pretreated with anthracyclines. Anticancer Res 22: 1131-1134, 2002.
15. Mantovani A, Allavena P, Sicla A, Balkwill F. Cancer-related inflammation. Nature 454:436-444, 2008.