Role of Metformin in Combination with Trastuzumab and Neoadjuvant Chemotherapy in Treatment of HER-2 Positive Breast Cancer

Ahmed Said Ahmed Said[*1], Hussein Ali Mustafa Abd Al-Mutteleb[2], Mohammed Ali Morsy Abdeen[3], Mohammed Abd Al Rahman Hassan[3], Soha Abdelrazek Abbas Ahmed[1]

Departments of [1]Clinical Oncology and Nuclear Medicine and

[2]General Surgery, Faculty of Medicine- Aswan University

Department of [3]Clinical Oncology and Nuclear Medicine, Faculty of Medicine- Cairo University

***Corresponding author:** Ahmed Said Ahmed Said, **Mobile:** (+20) 01125408705, **E-Mail:** ahmedsaidorman@yahoo.com

ABSTRACT

Backgound: HER2 overexpression is a good predictive marker of HER2 targeted therapy, which means that HER2 therapy will be very effective in reducing the size of HER2 positive breast cancers. Therefore, the size of HER2 positive breast cancer can easily be reduced in patients who wish to have a breast-conserving operation, and potentially improves the outcome of patients if pathologic complete response (PCR) can be achieved.

Objective: To determine whether metformin use with trastuzumab was associated with improvement in PCR rate in patients with breast cancer receiving neoadjuvant chemotherapy.

Patients and Methods: This prospective study was conducted at the Clinical Oncology Department, Aswan University and Upper Egypt Hospitals in the period between 1/7/2016 and 1/9/2019. This study included 30 patients divided into 2 groups, test group (metformin group) and standard group (non-metformin group). Histopathology was confirmed by tissue core biopsy.

Results: All patients in the two groups in our study achieved either pathological complete response or partial response. No patients developed disease progression or were still stable disease. Among the patients in test group (metformin group), 12 patients (80%) achieved pathological complete response (PCR) while 3 (20%) patients did not achieve PCR. However, among the patients in standerd group (non-metformin group) 9 patients (60%) achieved pathological complete response (PCR) while 6 (40%) patients did not achieve PCR. There was statistically significant difference between the two groups regarding the response with better PCR in metformin group (p value is 0.089).

Conclusion: The addition of metformin to tratuzumab in neoadjuvant chemotherapy has a significant impact on pathological complete response (PCR) in female patients with HER-2 positive breast cancer.

Keywords: Breast Cancer, Metformin, Neoadjuvant Chemotherapy.

INTRODUCTION

Preoperative systemic therapy (neoadjuvant) is becoming popular nowadays for early-stage or locally advanced breast cancer. In the last decade, pathological response for neoadjuvant chemotherapy, as an indicator for long term clinical benefit in breast cancer, was an evolving chance for more rapid incorporation of new drugs **(1)**.

Metformin, a biguanide derivative that reduces insulin levels, has long been a cornerstone in the treatment of type 2 diabetes (T2D). There is compelling evidence to incorporate metformin into the armamentarium against cancer, particularly breast cancer (BC). Notwithstanding the limitations of observational studies, many have consistently indicated that metformin can reduce the incidence, outcome, and mortality of BC in patients with T2D **(2)**. Moreover, preclinical studies have described a variety of molecular mechanisms through which metformin indirectly or directly inhibits the growth of BC cells in vitro and in vivo **(3)**.

The extensive clinical experience accumulated from patients with T2D prescribed metformin, together with its well characterized and modest toxicity profile, has significantly shortened the clinical evaluation path of metformin in cancer prevention and treatment **(4)**. Accordingly, many clinical studies, including proof-of-principle studies in the prevention setting and phase 2 trials in the adjuvant and metastatic settings, have been planned and/or are currently under way to test the causal nature of the suggested correlation between metformin and clinical benefit in cancer **(5)**.

To avoid overestimation of the potential effects of metformin in unselected populations of nondiabetic BC patients, preoperative translational studies are important to define specific BC subgroups more likely to benefit from metformin-based regimens. The neoadjuvant (preoperative) approach is known to maximize the capacity to test the benefits of drug combinations in the context of carefully designed clinical trials of early BC **(5)**. In this regard, a landmark retrospective study revealed that patients with T2D and BC who received metformin and neoadjuvant chemotherapy appeared to have a higher PCR rate than did those not receiving metformin, a hypothesis-

Received:6 /7 /2020
Accepted:5 /9 /2020

generating finding that warrants prospective evaluation [6].

