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Neoadjuvant Metformin Added to Systemic Therapy Increases Pathological Complete Response in Breast Cancer: A Cross-sectional Study, Mexico Hospital, Costa Rica

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ABSTRACT

Background: Metformin shows anti-proliferative effect on tumor cells. We studied the effect of metformin on achieving complete pathological response (pCR) in breast cancer patients receiving neoadjuvant therapy in a Latin American population.

Methods: We conducted a cross-sectional study in Mexico Hospital, Costa Rica, from January 2007 to December 2015. Women with early-stage or locally advanced breast cancer receiving neoadjuvant systemic treatment were recruited for the study. Univariate and multivariate models were used to compare the pCR rate with metformin plus standard therapy versus standard treatment alone.

Results: Of 53 included women with early-stage or locally advanced breast cancer were included, 14 received metformin with systemic therapy, and 39 had systemic therapy alone. Only 15% of the patients had diabetes mellitus. The pCR rate was in the metformin group was 64.3% compared with 23.1% in the systemic therapy-alone group (OR: 6.0, 95% CI: 1.60–22.53, P= 0.008). This finding was confirmed after adjustment for potential confounders, suggesting that the use of metformin increased the pCR likelihood regardless of breast cancer subtype (adjusted OR: 5.56, 95% CI: 1.27–24.3, P = 0.02). There was a trend of achieving pCR in patients with Ki-67 > 55%. However, it did not reach statistical significance when metformin was added, suggesting that probably a high Ki-67 level in the presence of metformin is not a predictor factor of pCR.

Conclusion: This is the first study conducted in a Latin American population showing that metformin with systemic therapy increases pCR regardless of the intrinsic molecular subtype or Ki-67 levels. These findings encourage prospective studies to evaluate the role of neoadjuvant metformin in this population.

Introduction

According to the World Health Organization, breast cancer (BC) is one of the leading causes of morbidity and mortality worldwide with approximately 14 million new cases in 2012.[1] In 2015, Costa Rica reported a BC incidence rate of 42.25 per 100 000 women.[2]

BC is considered not a single disease, but a group of different entities with diverse pathological features, clinical implications, and outcomes.3 Estrogen and progesterone receptors as well as HER2 expression (using immunohistochemistry), in conjunction with clinicopathological characteristics such as tumor grade, tumor size, and nodal involvement, are useful for establishing the management and prognosis of these patients.[4] Gene expression microarrays in BC have led to identifying of at least five molecular subtypes, labeled as luminal A, luminal B, HER2+, basal-like, and

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normal breast-like.[5] These BC subtypes have been associated with significant differences in overall survival as well as response to systemic therapy.[6,7]

A recent meta-analysis showed that patients with BC who attain a pathological complete response (pCR) in the breast and axilla after neoadjuvant systemic treatment might have a better survival rate, mainly in aggressive tumors such as HER2 or triple-negative (TN) subtypes.[8] Following this finding, the use of presurgical chemotherapy became very common, and biomarkers such as HER2, and hormone receptor (HR) status have been frequently used to indicate the implementation of this therapeutic approach. Furthermore, other markers have been explored to predict pCR, including Ki-67, which is a non-histone nuclear cortex protein expressed in the cell nucleus during the G1, S, G2, and M phases of the cell cycle. High Ki-67 levels have been associated with an increased chance of achieving pCR and therefore improved survival.[9-11]

It has been proposed that hyperinsulinemia, insulin resistance as well as obesity influence breast cancer prognosis. Various mechanisms have been hypothesized, including increased circulating plasma levels of estrogen, insulin, insulin-like growth factor, and other hormonal factors that act to promote tumor growth and increase the risk of metastatic disease.[12-15]

Metformin is an oral biguanide used for treatment of type 2 diabetes that inhibits hepatic gluconeogenesis and increases insulin sensitivity at peripheral level, thereby improving the glycemic control.[16] Taking into account these pharmacological properties and the increasing evidence for the antitumor effect of metformin,[17] we decided to conduct a cross-sectional study to evaluate the impact of metformin on achieving pCR in breast cancer patients who received neoadjuvant chemotherapy in a Central American population. To our knowledge, this is the first study conducted in this region.

