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Research Article

Efficacy of Combining Metformin with Neoadjuvant Chemotherapy on Pathologic Response in Non-diabetic Patients with Carcinoma Breast- A Randomized Controlled Trial

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ABSTRACT

Background: In India breast cancer forms the commonest malignancy after cervical cancer in females and is detected in 20 per 1,00,000 women. Metformin acts as oral hypoglycemia drug and anti-tumor drug. Mechanism of action of metformin is to inhibit cellular proliferation as well as to increases pathological Complete Response in breast cancer patients when used in addition to neoadjuvant chemotherapy.

Methods: This randomized control trial study was conducted on 54 patients to assess the effect of adding metformin to neoadjuvant chemotherapy in pathologic response in Breast Cancer patients as well as to establish safety and tolerance of metformin as a neoadjuvant drug in Breast cancer and to measure the effect of metformin on sex hormones, tumor and insulin resistance dated from November 2016 to June 2018. Study group received metformin along with neoadjuvant chemotherapy and Control group received neoadjuvant chemotherapy only. In every visit, side effects of metformin were assessed like nausea, vomiting, abdominal discomfort, dizziness. Pre NACT-BMI and Post NACT- BMI were calculated and differences were assessed. Any post-operative complication was looked for post-surgery. Data was analysed by SPSS version 19.

Results: Our study showed that DHEAS level decreased by 5.65 in study group while the fall in DHEAS in non-metformin arm was 2.1. 7.1% of participants in metformin group showed complete response, 78.6% participants showed partial response and 14.3% had progressive disease. In non-metformin group, complete, partial response and progressive disease were seen in 40.0%, 60.0% and 0.0% respectively. Patient in control group had higher complete response. However, the difference in pathologic complete response between metformin and non-metformin group has no statistical significance (p= 0.057).

Conclusion: Our study supports the view that patient without insulin resistance treated with NACT alone has higher pathologic complete response than the patient treated with NACT with metformin. However, sample size of present study is small to support the results.

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Introduction

In India breast cancer forms the commonest malignancy after cervical cancer in females and is detected in 20 per 1,00,000 women [1]. For selected high-risk breast cancers, tumor size ≥ 2cm and locally advanced, neoadjuvant approach to breast cancer is established as a therapeutic regimen [2]. The neoadjuvant therapy reduces the tumor size and can make patients candidates for surgical resection or breast-conserving surgery rather than mastectomy [3]. Metformin is the drug of choice in diabetic patients found to have promising result as antineoplastic drug [4]. It exerts its anti-tumor activity through insulin-independent direct actions on cancer cell [5, 6]. Several studies have been conducted to evaluate mechanism of action of metformin on breast cancer. Mechanism of action of metformin is to inhibit cellular proliferation as well as to increases pCR in breast cancer patients when used in addition to neoadjuvant chemotherapy [7-10].

Pathologic complete response, (pCR), BMI, Insulin resistance (HOMA IR), Proliferative index and HER2/neu status was found to be strong predictor of response of metformin in breast cancer [11-19]. No RCT studies have been done in past to assess the pathologic response in non-diabetics or diabetics with breast cancer. Single large retrospective study was done by Sao Jiralerspong *et al.* to observe pathologic response in diabetic patient taking metformin along Neoadjuvant chemotherapy [20]. The study showed metformin had higher complete response than non-metformin in diabetic patients and diabetic with metformin group had higher complete response than non-diabetic group. In this context randomized trial study was conducted to assess the effect of adding metformin to neoadjuvant chemotherapy in pathologic response in Breast Cancer patients as well as to establish safety and tolerance of metformin as a neoadjuvant drug in Breast cancer and to measure the effect of metformin on sex hormones, tumor, and insulin resistance.

Patient and Methods

I Study Design

The study design was randomized controlled trial.