Metformin was shown to suppress both the tyrosine kinase activity and the expression of the human epidermal growth factor receptor 2 (HER2) protein in in vitro models of HER2-overexpressing BC cells, in addition to prolonging survival in HER2-overexpressing transgenic BC mouse models [7]. Metformin treatment leads also to lower levels of circulating insulin and insulin-like growth factor (IGF-I), and to cell-autonomous inhibition of the mTOR pathway [8].

Such a multi-faceted capacity of metformin to target not only HER2 itself but also central mechanisms implicated in refractoriness to HER2-targeted therapies including both the IGF-I/mTOR signaling pathway and the self-renewal/proliferation of tumor-initiating cancer stem cells [9] provides strong experimental support to translate these pre-clinical findings into new metforminbased clinical management strategies that may benefit HER2-positive BC patients. However, most of the in-vitro models showing anti-HER2 activity of metformin used drug concentrations in the millimolar range, far higher than reported plasma metformin concentrations seen in diabetic patients treated with metformin [10], thereby leaving unanswered the question of whether metformin would have a clinical effect in patients suffering from HER2-positive BC.

AIM OF THE WORK

This prospective study aimed to determine whether metformin use was associated with improvement in pathologic complete response (PCR) rate in patients with HER-2 positive breast cancer receiving neoadjuvant chemotherapy.

PATIENTS AND METHODS

Our prospective study was conducted at the Clinical Oncology Department, Aswan University and Upper Egypt Hospitals in the period between 1/7/2016 and 1/9/2019. 30 female patients with breast cancer who were eligible to receive neoadjuvant chemotherapy were included. We divided female patients with breast cancer into 2 groups: 15 patients received neoadjuvant chemotherapy AC-Taxol +trastuzumab+ metformin (**test group)** and 15 patients received neoadjuvant chemotherapy AC-Taxol + trastuzumab (**standard group**) without metformin.

Inclusion Criteria: Female patients with histological and radiological proof of non-metastatic breast cancer. Age: from 18 to 70 years old. Female patients with clinical T2 Breast cancer or more and/or clinical positive LN with HER-2 positive status. Performance status: 0-2 WHO. Patients with adequate left ventricular ejection fraction (≥ 50%) and normal hematological, renal and hepatic functions.

Exclusion Criteria: Bilateral tumour. Patients submitted to excisional biopsy from breast mass. Patients who were pregnant or lactating. Patints with double malignancy. Presence of metastatic disease at diagnosis. Patients refuse to be conducted in the study. Patients that are developing severe complications from the use of treatment. Diabetic patients already on metformin therapy. Withdrawal of concent at any time during the study.

Pretreatment evaluation:

1. Medical history and physical examination.
2. Sono-mamography or MRI breast if needed.
3. CT chest, abdomen and pelvis.
4. Bone scan.
5. Echocardiography and left ventricular ejection fraction.
6. Fasting and postprandial blood sugar.
7. Baseline laboratory investigation (CBC, liver function tests (LFT) and kidney function tests (KFT).
8. Assesment of ER, PR and Her-2 neu status on pathology specimen.

During treatment evaluation:

1. Physical examination before each cycle.
2. CBC, LFT and KFT before each cycle.
3. Fasting and postprandial blood sugar.

Drug therapy:

1. Four cycles AC followed by 12 weeks paclitaxel 80 mg/m^2 plus trastuzumab and metformin 500 mg twice/day until the time of surgery versus four cycles AC followed by 12 weeks paclitaxel plus trastuzumab without metformin.
2. Adjuvant hormonal treatment in hormonal receptors-positive patients.

Surgery:

Two to three weeks after full course of chemotherapy.

