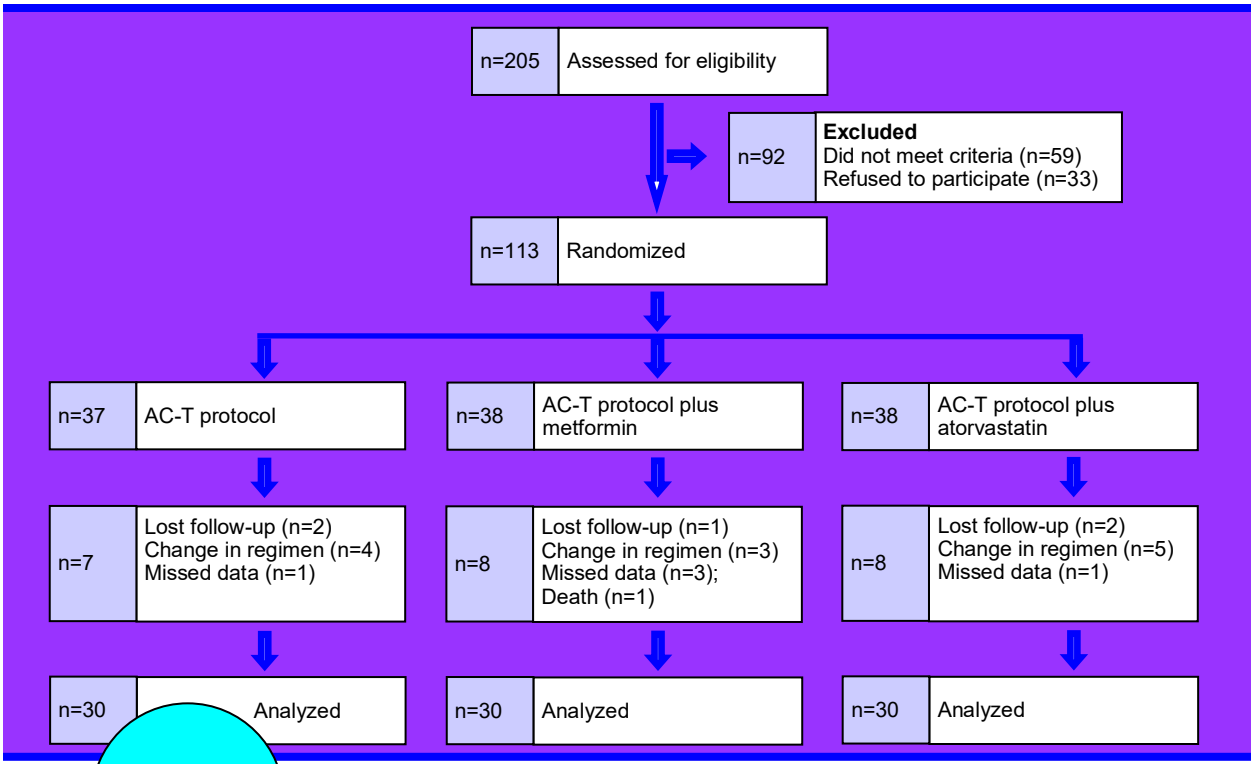


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Clinical Trial

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Clinical Trial

# Antitumor effects of metformin or atorvastatin as adjuvant therapies to neoadjuvant chemotherapy in non-metastatic breast cancer

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## Abstract

Breast cancer remains the most common life-threatening malignancy among women worldwide. This randomized, placebo-controlled study evaluated the antitumor effects of metformin or atorvastatin as adjuvant therapies combined with neoadjuvant chemotherapy in 90 patients with non-metastatic breast cancer. Patients received either the AC-T regimen (doxorubicin cyclophosphamide, and paclitaxel) with placebo, metformin (1,000 mg/day), or atorvastatin (20 mg/day) for 24 weeks. Serum levels of vascular endothelial growth factor (VEGF), Ki-67, and caspase-3 were assessed before and after treatment, and radiological and pathological responses were evaluated using RECIST 1.1 and Miller–Payne criteria. Either metformin or atorvastatin significantly reduced VEGF and Ki-67 levels and increased caspase-3 compared to the control group, indicating suppression of tumor proliferation and enhanced apoptosis. These effects were associated with improved radiological and pathological responses. Atorvastatin showed greater effects on VEGF reduction and caspase-3 elevation, in addition to cardioprotective activity against doxorubicin-induced toxicity. Both agents enhanced therapeutic outcomes in patients with non-metastatic breast cancer.