Methods

Study population

After receiving approval from the institutional ethics committee, we conducted a cross-sectional study from January 2007 to December 2015 in Mexico Hospital, San Jose, Costa Rica, to evaluate the pCR rate in patients with invasive breast cancer receiving neoadjuvant systemic therapy with or without metformin. We included all women with early-stage or locally advanced, biopsy-proven invasive breast cancer who received systemic treatment neoadjuvantly. The following exclusion criteria were used: metformin use in a different proposed dosage (< 1 g/day), pregnancy, unknown HR or HER2 status, ECOG > 2, intolerance or hypersensitivity to metformin, not giving informed consent, having secondary or concurrent malignancies except for controlled non-melanoma skin cancer or cervical carcinoma in situ.

Treatment and pathology assessments

Patients included were previously evaluated in the multidisciplinary tumor board. Overall, all patients received preoperative systemic therapy based on anthracylines (epirubicin) and cyclophosphamide with or without 5-fluorouracil, followed by taxanes (paclitaxel). In case of HER2 overexpression—determined using IHC or fluorescence in situ hybridization (FISH) trastuzumab was added to taxanes. Neoadjuvant hormonal treatment was allowed without concomitant chemotherapy. After systemic therapy, patients underwent surgery (radical mastectomy, simple mastectomy, or conserving surgery) according to initial stage of the disease and response to systemic treatment. Also, the axilla was managed with sentinel node biopsy or axillary lymph node dissection. Adjuvant radiotherapy was given according to standard practice. In HR-positive tumors, adjuvant endocrine therapy was used, and HER2-positive tumors had to complete one year of adjuvant trastuzumab. When oral metformin was incorporated, the permitted dosage was 500 mg twice a day in conjunction with systemic therapy.

Two experienced BC pathologists reviewed all the biopsies. The initial diagnosis was made using a breast or axillary node core needle biopsy. The report had to include a complete IHC (ER, PR, HER2, and Ki-67 percentage), tumor grade, and histologic subtype. In case of equivocal HER2 status by IHC, FISH would be performed. A pCR was defined as the absence of invasive carcinoma in breast or the axillary nodes in the surgical specimen (ypT0, ypN0).

Statistics

Demographic data are summarized and showed using descriptive statistics, and categorical variables are presented as percentages. Continuous variables are given as medians and ranges. The Fisher exact test was used to determine the association between metformin, immunophenotype, and pCR. A Multivariate logistic regression analysis was implemented to find potential predictors of pCR. We used the receiver operating characteristic (ROC) curve to establish the best cut-off point of Ki-67 for prediction of pCR. A P value less than 0.05 was considered statistically significant. Data were analyzed using SPSS (V. 20.0, Chicago, IL).

Results

We identified 81 women with invasive breast cancer who received neoadjuvant treatment. Fifty-three women with early-stage or locally advanced breast cancer fulfilled the inclusion criteria and were enrolled. Of them, 14 patients received metformin (500 mg bid PO) during chemotherapy, and 39 were