Patients were evaluated after the end of treatment according to RECIST criteria (Response Evaluation Criteria in Solid Tumors**):**

A. Evaluation of target lesions: (Breast and axillary lymph nodes):

1. **Complete Response (CR):** Disappearance of all target lesions and reduction in the short axis measurement of all pathologic lymph nodes to < 10 mm.
2. **Partial Response (PR):** ≥ 30% decrease in the sum of the longest diameter of the target lesions compared to baseline.
3. **Progressive Disease (PD):** ≥ 20% increase of at least 5 mm in the sum of the longest diameter of the target lesions compared to the smallest sum of the longest diameter recorded **OR** The appearance of new lesions, including those detected by FDG-PET.
4. **Stable Disease (SD):**
5. Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD [9]**.**
6. **Pathological complete response (PCR):** The core of definition of PCR is achieving no residual

histological evidence of tumor (Breast and axillary lymph nodes) after chemotherapy at the time of surgery [10].

B. Evaluation of non target lesions:

1. **Pathological Complete response (PCR):** Disappearance of all non-target lesions and normalization of tumor marker levels
2. **Incomplete Response (IR) or Stationary disease (SD):** Persistence of 1 or more non-target lesions and/or the maintenance of tumor marker levels above normal limits.
3. **Progressive Disease (PD):** The appearance of 1 or more new lesions or unequivocal progression. If patient has measurable disease, an increase in the overall level or substantial worsening in non-target lesions, such that tumor burden has increased, even if there is SD or PR in target lesions. If not measurable disease, an increase in the overall tumor burden comparable in magnitude with the increase that would be required to declare PD in measurable disease [9].

Ethical approval and written informed consent:

An approval of the study was obtained from Aswan University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of the operation.

Statistical analysis:

Recorded data were analyzed using the statistical package for the social sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as median, range, and mean ± standard deviation (SD). Qualitative data were expressed as frequency and percentage. Independent-samples t-test of significance was used when comparing between two means. Chi-square (x^2) test of significance was used in order to compare proportions between two qualitative parameters. The confidence interval was set to 95% and the margin of error accepted was set to 5%. The p-value significance was considered as the following: P-value ≤ 0.05 was considered significant. P-value < 0.001 was considered as highly significant. P-value > 0.05 was considered insignificant.

RESULTS

Epidemiological characterestics (Table 1):

There was no statistically significant difference between the two groups regarding epidemiological characteristcs and comorbidities.

Table (1): Epidemiological characterestics and comorbidities

		Test group		Standard group		P value
		Count	%	Count	%	
Section A						
Age (yrs.)	Median	45		42		
	Range	31-55		24-57		**>0.05**
Menopausal status	Premenopausal	10	66%	11	73%	**>0.05**
	Postmenopausal	5	34%	4	27%	
Section B						
		Test group		**Standard group**		**P value**
Weight (kg)	Mean	76.04		70.65		**>0.05**
	Range	54-110		42-101		
Height (cm)	Mean	156.8		155.5		**>0.05**
	Range	144-173		146-165		
BMI	Mean	30.9		28.8		**>0.05**
	Range	20.3-42.9		18.6-42		
Section C						
		Test group		**Standard group**		**P value**
DM	No	11	73.00%	12	80.00%	**>0.05**
	Yes	4	27.00%	3	20.00%	
HTN	No	12	80.00%	12	80.00%	**>0.05**
	Yes	3	20.00%	3	20.00%	

Pathological characterestics (Table 2):

All patients in the 2 groups were submitted to tissue core biopsy from suspicious malignant lesion and were evaluated according to pathological subtypes, hormonal receptor status and biological stratification. All patients in both groups were proved to have invasive breast cancer by tissue core biopsy. Among the patients in 2 groupes, pathological subtypes detected was as follows: Invasive duct carcinoma (IDCa), invasive lobular carcinoma (ILCa), mixed invasive duct carcinoma (mixed IDCa) and mixed lobular carcinoma (mixed ILCa).

There was no statistically significant difference between the two groups regarding pathological subtypes, ER or PR.

Table (2): Pathological characterestics

		Test group		Standard group		p value
		Count	**%**	**Count**	**%**	
Section A						
Pathology	IDCa	22	88.00%	21	84.00%	>**0.05**
	ILCa	1	4.00%	3	12.00%	
	mixed IDCa and ILCa	2	8.00%	1	4.00%	
Section B						
		Test group		**Standard group**		**p value**
ER	Negative	6	40.00%	7	46.00%	>**0.05**
	Positive	9	60.00%	8	54.00%	
PR	Negative	6	40.00%	7	46.00%	>**0.05**
	Positive	9	60.00%	8	54.00%	

Surgical intervention post neoadjuvant chemotherapy (Table 3):

All patients were submitted to either modified radical mastectomy (MRM) or conservative breast surgery (CBS).