## Introduction

Breast cancer is the most frequently diagnosed life-threatening malignancy among women and remains a major global health burden. In many less-developed countries, it represents the leading cause of cancer-related mortality in women (Sung et al., 2021).

Current treatment strategies for breast cancer include surgery, radiotherapy, chemotherapy, hormonal therapy, and targeted therapy using drugs such as tamoxifen, trastuzumab, doxorubicin, paclitaxel, and cyclophosphamide (Burstein et al., 2003).

In recent years, several non-oncologic drugs have also demonstrated potential antitumor properties. Among antidiabetic agents, metformin has shown possible anticancer effects in breast cancer models and clinical studies (Ma et al., 2014). Additionally, statins such as simvastatin have been reported to exert antiproliferative and proapoptotic effects across various breast cancer models (Koyuturk et al., 2007).

Metformin demonstrated antitumor activity through both indirect and direct mechanisms. Indirectly, metformin reduces insulin levels by activating adenosine monophosphate-activated protein kinase (AMPK), which suppresses hepatic gluconeogenesis and consequently decreases circulating glucose and insulin levels. This reduction in insulin signaling may limit tumor growth and proliferation (Foretz et al., 2014). Directly, metformin inhibits the mammalian target of rapamycin complex 1 (mTORC1), a central regulator of cancer cell metabolism, growth, and proliferation (Chiang and Abraham, 2007). In addition, AMPK activation has been shown to suppress vascular endothelial growth factor (VEGF), inhibit angiogenesis, and activate caspase-3, a

key mediator of programmed cell death (Zheng et al., 2020). Furthermore, metformin inhibits aromatase activity, thereby reducing estrogen synthesis and potentially limiting hormone-dependent breast tumor progression (Peng et al., 2017). Collectively, these mechanisms support the potential role of metformin as an adjunct therapeutic agent in breast cancer management.

Statins exert anti-cancer effects through multiple molecular pathways. They suppress angiogenesis, promote apoptosis, and reduce tumor invasiveness and metastatic potential (Barbalata et al., 2020). By lowering low-density lipoprotein cholesterol (LDL-C), statins may also affect carcinogenesis, as rapidly proliferating tumor cells require increased cholesterol for membrane synthesis (Giacomini et al., 2021). Moreover, inhibition of the mevalonate pathway reduces the production of farnesyl pyrophosphate and geranylgeranyl pyrophosphate, which are essential isoprenoids involved in post-translational modification of small G-proteins that regulate tumor growth and progression (Gazzerro et al., 2012). Statins further inhibit angiogenesis by reducing VEGF expression and capillary tube formation (Vincent et al., 2001). In addition, they induce apoptosis by modulating pro- and anti-apoptotic proteins and activating caspase-3, caspase-7, caspase-8, and caspase-9 (Cafforio et al., 2005). Statins also attenuate pro-inflammatory cytokine production, which plays a crucial role in tumor initiation and metastasis (Landskron et al., 2014). Together, these effects suggest that statins may influence both early carcinogenesis and later stages of tumor progression, thereby affecting cancer incidence, recurrence, and mortality.

Importantly, concomitant use of statins with cytotoxic chemotherapy has been associated with reduced cardiotoxicity (Calvillo-Argüelles et al., 2019). Moreover, statins may enhance the efficacy of anti-cancer agents by synergistically promoting cell-cycle arrest, apoptosis, and increased chemosensitivity, while also overcoming drug resistance mechanisms (Tilija Pun and Jeong, 2021).

Despite advances in breast cancer treatment, there is still a need for safe, cost-effective adjuvant therapies that enhance chemotherapeutic efficacy while minimizing toxicity. Metformin and atorvastatin have shown promising anti-cancer and cardioprotective effects through distinct but complementary molecular pathways; however, clinical evidence supporting their combined use with standard chemotherapy in non-metastatic breast cancer remains limited. We hypothesized that the addition of metformin or atorvastatin to standard neoadjuvant chemotherapy improves tumor biological response and treatment outcomes in patients with non-metastatic breast cancer compared with chemotherapy alone.

This study aimed to evaluate the antitumor effects of metformin and atorvastatin as adjuvant therapies in combination with standard neoadjuvant chemotherapy in patients with non-metastatic breast cancer.

## Materials and Methods

### *Study design and population*

This study was designed as a randomized, placebo-controlled, parallel-group clinical trial. A total of 90 female patients with histologically and radiologically confirmed non-metastatic breast cancer were recruited from the outpatient Oncology Clinic of Tanta Oncology Center, Tanta, Egypt, between September 2022 and September 2024.