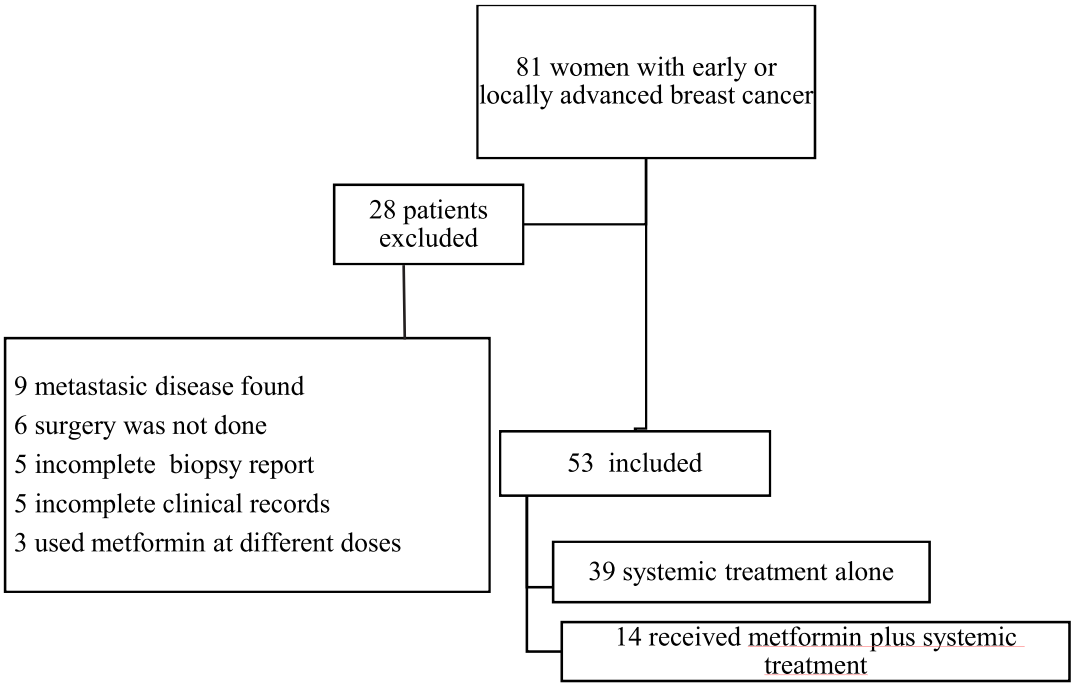

Figure 1. Consort Diagram

Table 1. Patient Demographics and Clinical Characteristics by Treatment Group

Characteristics	Systemic therapy + metformin (N=14)	Systemic therapy alone (N=39)
Age	50.3 (38–66)	53.1 (22–83)
<50 years	6 (42.8%)	14 (35.9%)
≥50 years	8 (57.2%)	25 (64.1%)
Clinical stagea		
I	0 (0.0%)	0 (0.0%)
II	7 (50.0%)	17 (43.6%)
III	7 (50.0%)	22 (56.4%)
Tumor IHC		
HER2+	5 (35.7%)	12 (30.8%)
HER2-	9 (64.3%)	27 (69.2%)
RE+	3 (21.4%)	19 (48.7%)
RE-	11 (78.6%)	20 (51.3%)

[a] *TNM Stage according to AJCC Cancer Staging Manual 7th Ed.*

treated with chemotherapy alone. (Figure 1)

Patient demographics and clinical characteristics of the groups are presented in Table 1. The mean age of the patients was 52.48 (range: 22–83) years. Overall, 56.6% of the patients did not have any comorbidities, 28.3% had hypertension, and 15.1% had type 2 diabetes mellitus. Two patients reported dyslipidemia, 1 patient had colon carcinoma more than five years before accrual, 1 patient had hypothyroidism, and 1 patient suffered from anxiety syndrome.

Infiltrating ductal carcinoma was the most common histology (n = 49, 92.45%), followed by infiltrating lobular carcinoma (n = 2, 3.77%), medullar carcinoma (n = 1, 1.89%), and poorly differentiated carcinoma (n = 1, 1.89%). We found that 21 patients (40%) had luminal subtype, 20 (38%) had TN tumors, 7 (13%) coexpressed estrogen receptor and HER2, and 10 cases (19%) were HER2+/HR-. Twenty-eight patients (53%) had grade3 tumors, 22 (43%) exhibited grade 2 invasive breast cancer, and only 3 patients (4%) had grade 1 differentiation. In total, 35 women (67%) had lymphovascular invasion.