There was no statistically significant difference between the two groups regarding types of surgery.

Table (3): Surgical intervention

		Metformin		No metformin		
		Count	**%**	**Count**	**%**	**p value**
Surgery	MRM	9	60.00%	11	73.00%	>**0.05**
	CBS	6	40.00%	4	27.00%	

Response (Table 4):

1- Clinical response:

All patients in the 2 groups were evaluated clinically after the end of chemotherapy. No patients developed clinical progressive disease or stable disease.

There was no statistically significant difference between the two groups regarding T staging postneoadjuvant chemotherapy (p value 0.393). There was no statistically significant difference between the two groups regarding N staging post neoadjuvant chemotherapy (p value 0.343).

2- Radiological response:

All patients in the 2 groups were evaluated radiologically by breast ultrasound and mammography after the end of chemotherapy to detect any residual suspicious breast mass or residual suspicious lymph nodes. All patients achieved either complete remission or regressive disease. No patients developed radiological progressive disease or stable disease.

There was no statistically significant difference between the two groups regarding residual breast mass postneoadjuvant chemotherapy (p value 0.156). There was no statistically significant difference regarding radiological residual LNs postneoadjuvant chemotherapy (p value 0.417).

3- Pathological response:

All patients in 2 group achieved either pathological complete response or partial response.

There was statistically insignificant difference between the two groups regarding the response with better PCR in metformin group.

Table (4): Clinical, radiological and pathological responses

		Test group		Standard group		
		Count	**%**	**Count**	**%**	**p value**
Clinical assessment:						
T stage	T0	13	86.00%	12	80.00%	**>0.05**
	T1	2	14.00%	3	20.00%.	
LN stage	N0	13	86.00%	12	80.00%	**>0.05**
	N1	2	14.00%	3	20.00%	
Radiological assessment:						
Residual breast mass	No	12	80.00%	9	60.00%	**>0.05**
	Yes	3	20.00%	6	40.00%.	
Residual LN	No	12	80.00%	11	73.00%	**>0.05**
	Yes	3	20.00%	4	27.00%	
CR		**12**	**80%**	**9**	**60%**	**>0.05**
PR		**3**	**20%**	**6**	**40%**	

Correlation between PCR and different criteria (Table 5):

Table (5): Correlation between PCR and different criteria

Criteria		**PCR in Metformin**	**PCR in Non metformin**	**P value**
Age	<50	40%	24%	
	>50	20%	12%	**>0.05**
Menopause	Pre-menopause	60%	54%	**>0.05**
	Post-menopause	20%	14%	
Site of disease	LOQ Retroareolr UIQ UOQ LIQ Axilla	4% 28% 0% 20% 4% 4%	12% 4% 4% 8% 4% 4%	**>0.05**
Clinical staging	IIIA	48%	32%	**>0.05**
	IIIB	12%	4%	
Preoperative T	T2	4%	4%	**>0.05**
	T3	44%	28%	
	T4	12%	4%	
Preoperative N	N1	28%	20%	**>0.05**
	N2	32%	16%	
Pathology	IDCa	52%	32%	**>0.05**
	ILCa	4%	0%	
	Mixed	4%	4%	
Comorbidities	DM	12%	8%	
	HTN	16%	4%	**>0.05**
	No	40%	28%	
ER	+ve	54%	26%	**0.004**
	-ve	26%	34%	
PR	+ve	54%	26%	**0.004**
	-ve	26%	34%	

Regarding Adjuvant Hormonal Treatment (Table 6):

In test group: 9 patients (60 %) received adjuvant hormonal treatment while 6 patients (40%) not received adjuvant hormonal treatment. In standard group: 8 patients (54%) received adjuvant hormonal treatment while 7 patients (46%) did not receive adjuvant hormonal treatment.

Among the patients that received adjuvant hormonal treatment:

In test group, 7 patients (28%) received aromatase inhibitors (AI) while 11 patients (44%) received tamoxifen.

In standard group, 4 patients (16%) received aromatase inhibitors (AI) while 11 patients (44%) received tamoxifen.