All enrolled patients were receiving neoadjuvant chemotherapy according to the AC-T protocol, which consists of doxorubicin (Adriamycin®) at a dose of 60 mg/m² intravenously combined with cyclophosphamide (Endoxan®) at a dose of 600 mg/m² intravenously every 3 weeks for four cycles, followed by paclitaxel (Taxol®) at a dose of 80 mg/m² weekly for 12 weeks. Patients were randomly allocated using a sealed-envelope method into three equal groups (n=30 each): the control group received the AC-T protocol plus placebo; the metformin group received the AC-T protocol plus metformin (1,000 mg/day); and the atorvastatin group received the AC-T protocol plus atorvastatin (20 mg/day). The total duration of the intervention was 24 weeks.

### *Inclusion criteria*

Eligible participants were female patients who met all of the following criteria: histologically and radiologically confirmed breast cancer, clinical stages I, II, or III according to the American Joint Committee on Cancer TNM staging system (Amin et al., 2017), age ≥ 18 years, Eastern Cooperative Oncology Group (ECOG) performance status <2, and patients planned for or receiving neoadjuvant chemotherapy according to the AC-T protocol.

### *Exclusion criteria*

Patients were excluded if they met any of the following criteria: metastatic breast cancer (stage IV), pregnancy or lactation, presence of significant hepatic disease, renal impairment, myopathy or known statin intolerance, and neurological or psychiatric disorders (including dementia or intellectual disability) that could impair understanding of or compliance with informed consent procedures. Patients with HER2-positive breast cancer were excluded to ensure biological and treatment homogeneity of the study population.

### *Clinical and demographic assessment*

At baseline, a full medical history was obtained for all participants, followed by comprehensive physical and

clinical examinations. Performance status was assessed using the Eastern Cooperative Oncology Group (ECOG) performance status scale. In addition, demographic characteristics were recorded, and anthropometric measurements, including weight and height, were obtained. Body mass index and body surface area were subsequently calculated.

### *Clinical and radiological evaluation*

All patients underwent radiological and pathological assessments at baseline and after 24 weeks of treatment. Radiological evaluation included diagnostic mammography and magnetic resonance imaging, while treatment response was assessed using the Response Evaluation Criteria in Solid Tumors (RECIST 1.1(Eisen-hauer et al., 2009). Pathological response was evaluated using the Miller–Payne grading system, which quantifies tumor response to chemotherapy (Ogston et al., 2003).

### *Blood sampling and biochemical analysis*

Venous blood samples (7 mL) were collected from each patient after 10–12 hours of fasting between 09:00 and 11:00 hours at baseline and after completion of the treatment period. Of this volume, 2 mL was used for a complete blood count, which was analyzed using an automated Cobas® e411 hematology analyzer (Roche, Germany).

The remaining 5 mL was transferred into plain tubes, allowed to clot, and centrifuged at 3,000 rpm for 10 min using a Hettich Zentrifugen EBA 20 centrifuge. Serum was separated and divided into two aliquots. The first aliquot was used immediately for routine biochemical analyses, including alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, serum creatinine, total bilirubin, and lipid profile, all measured using a fully automated Beckman Coulter/ Olympus AU680 Chemistry Analyzer (Japan).

The second aliquot was coded and stored at -80°C for subsequent analysis of VEGF and caspase-3 levels. VEGF was measured using ELISA kits (Shanghai Sunred Biological Technology Co., Ltd, China; Catalogue No: 201-12-008), while caspase-3 was determined using ELISA kits from the same manufacturer (Catalogue No: 201-12-0970).

### *Assessment of Ki-67 and hormonal receptors*

Immunohistochemical evaluation of Ki-67 (marker related to proliferation; Antigen Kiel-67), progesterone receptor, estrogen receptor, and human epidermal growth factor receptor 2 (HER2) was performed using an immunoassay analyzer (PEICH MARK/ULTRA, Germany; Catalogue No: 60222-MED-20257-1).

### *Assessment of treatment adherence and safety*

Patients were followed up weekly throughout the study period to monitor treatment adherence and document any adverse drug reactions. Adverse events were classified according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE), version 5.0 (2018).

### *Study outcomes*

The primary outcomes were overall response rate and pathological response, assessed at the end of the study using RECIST 1.1 criteria and the Miller–Payne grading system, respectively. The secondary outcomes included changes in serum biomarker levels, specifically VEGF, caspase-3, and Ki-67.