II Patient Selection

The sampling population was non-diabetic breast cancer women planned for neoadjuvant chemotherapy presented to JIPMER. All Non-diabetic Breast cancer ladies eligible for research were placed in two groups by randomization. All non-diabetic Breast cancer women with BMI more than 22 planned for neoadjuvant chemotherapy were included in the study. Patients with Renal disease, Hepatic disease, alcohol abuse, psychiatric disorder, pregnancy were excluded from the study.

III Sample Size

Since no data was available on the difference in the pathologic response between the patients who received and not received metformin along with chemo in NACT settings, the sample size was considered based on the logistics and feasibility. It was expected from the past records that about 100-120 non-diabetic breast cancer patients will receive neoadjuvant chemotherapy during this period. Therefore, we decided to include 54 participants.

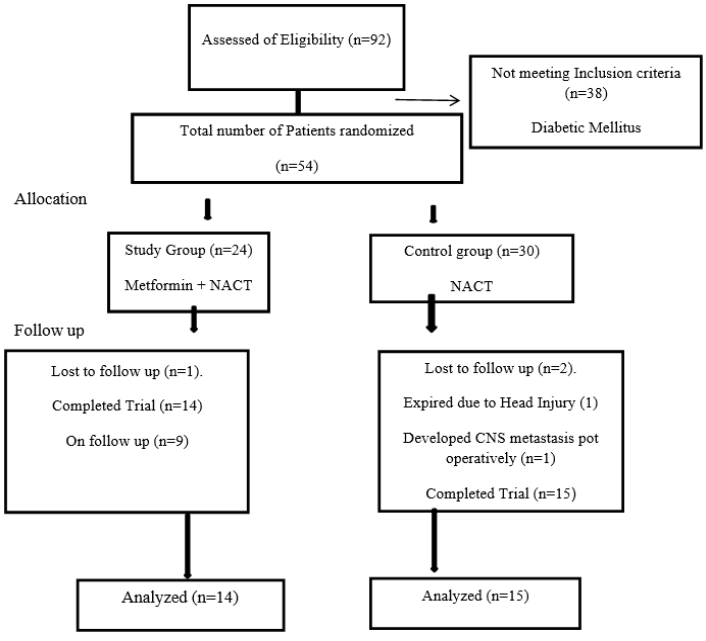

Figure 1: Consort Diagram of the study population.

IV Randomization

Block randomization with varying block size generated through computer was used to randomize the participants into groups. Blocks of size 4 and 6 were used. Allocation of group was done by SNOSE (Serially numbered opaque sealed envelope) technique, which was done immediately after plan for neoadjuvant chemotherapy was made. The consort diagram of study is presented in (Figure 1).

V Treatment

Study group received metformin along with neoadjuvant chemotherapy and Control group received neoadjuvant chemotherapy only. Participants in Study group were started with Metformin from day 1 of neoadjuvant chemotherapy up to the 7th cycle of neoadjuvant chemotherapy. Three cycles of FEC and four cycles of Docetaxel were given as a standard neoadjuvant therapy in this study. Metformin is known to cause hypoglycemia in few patients, so metformin was started initially with 500mg OD for 2 weeks and then 500mg BD for next 14 days and then 500mg TDS till 7th cycle of chemotherapy. Clinical staging and Bilateral Breast Imaging was done with CT scan and Ultrasonography (USG) 3 days after last cycle of neoadjuvant chemotherapy. After starting chemotherapy, 5 ml blood was collected. 1 ml of blood was sent to biochemical laboratory for fasting blood sugar. 4 ml of blood was then centrifuged at 3000 rotation per min for 3 minutes to collect plasma and was preserved in deep freezer. Participants underwent surgery (either modified radical mastectomy or breast conservation surgery). To ensure that participants are taking metformin regularly, pill count and calendar method was used in every visit. In pill count method fixed number of metformin was given to the participants and compliance was assessed in each visit. In calendar method, a form with date and time of intake column was given which the participants had to mark after having each pill.