Regarding Targeted therapy (Trastuzumab):

All patients in the two groups received neoadjuvant and adjuvant herceptin (complete 1 year) treatment. No patients stopped targeted therapy (Trastuzumab) either in neoadjuvant or adjuvant setting

Table (6): Adjuvant hormonal and target therapy

		Metformin		No metformin		
		Count	%	Count	%	p value
Adjuvant Hormonal Treatment	No	6	40.00%	7	46.00%	**>0.05**
	Yes	9	60.00%	8	54.00%	
Adjuvant Hormonal Treatment	AI	2	14.00%	2	14.00%	**>0.05**
	Tamoxifen	7	46.66%	6	40.00%	
Adjuvant Trastuzumab	No	0	00.00%	0	00.00%	**>0.05**
	Yes	15	100.00%	15	100.00%	

DISCUSSION

The role of metformin with neoadjuvant chemotherapy in breast cancer had been studied frequently. In a retrospective study that involved 2529 patients who received neoadjuvant chemotherapy for early-stage breast cancer between 1990 and 2007 **[6],** patients were compared by groups: 68 diabetic patients taking metformin, 87 diabetic patients not taking metformin, and 2374 non-diabetic patients who did not receive metformin. Pathological complete response (PCR) rate was 24% in the metformin group, 8.0% in the non-metformin group, and 16% in the non-diabetic group, i.e. diabetic patients with breast cancer who have received metformin and neoadjuvant chemotherapy had a significantly higher PCR rate than did diabetics not receiving metformin.

In another cross sectional study that involved 53 patients who received neoadjuvant chemotherapy for early-stage or locally advanced breast cancer receiving neoadjuvant systemic treatment from January 2007 to December 2015 **[11]**, patients were divided into two groups: 14 received metformin with systemic therapy, and 39 had systemic therapy alone. The PCR rate in the metformin group was 64.3% compared to 23.1% in the systemic therapy-alone group.

In another study, a phase 2 trial of neoadjuvant metformin in combination with trastuzumab and chemotherapy, **Martin-Castillo *et al.* [12]** studied 58 patients who received neoadjuvant chemotherapy in women with early HER2-positive breast cancer between June 2012 and March 2016. The patients were divided into two groups: 29 received metformin with systemic therapy plus trastuzumab, and 29 had systemic therapy plus trastuzumab without metformin. PCR rate was numerically higher in the metformin-containing arm A 65.5% (19 of 29 patients) than in arm B 58.6% (17 of 29 patients).

In our study, the median age in the test group was 45 years old (ranging from 31-55 years) while the median age in the standerd group was 42 years old (ranging from 24-57 years).

The median age in the test group in our current study is younger than metformin groups in **Jiralerspong *et al.* [6]** , **Van der Laata *et al.* [11]** and **Martin-Castillo *et al.* [12]**studies (45 in test group of our current study while 57.5 , 50.3 and 47.2 years respectively in their studies).

The median age in the standard group was younger than the median age of non-metformin groups in **Jiralerspong *et al.* [6]**, **Martin-Castillo *et al.* [12]** and **Van der Laata *et al.* [11]** studies (42 in standard group of our current study while 57, 49, 53.1 and 48 years respectively in the other studies**).**

In our current study, majority of patients were premenopausal (66 % in test group and 73 % in standard group). This percent is higher than percent of premenopusal patients in **Jiralerspong *et al.* [6]** study (22 % in metformin group and 16% and 49% in non-metformin group). While, in **Martin-Castillo *et al.* [12]** study, percent of premenopusal patients was 64% in metformin group and 58% in non-metformin group.

The percent of postmenopausal patients (34% in test group and 27 % in standard group. This

percent is lower than percent of postmenopusal patients in **Jiralerspong *et al.* [6]** study (78 % in metformin group and 48% and 51% in non-metformin group). While, in **Martin-Castillo *et al.* [12]** study percent of postmenopusal patients was (36% in metformin group and 42% in non-metformin group).

The mean body mass index (BMI, kg/m 2) in test group was 30.9 kg/m^2 while in standerd group was 28.8 kg/m^2. The mean BMI in the test group in our current study was lower than BMI in two groups in **Jiralerspong *et al.* [6]** study. While, it was higher than mean BMI in third group in **Jiralerspong *et al.* [6]** study (33.8, 32.8 and 26.9 respectively). The mean BMI in standard group was lower than BMI in two groups and higher than mean BMI in third group in **Jiralerspong *et al.* [11]** (33.8, 32.8 and 26.9 respectively).