### *Sample size calculation*

The sample size was calculated using Stephen Thompson's equation (Thompson, 2012):

$$\mathbf{n} = \frac{\mathbf{N}\times\mathbf{P}(\mathbf{1}-\mathbf{P})}{\left[\left[(\mathbf{N}-\mathbf{1})\times\left(\frac{\mathbf{d}^2}{\mathbf{z}^2}\right)\right]+\mathbf{P}(\mathbf{1}-\mathbf{P})\right]}$$

Where N is the assumed population size (100,000,000), P is the probability value (0.063), d is the alpha error (0.05), and z is the z score (1.96) at 95% confidence level

The calculated total sample size (n) is 90 patients for all study groups.

### *Statistical analysis*

Statistical analysis was performed using IBM SPSS Statistics version 24.0 (SPSS Inc., USA, 2016). Data normality was assessed using the Shapiro–Wilk test. Categorical variables were analyzed using the chi-square test. Within-group comparisons between baseline and post-treatment values were performed using paired t-tests. Between-group comparisons were conducted using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple pairwise comparisons. Data were expressed as mean ± standard deviation (SD), frequency, and percentage as appropriate. A p-value $<0.05$ was considered statistically significant.

## Results

Out of 205 patients with non-metastatic breast cancer screened for eligibility, 92 patients were excluded either secondary to not meeting the inclusion criteria (n=59) or declined to participate (n=33). Therefore, only 113 patients were selected, randomized, and allocated into the three study groups. During the follow-up period, 23 patients in the three study groups dropped out secondary to loss of follow-up, change of regimen, missed data, or death. Hence, the final analysis included 90 patients (30 in each group; Figure 1).

### *Demographic and clinical characteristics*

The three study groups were matched for demographic and anthropometric data (age, weight, height, body mass index, and body surface area). The three study