VI Assessment

Before starting Chemotherapy, 5 ml blood was collected. One ml of blood was sent to biochemical laboratory for fasting blood sugar and remaining 4 ml of blood was then centrifuged at 3000 rotation per min for 3 minutes to collect plasma and was preserved in deep freezer minus 20C till biochemical parameters were measured. Estradiol, Testosterone, Dehydroepiandrosterone, Serum insulin, SHBG were measured with reagent and calibers (DHEA-S, Estradiol 2x 50 Det, SHBG, Testosterone, Ultrasensitive insulin, DHEA-s Cals S0- S5, Estradiol Cals S0-S5, SHBG Cals S0-S5, testosterone Cals S0-S5, Ultrasensitive) of Sabari Beckman Quilter, Chennai. Biochemical Technician doing the test was blinded. Serum Hormone levels and HOMA IR were compared in pretreatment and post treatment serum sample. Ki67 levels were measured from the trucut biopsy specimen pre NACT and from specimen after surgery. Ki67 reagent, a monoclonal antibody supplied by Sri Seikdant Company, Chennai was used to assess Ki67 level. Associate Professor from Department of Pathology JIPMER who was blinded for the study assessed the change in Ki67 levels and pathological response. Ki67 level was measured from trucut specimen taken before starting chemotherapy and from the specimen after definitive surgery.

Ki67 level were measured in all participants preoperatively and in case of post-operative, Ki67 level was measured in participants who developed metastasis and only if trucut biopsy was indicated as per treatment plan. Due to technical issue, Ki67 staining could not be performed in all samples. So Ki67 analysis could not be done. The pathological response was recorded and categorized as partial response, complete response and progressive disease based on the extent of residual tumor on histopathological examination. Pathologic complete response was defined as complete absence of tumor in breast and axilla in post-surgery specimen. Patients who developed metastasis were taken as progressive disease in pathologic response. A RECIST 1.1 criterion was used to evaluate the response from USG and CT findings. Assistant Professor from Department of Radiology who was blinded for the study assessed response based on RECIST 1.1 criterion. Response was categorized into partial, complete and stable/progressive disease. Clinical response was categorized into partial, complete and stable/progressive. Patient who developed metastasis post treatment was categorized into progressive disease in clinical response, response based on USG findings/ CT findings and pathologic response.

ER and PR were assessed using standard Immunohistochemistry with monoclonal antibody. The nuclear staining > 10% was taken as positive. HER2/neu was assessed using Immunochemistry with monoclonal antibody. 1+ is taken as negative, 2+ is taken as equivocal and 3+ is taken as positive. In every visit, side effects of metformin were assessed like nausea, vomiting, abdominal discomfort, dizziness. Pre NACT-BMI and Post NACT-BMI were calculated, and differences were assessed. Any Post-operative complication was looked for post-surgery.

VII Statistical Analysis

Data was analysed with SPSS version 19. The distribution of Categorical data such as clinical characteristics, co-morbidities, receptor status (ER, PR, and HER2/neu), pCR, response based on clinical, CT scan and USG findings, side effects of Metformin were expressed as frequency and percentages. The continuous data such as age, change in BMI, change in hormone levels and change in HOMA IR were expressed as median with range (95% CI). The comparison of the categorical variables mentioned above between the groups were carried out by using Chi square test (X^2) or fishers exact test. The comparisons of the continuous data in relation to the categorical variation were carried out using Mann Whitney test. The comparison of the clinical and pathological response between the groups were carried out by using X^2 or Fishers exact test whichever was appropriate. All statistical analysis was carried out at 5% level significance and p value < 0.05 was considered significant.