After neoadjuvant therapy (Table 2), 30 women (57%) had breast-conserving surgery and 23 (43%) had radical mastectomy. Eighteen patients (34%) achieved a pCR. We analyzed the pCR rate according to tumor subtype determined by IHC (Table 3). HER2-overexpressing tumors tended to predict pCR (OR: 3.88, P = 0.071), in contrast to luminal tumors, which did not achieve a pCR (OR 0.09; P = 0.01). When metformin was added to systemic therapy, a pCR rate of 64.3% was achieved, while the pCR rate in the systemic therapy-alone arm was 23.1% (OR: 6.0, 95% CI: 1.60–22.53, P = 0.008). After adjustment for potential confounders (e.g., intrinsic breast cancer subtype assessed using immunohisto-

Table 2. Neoadjuvant Systemic Treatment Used

	N	percentage
FEC* 75 followed by weekly paclitaxel.	6	11.32%
FEC/EC 100 followed by weekly paclitaxel	21	39.62%
Epirubicin followed by weekly paclitaxel + trastuzumab	14	26.42%
Weekly paclitaxel + trastuzumab	1	1.89%
Weekly paclitaxel alone	1	1.89%
Dose-dense (epirubicin followed by paclitaxel)	6	11.32%
Platinum/epirubicin/paclitaxel	2	3.77%
Anastrozole	2	3.77%
TOTAL	53	1%

**F= 5-Fluorouracil, E= Epirubicin, C= Cyclophosphamide*

Table 3. Association between IHC-determined tumor subtype and pathological complete response rate

Subtype	With pCR, n (%)	Without pCR, n (%)	OR (95% CI)
HR+/HER2-	1 (6.7)	14 (93.3)	0.09 (0.01–0.74) P = 0.01
HR+/HER2+	2 (28.6)	5 (71.4)	0.75 (0.13–4.31) P = 0.555
HR-/HER2+	6 (60)	4 (40)	3.88 (0.93–16.19) P = 0.071
Triple-negative	9 (42.9)	12 (57.1)	1.92 (0.60–6.10) P = 0.37
Total	18	35	-

Table 4. Multivariate Analysis of Potential Predictors of Pathological Complete Response

Variable	OR (95% CI)	P
Metformin	5.56 (1.27–24.3)	0.02
RE+	0.16 (0.01–2.39)	0.18
RE-/HER2+	3.23 (0.837–29.16)	0.28
Triple-negative	1.27 (0.17–9.31)	0.17

Table 5. Comparison of pCR by Ki-67 Percentage

Ki-67 (number)	With pCR, n (%)	Without pCR, n (%)	OR (95 CI %), P
<55% (14)	2 (14.3%)	12 (85.7%)	8.0 (1.42–45.06), 0.013
>55% (21)	12 (57.1%)	9 (42.9%)	

chemistry), metformin use was independently associated with a higher likelihood of pCR (OR: 5.56, 95% CI: 1.27–24.3, P = 0.02) (Table 4). We also noted a trend for achieving a higher pCR rate in TN tumors when metformin was incorporated (OR: 10, 95% CI: 1.03–67.1, P = 0.053).

We decided to incorporate Ki-67 as a predictor marker for achieving pCR, with an 86% sensibility and 57% specificity when the cut-off was set at 55% (corresponding to the highest area under the ROC curve = 0.755). We found a higher chance of obtaining a pCR when Ki-67 percentage was greater than 55% (Table 5); however, when metformin was added to this model, we did not find a statistically significant increase in the rate of pCR in patients with Ki-67 > 55% (OR: 4.0, 95% CI: 0.36–44.1, P = 0.26).

We did not register any serious adverse events with metformin. Mild diarrhea was observed in 3 patients, which was controlled with short-term antidiarrheal medications. No other side effects were found.