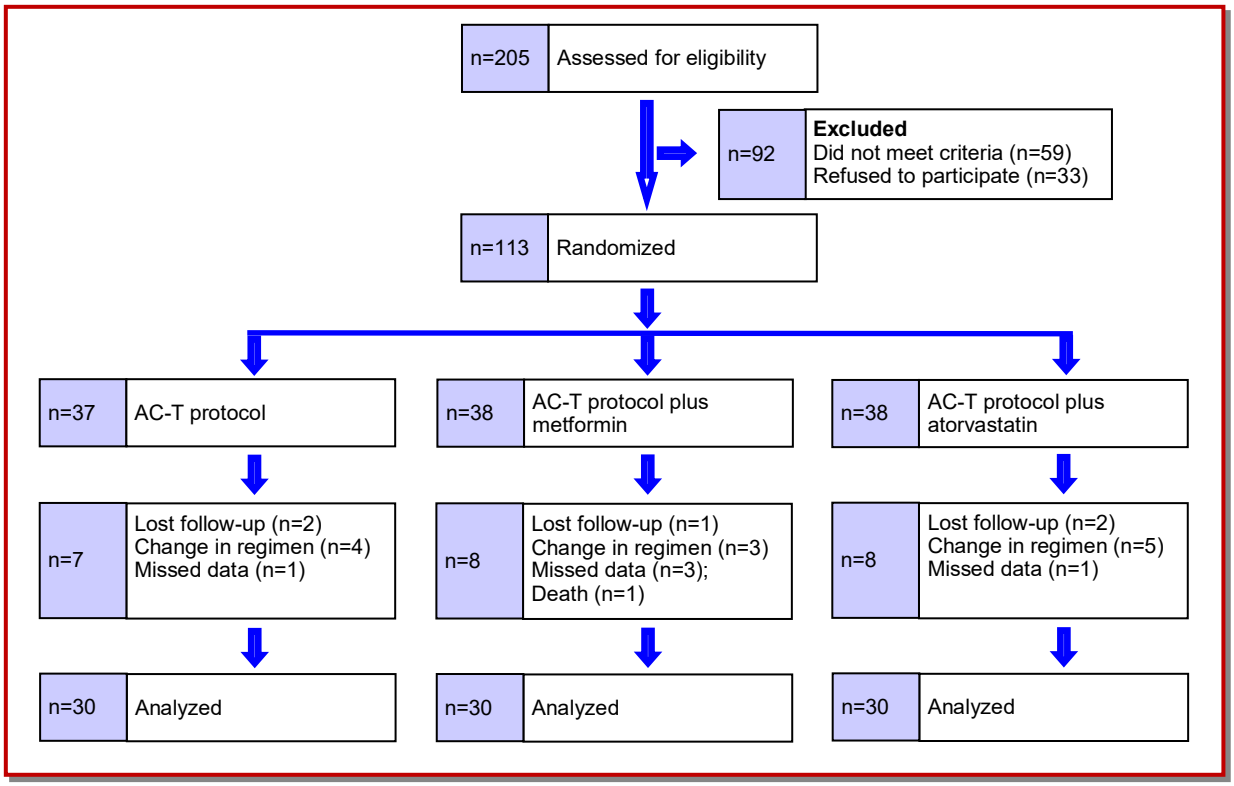


Figure 1: Participants flow chart

groups were matched in clinical characteristics (performance status, stage of disease, and hormonal status; Table I).

***Hematological parameters***

White blood cell count, absolute neutrophil count, absolute lymphocyte count, platelet count, red blood cell count, and hemoglobin concentration showed statistically non-significant variations among all study groups both at baseline and 24 weeks after the treatment (ANOVA, $p>0.05$). However, 24 weeks after intervention, these aforementioned hematological parameters showed a statistically significant decrease as compared to baseline data (paired t-test, $p<0.001$).

***Liver and kidney function parameters***

The comparison of the three study groups before and 24 weeks after the treatment revealed no significant differences in total bilirubin, alanine transaminase, aspartate transaminase, blood urea nitrogen, and serum creatinine levels ($p>0.05$).

In the control group, significant increases were observed in bilirubin, blood urea nitrogen, and serum creatinine after the intervention compared to baseline ($p<0.05$), accompanied by non-significant changes in alanine transaminase and aspartate transaminase ($p>0.05$).

In the metformin and atorvastatin groups, some liver and kidney function parameters showed statistically significant increases after the intervention compared to baseline; however, all values remained within the normal physiological range.

***Effects on selected proliferative and apoptotic markers***

At baseline and before the initiation of intervention, the levels of VEGF, caspase-3, and Ki-67 showed non-significant differences between the three studied groups (ANOVA test, $p>0.05$; Table II).

Twenty four weeks after the intervention, VEGF, caspase-3, and Ki-67 levels showed significant differences among the three study groups (ANOVA test, $p<0.05$). The three groups showed a highly significant decrease in VEGF and Ki-67 levels, which was associated with a highly significant increase in caspase-3 levels as compared to baseline levels (paired t-test, $p<0.01$).

Furthermore, an ANOVA analysis was used to compare the percentage mean changes of proliferative and apoptotic markers from baseline to the end of treatment among the three studied groups. The three groups were significantly different in the percentage mean changes of caspase-3 and Ki-67 levels and highly significantly different in the percentage mean changes of VEGF levels.

***Effects on radiological and pathological response***

After the intervention, the RECIST criteria used to evaluate radiological response showed that seven patients (23.3%) in the metformin group, six patients (20%) in

**Table I**

**Demographic and clinical characteristics**

| Parameters | Control (n=30) | Metformin (n=30) | Atorvastatin (n=30) | ANOVA (p-value) |
|---|---|---|---|---|
| Age (years) | 50.1 ± 8.8 | 49.2 ± 9.2 | 47.1 ± 9.4 | 0.425 |
| Weight (kg) | 69.6 ± 6.5 | 70.6 ± 5.6 | 70.2 ± 6.9 | 0.837 |
| Height (cm) | 165.0 ± 5.2 | 164.7 ± 5.0 | 163.9 ± 5.0 | 0.713 |
| Body mass index (kg/m$^2$) | 25.6 ± 2.2 | 26.1 ± 2.3 | 26.2 ± 2.5 | 0.635 |
| Body surface area (m$^2$) | 1.8 ± 0.1 | 1.8 ± 0.1 | 1.8 ± 0.1 | 0.893 |
| ECOG (Eastern Cooperative Oncology Group) score | | | | Chi-Square (p-value) |
| 0 | 19 | 18 | 18 | 0.999 |
| 1 | 9 | 10 | 10 | |
| 2 | 2 | 2 | 2 | |
| Stage | | | | |
| I | 5 | 6 | 2 | 0.941 |
| II | 19 | 17 | 7 | |
| III | 6 | 7 | 18 | |
| Estrogen receptor | | | | |
| Positive | 22 | 21 | 21 | 0.947 |
| Negative | 8 | 9 | 9 | |
| Progestin receptor | | | | |
| Positive | 18 | 18 | 18 | 1.000 |
| Negative | 12 | 12 | 12 | |

Data are mean ± SD; metformin 1,000 mg/day; atorvastatin 20 mg/day

the atorvastatin group, and only one patient (3.3%) in the control group exhibited a complete response. Furthermore, no one (0%) in both the metformin and atorvastatin groups, versus 2 patients in the control group (6.7%), developed progressive disease. The overall/objective response rate [sum of complete response and partial response] was 27 patients (90%) in both the metformin and atorvastatin groups, versus 19 patients (63.3%) in the control group. Furthermore, RECIST criteria and objective response rate showed a significant difference among the three groups (p=0.036, p=0.010, respectively; Table III).