Result

Between November 2016 and June 2018, a total of 92 locally advanced breast cancer patients were enrolled into the study. Of these 38 patients were illegible. After excluding these 38 patients, a total of 54 were included in the study. Among them, 23 were in study group and 27 were in control group after randomization. One patient from study group and two patients from control group lost to follow up. One patient in study group expired from head injury. Nine patients from study group and eleven patients from control group are on follow up. A total of 14 patients from study group and 16 patients from control group completed the trial. One patient from control group had complete response and underwent surgery but she developed CNS metastasis post operatively. We could not rule out presence of metastasis pretreatment, so decided to exclude her from the study.

I Patients Demographic and Clinical Characteristics

The mean age of participants in present study was 46.9 years (SD 8.351). In our study, 55.2% of participants belonged to stage IIIA, 41.4% were in IIIB and remaining of 3.4% were in IIIC. Similarly, 6.9% fell in T2,

48.3% had T3 tumors, 3.4% had T4a and remaining 41.4% participants had T4b. Before treatment, 17.2% had N0 disease, 58.6% of participants had N1 disease, 20.7% of participants had N2 disease and remaining 3.4% had N3 disease. HER2/neu positive tumor was present in 27.6% of participants, 62.1% had HER2/neu negative receptor status and remaining 10.3% had HER2/neu equivocal. ER positive tumors were present 72.4% of participants while 27.6% of participants had ER negative tumor. Grade 1 tumor was present in 20.7% of participants and 62.1% participants had grade2 tumor and the remaining 17.2% had grade 3 tumor.

II Comparison of Demographic and Clinical Characteristics

The median age in metformin group was 44.50 (15) and in non-metformin group was 45 (8). However, the difference was not statistically significant (0.895). Our results showed that decrease in BMI between metformin and non-metformin group was not statistically significant (0.427). The difference in clinical staging, Histology and grade of tumor between two groups was insignificant (0.579, 0.483 and 0.250 respectively). In study group 71.4% were ER positive tumor while ER positive tumors were 73.3% in control group. In addition, 28.6% were HER2/neu positive, 64.3% were HER2/neu negative and 7.1% were HER2/neu equivocal in metformin arm. In non-metformin arm HER2/neu positive, HER2/neu negative and HER2/neu equivocal were 26.7%, 60% and 13.3% respectively. The difference in ER receptor status, PR receptor status and HER2/neu status in two groups was statistically insignificant (1.000, 0.710 and 1.000 respectively) (Table 1).

Table 1: Comparison of Demographic and Clinical Characteristics (N=29).

	Metformin (%)	**Non metformin (%)**	**p value**
Age in median years (IQR)	45.50 (15)	45 (8)	0.895
BMI in median (IQR)			
Baseline			
Post treatment			
	24.1 (1.45)	24.0 (2.1)	0.676
	23.8 (1.35)	23.2 (2)	0.930
Comorbidities			0.159
CAD	7.1	0	
Hypertension	28.6	6.7	
Hypothyroid	0	6.7	
Clinical stage		0.579	
IIIA	64.3	46.7	
IIIB	35.7	46.7	
IIIC	0.00	6.6	
T Stage		0.462	
T2	14.3	0	
T3	50	46.7	
T4a	0	6.6	
T4b	35.7	46.7	
N stage		0.762	
N0	14.3	20	
N1	57.1	60	
N2	28.6	13.3	
N3	0	6.7	
Tumor Histology		0.483	
IDC	92.9	100	
Medullary	7.1	0	
Grade		0.250	
1	7.2	33.3	
2	71.4	53.3	
3	21.4	13.4	
ER		1.000	
Positive	71.4	73.3	
Negative	28.6	26.7	
PR		0.710	
Positive	64.3	53.3	
Negative	35.7	46.7	

HER2/neu		1.000
Positive	28.6	26.7
Negative	64.3	60
Equivocal	7.1	13.3

Age and BMI are expressed in median. All other variables are expressed in percentage.