Most of the patients in our study were free of comorbidities as only 4 patients in the test group and 3 patients in standerd group had history of comorbidities (D.M. and Hypertension).

In our study, all the patients were presented by more advanced local disease than the other groups having much higher percent of clinical stage III (100%) compared to retrospective and cross sectional studies (44%, 43% and 40% were stage III in retrospective study while 50% and 56 % in cross sectional study). No patients in our current study presented by stage I or stage II disease but in the other studies, percent of stage I disease at presentation was 0%, 1% and 5 % in the **Jiralerspong *et al.* [6]** study and 0 % in **Van der Laata *et al.* [11]** study. While, percent of stage II disease at presentation was 56%, 56% and 55 % in **Jiralerspong *et al.* [6]** study, and 50 % and 44% in **Van der Laata *et al.* [11]** study.

Esterogen and progesterone receptors (ER, PR) were detected among the patients in our current study. In the test group, ER and PR were positive in 9 patients and negative in 6 patients. In the standerd group, ER and PR were positive in 8 patients and negative in 7 patients.

Although all the patients achieved very good clinical and radiological response with marked reduction of primary lesion, majority of patients were submitted to modified radical mastectomy (MRM). That could be explained by the knowledge of the benefit of mastectomy among the patients at Upper Egypt as majority of patients think that mastectomy prevent recurrence but the disease may recurs in conservative surgery. Another point, some patients believe that conservative surgery has postoperative complications such as pain and breast edema. There was no statistically significant difference between the two groups regarding types of surgery.

All patients in the two groups in our study achieved either pathological complete response or partial response. No patients developed disease progression or were still stable disease. Among the patients in test group (metformin group), 12 patients (80%) achieved pathological complete response (PCR) while 3 patients (20%) did not achieve PCR. However, among the patients in standerd group (non-metformin group), 9 patients (60%) achieved pathological complete response (PCR) while 6 patients (40%) did not achieve PCR. In our current study, the rate of PCR in the test group was higher than that of the metformin group in **Jiralerspong *et al.* [6]** , **Van der Laata *et al.* [11]** and **Martin-Castillo *et al.* [12]** studies (80% versus 24 %, 64.3 and 65.5 respectively). Also, the rate of PCR in the standard group was higher than that of the non-metformin group in **Jiralerspong *et al.* [6]** and **Van der Laata *et al.* [11]** studies (60% versus 8%, 16% and 23.1% respectively, but lower than PCR rate in **Martin-Castillo *et al.* [12]** study (36 % versus 58.6%).

In our current study, there was statistically insignificant difference between the test and standard groups regarding the response with better PCR in metformin group. In **Jiralerspong *et al.* [6]** study, the rate of PCR was 24% in the metformin group, 8.0% in the non-metformin group and 16% in the nondiabetic group (P value is 0.02). Pairwise comparisons between the metformin and nonmetformin groups (P value is .007) and the nonmetformin and nondiabetic groups (P value is 0.04) were significant. Comparison of the PCR rates between the metformin and nondiabetic groups did not meet significance (P value is 0.1). This difference could be explained by difference in number of patients in the two studies (our current study and **Jiralerspong *et al.* [6]** study). In our current study, only 30 patients were included and were divided into 2 groups (test and standard), with equal number in each groups (15 patients for each). In **Jiralerspong *et al.* [6]** study, 2529 patients were included. Thus, we notice the big difference in total number of population in comparison to our current study and unequality of number of patients in 3 groups in retrospective study.

Another point, although number of patients in test group of our current study was lower than that of metformin group in the retrospective study of **Jiralerspong *et al.* [6]** (15 patients versus 68 patients), percent of premenopausal patients was higher in our study than that study (66% versus 22%). In addition, initial clinical stage was more advanced in the test group of our study than that of retrospective study (100% stage III versus 56% stage II and 44% stage III in retrospective study). PCR rate in our current study was higher than that of retrospective study (80% versus 24 %).