Regarding Miller-Payne's criteria for grading pathological response, there was a significant difference among the three study groups (p=0.033). Five patients (16.7%) in the control group showed grade I (no change in malignant cells), compared with 1 patient (3.3%) in both the metformin and atorvastatin groups with grade I. One patient (3.3%) in the control group showed grade V (no malignant cells identifiable), and five patients (16.7 %) showed Grade IV (more than 90% loss of tumor cells). Regarding the metformin group, 5 patients (16.7%) showed Grade V and 11 patients (36.7%) showed Grade IV. Regarding the atorvastatin group, four patients (13.3%) showed Grade V, and 11 patients (36.7%) showed Grade IV. The detailed results of the RECIST criteria for radiological response and the Miller -Payne criteria for grading response are postulated in Table III.

### *Safety and tolerability of study medications*

All patients were asked and evaluated for drug-related adverse effects according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE, version 5, 2018). The safety and tolerability profiles of both treatment arms were fairly similar. Overall, the reported side effects were manageable, and all of them were mild and moderate (Grade I-II). The three study groups did not report significant toxicity (grade 3 or 4). Gastrointestinal side effects were the most frequent toxicity, particularly nausea and vomiting, and then diarrhea. There was no significant difference in the incidence of adverse events (leukopenia/neutropenia, thrombocytopenia, anemia, nausea/vomiting, diarrhea, alopecia, mucositis, difficult breathing/cough, myalgia, and elevated liver enzymes) among the three study groups (Table IV). However, a significant increase in the incidence of cardiac toxicity was reported in both the control and metformin groups [7 (23.3%) and 6 (20.0%), respectively] as compared to the atorvastatin group [1 (3.3%); p=0.041].

**Table II**

**Selected proliferative and apoptotic markers**

| Parameters | Control (n=30) | Metformin (n=30) | Atorvastatin (n=30) | ANOVA p-value |
|---|---|---|---|---|
| *VEGF (ng/L)* | | | | |
| At baseline | 2061.2 ± 783.6 | 1931.2 ± 884.6 | 2059.5 ± 902.9 | 0.8 |
| After 24 weeks | 1766.3 ± 634.5 | 1286.9 ± 546.8a** | 1156.3 ± 441.0b** | <0.001** |
| Paired test (p-value) | 0.003** | <0.001** | <0.001** | |
| *Caspase-3 (ng/mL)* | | | | |
| At baseline | 4.8 ± 3.5 | 4.4 ± 3.5 | 2.9 ± 2.3 | 0.055 |
| After 24 weeks | 6.3 ± 4.1 | 6.3 ± 4.1 | 4.0 ± 2.0b*, c* | 0.0185* |
| Paired test (p-value) | <0.001** | <0.001** | <0.001** | |
| *Ki-67 (%)* | | | | |
| At baseline | 47.7 ± 21.6 | 50.7 ± 22.3 | 48.0 ± 22.8 | 0.8 |
| After 24 weeks | 26.7 ± 12.1 | 18.7 ± 8.2a** | 18.3 ± 8.3b** | 0.001** |
| Paired test (p-value) | <0.001** | <0.001** | <0.001** | |
| | | | | ANOVA p-value |
| VEGF (ng/L) | -10.8 | -27.8a** | -37.5b** | <0.001** |
| Caspase-3 (ng/mL) | 40.9 | 67.8 | 78.4b* | 0.028* |
| Ki67 (%) | -38.3 | -54.2a* | -54.6b* | 0.030* |

Data are mean ± SD; VEGF: Vascular endothelial growth factor; *p<0.05; **p<0.01; a*: Significant difference comparing group 2 with group 1 (Post hoc test); b*: Significant difference comparing group 3 with group 1 (Post hoc test); c*: Significant difference comparing group 3 with group 2 (Post hoc test)