III Effect of Metformin in Hormone Levels and Insulin Sensitivity

Present study revealed that DHEAS level decreased by 5.65 in study group while the fall in DHEAS in non-metformin arm was 2.1. The serum testosterone level decreased in both groups post treatment (-5.5 in metformin vs. -9 in non-metformin). The serum estradiol decreased in both groups post treatment (-0.5 in metformin vs. -23 in non-metformin). The change in SHBG level shows opposite trend. The SHBG level increased in metformin groups and decreased in non-metformin group (9.42 vs. - 6.15 respectively). The difference in change in DHEAS, Testosterone, Estradiol and SHBG was statistically insignificant. The HOMA IR level increased in both groups post treatment (0.06 in metformin vs. 0.01 in non-metformin) and the difference between two groups was non-significant (p=0.861) (Table 2).

Table 2: Effect of Metformin in Hormone levels, Insulin Sensitivity (N=29).

	Metformin(n=14) Median (IQR)	Non metformin (n=15) Median (IQR)	Statistical significance p value
HOMA IR			
Baseline	0.69 (0.57)	0.38 (0.68)	0.513
Post Treatment	0.66 (0.51)	0.46 (0.94)	0.600
Testosterone (ng/dl)			
Baseline	46.00 (63)	25.00 (27)	0.285
Post Treatment	41.50 (48)	19.00 (32)	0.293
Estradiol (pg/ml)			
Baseline	40 (39.50)	51 (92.00)	0.395
Post Treatment	36 (22.75)	40 (30.00)	0.727
DHEAS (ug/dl)			
Baseline	54.95 (93.48)	56.4 (64.50)	0.793
Post Treatment	48.15 (84.93)	51.4 (81.60)	0.896
SHBG (nmol/L)			
Baseline	33.10 (25.42)	41.00 (26.60)	0.275
Post Treatment	39.65 (49.75)	38.00 (38.90)	0.662

Table 3: Effect of Metformin on Pathologic response, Clinical response and response based on CT and USG findings (N=29).

	Metformin n=14(%)	Non metformin n=15(%)	p value
Clinical			0.203
Partial	8(57.2)	5(33.3)	
Complete	3(21.4)	8(53.3)	
Stable/Progressive	3(21.4)	2(13.4)	
CT(RECIST 1.1)			0.370
Partial	7(50.0)	9(60.0)	
Complete	2(14.3)	4(26.7)	
Stable/progressive	5(35.7)	2(13.3)	
USG			0.044*
Partial	4(28.6)	10(66.7)	
Complete	4(28.6)	4(26.7)	
Stable/Progressive	6(42.8)	1(6.6)	
Pathologic			0.057
Partial	11(78.6)	9(60.0)	
	1(7.1)	6(40.0)	
Complete			
Progressive	2(14.3)	0(0.0)	

All values are expressed in percentage. *Statistical significance.

IV Response Based on Clinical Examination, CT Imaging and USG Imaging

The differences in response based on CT findings and Clinical examination was not statistically significant (p=0.370 and p=0.203 respectively). Based on USG findings, 42.8% had stable/progressive disease, 28.6% had complete response had complete response and 28.6% had partial response in metformin group. In control group 6.6% had stable/progressive disease, 26.7 had complete response and 66.7% had partial response. The difference in response based on USG findings was statistically significant (0.044). Based on USG findings, metformin group had higher stable/progressive disease compared to non-metformin group and control group. Patient who developed metastasis post treatment are categorized as progressive disease in all response (Table 3).

V Effect of Metformin on Pathologic Response

In our study, 7.1% of participants in metformin group showed complete response, 78.6% participants showed partial response and 14.3% had progressive disease. In non-metformin group, complete, partial response and progressive disease were seen in 40.0%, 60.0% and 0.0% respectively. Patient in control group had higher complete response. However, the difference in pathologic complete response between metformin and non-metformin group has no statistical significance (p= 0.057) (Table 3).

VI Post-Operative Complications

The results showed that 21.4% patient had surgical site infection (SSI) and 14.3% had seroma formation in study group while in non-metformin group, 6.7% had SSI and 6.7% had seroma formation. However, the difference in post-operative complication between two groups was not statistically significant (0.177). In Metformin group side effects of metformin could not be assessed because patients received chemotherapy concurrently.