In **Van der Laata *et al.* [11]** study, that involved 53 patients who received neoadjuvant chemotherapy

for early-stage or locally advanced breast cancer, patients were divided into two groups: 14 received metformin with systemic therapy, and 39 had systemic therapy alone. The PCR rate in the metformin group was 64.3% compared to 23.1% in the systemic therapy-alone group (P = 0.008). In comparison to our current study, PCR rate in metformin group in **Van der Laata *et al.* [(11)]** study was lower that that of our current study (80 % versus 64.3%) with significant difference between metformin and non metformin groups (P value was 0.008). The difference in PCR rate could be explained by unequality of number of patients in metformin and non-metformin groups (14 patients in metformin group versus 39 patients in non-metformin group in cross sectional study while 15 patients in each group in our current study). There was more advanced initial clinical stage in the 2 groups in our study than that of the cross-sectional study (100% stage III in whole population in our current study versus 50% stage II and 50% stage III in metformin group and 43.6% stage II and 56.4% stage III in non-metformin group in the cross sectional study).

In **Martin-Castillo *et al.* [(12)]** study, 58 patients with early HER2-positive breast cancer received neoadjuvant chemotherapy. They were divided into two groups: 29 received metformin with systemic therapy plus trastuzumab and 29 had systemic therapy plus trastuzumab without metformin. The number of patients in our current study and **Martin-Castillo *et al.* [(12)]** study were comparable (30 versus 58) with equal number of patients in each group in the 2 studies. The rate of PCR in **Martin-Castillo *et al.* [(12)]** study was lower than that of our current study, the difference in PCR rate between the 2 groups in **Martin-Castillo *et al.* [(12)]** study did not meet statistically significant value (P value was 0.589).

CONCLUSION

The addition of metformin to trastuzumab and neoadjuvant chemotherapy has a nearby significant impact on pathological complete response (PCR) in female patients with advanced breast cancer with no significant increased toxicity.

REFERENCES

1. **Cortazar P, Geyer C (2015):** Pathological complete response in neoadjuvant treatment of breast cancer. Ann Surg Oncol., 22: 1441-1446.
2. **Decensi A, Puntoni M, Goodwin P *et al.* (2010)**: Metformin and cancer risk indiabetic patients: a systematic review and meta-analysis. Cancer Prev Res (Phila), 3:1451–1461.
3. **Foretz M, Guigas B, Bertrand L *et al.* (2014):** Metformin: from mechanisms of action to therapies. Cell Metab., 20:953–966.
4. **Goodwin P, Parulekar W, Gelmon K *et al.* (2015):** Effect of metformin vs placebo on and metabolic factors in NCIC CTG MA.32. J Natl Cancer Inst., 107(3):1-8.
5. **Cortazar P, Zhang L, Untch M *et al.* (2014):** Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet, 384:164–172.
6. **Jiralerspong S, Palla S, Giordano S *et al.* (2009):** Metformin and pathologic complete responses to neoadjuvant chemotherapy in diabetic patients with breast cancer. J Clin Oncol., 27: 3297– 3302.
7. **Anisimov V, Egarmin P, Piskunova T *et al.* (2010):** Metformin extends litespan of HER-2/neu transgenic mice and in combination with melatonin inhibits growth of transplantable tumors *invivo*. Cell Cycle, 9:188–197.
8. **Pollak M (2008):** Lnsulin and insulin-like growth factor signaling in neoplasia. Nat Rev Cancer, 8:915–928.
9. **Zhu P, Davis M, Blackwelder A *et al.* (2014)** Metforminselectively targets tumor-initiating cells in ErbB2-overexpressing breast cancer models. Cancer Prev Res (Phila), 7:199–210.
10. **He L, Wondisford F (2015):** Metformin action: concentrations matter. Cell Metab., 2015; 21:159–162
11. **Van der Laata A, Ramos-Esquivelb A, Landaverde D (2018):** Neoadjuvant Metformin Added to Systemic Therapy Increases Pathological Complete Response in Breast Cancer: A Cross-sectional Study, Mexico Hospital, Costa Rica. Arch Breast Cancer, 5 (1): 32-37.
12. **Martin-Castillo B, Pernas S, Dorca J *et al.* (2018):** A phase 2 trial of neoadjuvant metformin in combination with trastuzumab and chemotherapy in women with early HER2-positive breast cancer: the METTEN study. Oncotarget, 9 (86): 35687-35704.