**Table III**

**RECIST criteria and Miller-Payne criteria for grading pathological response**

| | Parameters | Control (n=30) | Metformin (n=30) | Atorvastatin (n=30) | Chi-Square (p-value) |
|---|---|---|---|---|---|
| RECIST criteria | Complete response | 1 | 7 | 6 | |
| | Partial response | 18 | 20 | 21 | |
| | Stable disease | 9 | 3 | 3 | 0.036* |
| | Progressive disease | 2 | 0 | 0 | |
| | Objective response rate | 19 | 27 | 27 | 0.010* |
| Miller-Payne grading | Grade I | 5 | 1 | 1 | |
| | Grade II | 12 | 4 | 4 | 0.033* |
| | Grade III | 7 | 9 | 10 | |
| | Grade IV | 5 | 11 | 11 | |
| | Grade V | 1 | 5 | 4 | |

Data are mean ± SD; RECIST: Response evaluation criteria in solid tumors

## Discussion

The present study evaluated the potential antitumor effects of metformin or atorvastatin as adjuvant therapies in combination with standard neoadjuvant chemotherapy (AC-T protocol) in patients with non-metastatic breast cancer. At the molecular level, metformin significantly modulated tumor-related biomarkers, characterized by reduced expression of VEGF and Ki-67 and increased levels of caspase-3. These findings suggest a simultaneous suppression of angiogenic signaling, a reduction in cellular proliferation, and activation of apoptotic pathways. Mechanistically, metformin exerts its antitumor effects primarily through the activation of AMP-activated protein kinase (AMPK), which leads to inhibition of the mTOR pathway, a central regulator of cancer cell growth and metabolism. Additionally, AMPK activation suppresses downstream oncogenic signaling pathways involved in angiogenesis and tumor progression, including VEGF-mediated vascular development (Wang et al., 2015). It has been demonstrated that metformin inhibits VEGF expression in breast cancer cells (Farahi et al., 2021). Furthermore, present results align with previous clinical trials evaluating the

**Table IV**

**Selected proliferative and apoptotic markers**

| Adverse effects | Control (n=30) | Metformin (n=30) | Atorvastatin (n=30) | Chi-Square p-value |
|---|---|---|---|---|
| *Hematological toxicity* | | | | |
| Leukopenia/neutropenia | 6 | 7 | 5 | 0.812 |
| Thrombocytopenia | 3 | 4 | 3 | 0.894 |
| Anemia | 5 | 6 | 4 | 0.787 |
| Febrile neutropenia | 0 | 0 | 0 | |
| *Non-hematological toxicity* | | | | |
| Nausea/vomiting | 20 | 19 | 19 | 0.953 |
| Diarrhea | 9 | 11 | 7 | 0.530 |
| Alopecia | 6 | 5 | 4 | 0.787 |
| Mucositis | 5 | 5 | 5 | 1.000 |
| Cardiac toxicity | 7 | 6 | 1 | 0.041* |
| *Metformin related toxicity* | | | | |
| Lactic acidosis | 0 | 0 | 0 | |
| Cough | 2 | 2 | 2 | 1.000 |
| *Statin related toxicity* | | | | |
| Myalgia | 3 | 4 | 7 | 0.333 |
| Rhabdomyolysis | 0 | 0 | 0 | |
| Elevated liver enzymes | 4 | 5 | 6 | 0.787 |

effect of metformin on 40 patients with early-stage breast cancer and 30 patients with operable early-stage breast cancer. These trials revealed that metformin decreased Ki-67 levels and reduced the proliferation rate (Dowling et al., 2015; Rattan et al., 2012).

Furthermore, the increase in caspase-3 supports a pro-apoptotic effect, which may be mediated through mitochondrial dysfunction and energy stress–induced apoptotic signaling, as previously demonstrated in experimental breast cancer models (Fatehi et al., 2023).

In this study, patients in the atorvastatin group exhibited a highly significant increase in proliferative markers (VEGF and Ki-67) and a significant decrease in apoptosis markers (caspase-3). The present findings align with previous reports indicating that elevated LDL levels promote breast cancer cell proliferation and induce genetic changes that negatively impact breast cancer prognosis. This may explain the beneficial effects observed with atorvastatin in the current study. These results are consistent with those of other researchers who reported that atorvastatin reduces proliferation, metastasis, and VEGF protein expression in ovarian cancer (Jones et al., 2017). Additionally, simvastatin has been shown to exert antiproliferative and antimetastatic effects in endometrial and breast cancers, respectively (Schointuch et al., 2014; Wolfe et al., 2015). The present findings also concur with previous studies demonstrating that statins induce apoptosis through activation of caspase-3 (Campbell et al., 2006). It has been proposed that statins decrease tumor cell proliferation (as evidenced by reduced Ki-67 staining) and enhance apoptosis (indicated by increased cleaved caspase-3 staining) in breast cancer (Yulian and Siregar, 2021). Furthermore, simvastatin has been reported to induce apoptosis (increased cleaved caspase-3) in endometrial cancer (Schointuch et al., 2014).