Discussion

Present study revealed that 7.1% of participants in metformin group showed complete response, 78.6% participants showed partial response and 14.3% had progressive disease. In non-metformin group, complete, partial response and progressive disease were seen in 40.0%, 60.0% and 0.0% respectively. The difference in pathologic complete response between metformin and non-metformin group was noted to approach statistical significance (p= 0.057). In accordance to that retrospective study was done analysing pathologic outcomes in 3 groups- metformin, non- metformin and non-diabetic which observed pCR in metformin, non-metformin and non-diabetic group were 24%, 8% and 16% respectively (p=0.02). Analysing effects of metformin in hormone levels and in insulin resistance showed the HOMA IR level increased in both groups post treatment (0.06 in metformin vs. 0.01 in non-metformin) and the difference between two groups was non-significant (p=0). Metformin reduces insulin levels by 22% in non-diabetics with early-stage breast cancer and hence decreases insulin resistance [21].

In retrospective study done by Sao Jiralerspong *et al.*, 95% in metformin group had BMI>25 kg/m^2, 86% in non-metformin diabetic group had BMI> 25 kg/m^2 and 65% in diabetic group had BMI> 25 kg/m^2. In our study, all patients had BMI< 25 kg/m^2. It supports our findings that BMI< 25 kg/m^2 can have opposite effect in pathologic response than previous study. HER2/neu positive tumors were 31%, 19% and 25% in metformin, non-metformin, and diabetic group in Sao Jiralerspong study [4]. They also found that odd ratio of HER2/neu positive to HER2/neu negative in relation to complete pathologic response was 2.38 (p< 0.001). In the present study 28.6% and 26.7% in metformin and non-metformin group had HER2/neu positive tumors respectively (1.000). HER2/neu equivocal tumors were 7.15 and 13.35 in metformin and non-metformin group respectively. All five patients who had stable/progressive disease in metformin group were HER2/neu negative tumors. Patient who had complete response in metformin group had HER2/neu equivocal.

Present study showed that the serum testosterone level decreased in both groups post treatment likewise study conducted by Campagnoli *et al*. observed significant decrease in insulin level (-25%), HOMA (-29%), free testosterone (-29%), estradiol (-38%) [15]. No RCT studies have been done in past to assess the pathologic response in non-diabetics or diabetics with breast cancer. Single large retrospective study was done by Sao Jiralerspong *et al.* to observe pathologic response in diabetic patient taking metformin along neoadjuvant chemotherapy.

Conclusion

Present study was randomized control Trial to explore the efficacy of metformin. In non-diabetic with breast cancer showed Metformin has no antitumor effect in patient without Insulin Resistance (HOMA<2.5), HER2/neu negative tumors and patient with BMI < 25 kg/m^2. In fact, a patient without insulin resistance who is treated with metformin along with NACT found to have more stable/Progressive disease. Patient without insulin resistance treated with NACT alone has higher pathologic complete response than the patient treated with NACT with metformin. However, sample size of present study is small to support the results.

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Author Contributions

Dr. Niraj and Dr. Kadambari conceived of the study and participated in its coordination. Ms. Nisha framed the design of the study. Dr. Niraj contributed to the gathering of the data. Dr. Pampa, Dr. Sunitha, Dr. Sandhiya, Mrs. Nisha Ghimire and Dr. Bobby contributed to the gathering of the data and interpretation. Ms. Nisha carried out the literature review. Dr. Niraj, Ms. Nisha, Dr. Kadambhari contributed to the preparation of the manuscript. Dr. Niraj, Dr. Sanmugam, Dr. Bobbyy

and Ms. Nisha contributed to the refinement of the Manuscript. All authors have approved the final submission.

Conflicts of Interest

None.

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