Discussion

Preoperative systemic therapy (neoadjuvant) is becoming popular nowadays for early-stage or locally advanced breast cancer. When this modality of treatment is given, the main purpose is to downstage the tumor for a less aggressive surgery with less postoperative complications and better cosmetic outcomes.[18] Neoadjuvant treatment also allows prompt tumor response evaluation, and the residual disease can be a prognostic factor of tumor recurrence and overall survival, mainly in high-risk histologies such as TN tumors or HER2+ invasive breast carcinomas.[6, 8] For this reason, there is a particular interest in exploring new therapies to increase the pCR rate. We decided to look at the effect of metformin plus standard therapy on the rate pCR. All the included patients were discussed in our institutional tumor board. As a result, there was a clear trend toward including more HER2+, TN, or HR-positive invasive breast cancer with high-risk features such as advanced disease or high histologic grade. This modality of treatment was also preferred in young patients, which explains the median age of 52.48 (±13.41) years. Most of the patients in our study were healthy (56.6%). This fact is very

relevant because other retrospective studies using metformin in this setting had evaluated diabetic patients.[19]

Overall, the pCR rate (ypT0/is ypN0) obtained in our research was 34%, which is higher than that reported in other studies (ranging from 14 to 19.8%).[20] Nevertheless, we have to emphasize that our study population was selected deliberately, including very responsive tumor subtypes. We found that the pCR rate was significantly higher when metformin was added to standard systemic therapy, even after adjustment for the tumor subtype, meaning that adding metformin to systemic therapy increased the likelihood of achieving pCR 5.56 times.

Metformin has shown anticancer properties, including activation of the AMP activated protein kinase (AMPK) pathway,[21] antioxidant activity, induction of apoptosis, and many others.[22] Patients with type 2 diabetes are hyperinsulinemic, and there is evidence that this condition contributes to tumorigenesis. In this scenario, metformin reverses, at least partially, hyperinsulinemia and exhibits antiproliferative properties, which might increase the effect of chemotherapy in a synergy. Preclinical research has demonstrated that metformin can sensitize tumor cells to chemotherapy through inhibition of the expression of PI3K/AKT proteins[23] and, consequently, controlling the activity of the mammalian target of rapamycin (mTOR). The combination of metformin with chemotherapy appears to inhibit mTOR activity, inducing cell cycle arrest.[24] Our study, although limited by its cross-sectional design and sample size, provides clinical evidence that metformin in conjunction with chemotherapy increases the pCR rate in the context of non-diabetic Latin American patients, which is relevant to planning prospective studies.

Ki-67 is a non-histone nuclear cortex protein expressed in all cell cycle phases except in the quiescent phase G0, reaching the peak level during mitosis. Ki-67 can serve as an indicator of tumor proliferative activity.[10, 25, 26] Higher level of Ki-67 has been hypothesized to be a predictor marker of pCR.[27, 28] A recent meta analysis involving 53 studies (10 848 patients) showed that tumors with high Ki-67 score had a better chance to respond to neoadjuvant treatment, although there was no clear cut-off.[29] In our study, there was a higher chance of obtaining a pCR when Ki-67 index was greater than 55% using ROC curve; however, when metformin was added, we were unable to find a specific Ki-67 cut-off. This might mean that, regardless of Ki-67 levels, the most important factor to obtain the pCR was metformin. This finding must be confirmed by future studies since this has not been evaluated in this specific approach, as far as we know, either in retrospective or prospective studies.

In conclusion, this is the first cross-sectional study conducted in a Latin American population showing that the use of metformin in combination with systemic therapy can increase the pCR rate in locally advanced or early breast cancer, irrespective of the histologic subtype, grade, or Ki-67 levels. These findings can encourage prospective studies using metformin in the neoadjuvant setting in our population.

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Conflict of Interest

We have read and understood the journal's policy on disclosing conflicts of interest and declare that we have none.

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