The findings of the current study revealed significant improvements in both radiological and pathological responses in patients treated with metformin and atorvastatin. Seven patients (23.3%) in the metformin group and six patients (20%) in the atorvastatin group, compared to one patient (3.3%) in the control group, showed a complete response. Regarding pathological evaluation using the Miller-Payne grading system, five patients (16.7%) in the metformin group and four patients (13.3%) in the atorvastatin group, compared with one patient (3.3%) in the control group, demonstrated grade V (no identifiable malignant cells). These findings from the current study align with a previous study that reported an improvement in the pathological complete response (pCR) rate in breast cancer patients, with or without diabetes, who received metformin (Jiralerspong et al., 2009). Additionally, our results are consistent with prior research showing that the addition of simvastatin to neoadjuvant chemotherapy improved the objective response rate and pathological complete response (pCR) according to Miller-Payne criteria, while also producing a cardioprotective effect in patients with early-stage breast cancer (Yulian and Siregar, 2021). Similarly, a retrospective multivariate analysis of 349 patients with rectal cancer revealed that the complete response rate among statin users was four times higher than that of non-

users (Katz et al., 2005).

The overall findings of the present study revealed greater improvement in patients with breast cancer who received either metformin or atorvastatin in combination with chemotherapy compared to those who received chemotherapy alone. This improvement may be attributed to the synergistic or additive antitumor effects of metformin and atorvastatin. Consistent with present findings, several studies have reported that metformin, when combined with other cytotoxic drugs, acts in an additive or synergistic manner to enhance anti-cancer effects (Peng et al., 2017). Additionally, it has been demonstrated that statins, in combination with cytotoxic drugs, also act synergistically or additively to enhance anti-cancer efficacy (Tilija Pun and Jeong, 2021). Furthermore, statins have been reported to counteract resistance developed against various anti-cancer drugs (Sethunath et al., 2019; Tilija Pun and Jeong, 2021).

Additionally, both agents are widely used, inexpensive, and have well-established safety profiles, making them attractive candidates for drug repurposing in oncology. Integrating these agents into standard chemotherapy regimens could offer a practical and rapidly translatable approach to improving patient outcomes, particularly in resource-limited settings.

Regarding safety, both interventions were generally well tolerated, with no serious adverse events reported. Most adverse effects were mild and transient. Importantly, a lower incidence of cardiotoxicity was observed in the atorvastatin group compared to the other groups. This finding is clinically significant, given the known cardiotoxic potential of doxorubicin in the AC-T regimen. The cardioprotective effect of atorvastatin may be attributed to its antioxidant, anti-inflammatory, and endothelial-protective properties, as previously documented (Calvillo-Argüelles et al., 2019).

The strengths of this study include its randomized, placebo-controlled design; the use of standardized chemotherapy across all groups; the comprehensive assessment of molecular biomarkers alongside radiological and pathological outcomes; and a relatively adequate follow-up duration. Additionally, the use of consistent pharmaceutical formulations throughout the study enhanced internal validity.

Several limitations include a) relatively small sample size, b) different dose levels of metformin and atorvastatin were not evaluated, and c) the investigational drugs were administered only during the neoadjuvant setting, precluding a more comprehensive assessment of potential dose–response relationships and long-term therapeutic effects.

## Conclusion

Both metformin and atorvastatin groups showed decreased proliferation and increased apoptosis of cancer cells, which was associated with improved radiological and pathological response in patients with breast cancer. Atorvastatin produced a significant decline in the incidence of doxorubicin-related cardiotoxicity secondary to its cardio-protective effect. Metformin or atorvastatin could represent a useful adjuvant therapy with cytotoxic drugs in patients with early-stage breast cancer.

## Financial Support

Self-funded

## Ethical Issue

The study was approved by the National Research Ethics Committee of Tanta University (Approval Code: 35557/6/22) and registered as a clinical trial at ClinicalTrials.gov (Identifier: NCT05507398). Written informed consent was obtained from all participants before enrollment. The study was conducted in accordance with the principles of the Declaration of Helsinki.

## Conflict of Interest

Authors declare no conflict of interest

## Acknowledgement

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