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ORIGINAL RESEARCH ARTICLE

The Effect of Metformin on Chemotherapy-Induced Toxicities in Non-diabetic Breast Cancer Patients: A Randomised Controlled Study

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Abstract

Background and Objective Breast cancer patients treated with adriamycin-cyclophosphamide plus paclitaxel (AC-T) are often challenged with serious adverse effects for which no effective therapies are available. Here, we investigated whether metformin, an antidiabetic drug with additional pleiotropic effects could favourably offset AC-T induced toxicities.

Patients and Methods Seventy non-diabetic breast cancer patients were randomised to receive either AC-T (adriamycin 60 mg/m^2 + cyclophosphamide 600 mg/m^2 × 4 cycles Q21 days, followed by weekly paclitaxel 80 mg/m^2 × 12 cycles) alone or AC-T plus metformin (1700 mg/day). Patients were assessed regularly after each cycle to record the incidence and severity of adverse events based on the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE), version 5.0. Moreover, baseline echocardiography and ultrasonography were done and repeated after the end of neoadjuvant therapy.

Results Addition of metformin to AC-T resulted in significantly less incidence and severity of peripheral neuropathy, oral mucositis, and fatigue ($p < 0.05$) compared to control arm. Moreover, the left ventricular ejection fraction (LVEF%) in the control arm dropped from a mean of 66.69 ± 4.57 to 62.2 ± 5.22% ($p = 0.0004$) versus a preserved cardiac function in the metformin arm (64.87 ± 4.84 to 65.94 ± 3.44%, $p = 0.2667$). Furthermore, fatty liver incidence was significantly lower in metformin compared with control arm (8.33% vs 51.85%, $p = 0.001$). By contrast, haematological disturbances caused by AC-T were preserved after concurrent metformin administration ($p > 0.05$).

Conclusion Metformin offers a therapeutic opportunity for controlling toxicities caused by neoadjuvant chemotherapy in non-diabetic breast cancer patients.

Trial Registration This randomised controlled trial was registered on November 20, 2019 in ClinicalTrials.gov under registration number: NCT04170465.

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Key Points

Metformin reduces the incidence and severity of AC-T-evoked peripheral neuropathy, oral mucositis, fatigue, and cardiac and hepatic complications.

Haematological and gastrointestinal complications of AC-T therapy are preserved following metformin administration.

1 Introduction

Breast cancer (BC) is the most common type of cancer encountered in females in Egypt and worldwide. In 2020, the International Agency for Research on Cancer GLOBOCAN 2020 reported that the prevalence of BC in Egypt was 121 per 100,000 Egyptian females [1]. Although chemotherapy is fundamental in the management of BC, the erupting adverse events (AEs) sometimes necessitate the interruption of therapy and mandate the use of less effective regimens [2–4]. Adriamycin (doxorubicin) combined with cyclophosphamide, followed by weekly paclitaxel (i.e., AC-T) regimen is commonly used in Egypt in the neoadjuvant and adjuvant treatment of BC [5, 6]. A frequently reported AE encountered in paclitaxel-treated patients is peripheral neuropathy [7]. Paclitaxel-induced peripheral neuropathy (PIPN) affects the quality of life of patients, and is also a dose-limiting adverse effect that can impede cancer therapy and affect cancer prognosis [8, 9]. Oral mucositis is another common toxicity of AC-T regimen that appears in 77% of patients [3, 10]. The combination of doxorubicin and cyclophosphamide causes a 3-fold increase in the risk of oral mucositis compared to individual drugs [11]. Oral mucositis reduces food intake and body immunity, making patients more liable to life-threatening systemic infections [12]. The AC-T regimen causes distressful fatigue in almost all BC patients, which is not relieved by rest [3, 13]. Cardiotoxicity is a major life-threatening AE of doxorubicin, which can progress to irreversible congestive heart failure [4]. Additionally, chemotherapy induces hepatic steatosis as a result of the oxidative stress generated in hepatocytes, which if left untreated, can progress to steatohepatitis and hepatocytes degeneration [14]. Among chemotherapeutic regimens used in BC treatment, the AC-T regimen accounts for more than 50% of fatty liver cases [2, 14].

To date, no effective pharmacologic therapies have been identified to prevent or treat the above-mentioned devastating chemotherapy-induced toxicities. Management of PIPN is limited to symptomatic treatment with pain killers [8]. Numerous medications have been proposed for chemotherapy-induced oral mucositis, but not one has proven completely beneficial [15]. Additionally, no current therapies have proven effective in alleviating cancer-related fatigue [16, 17]. On the other hand, dexrazoxane has been the only FDA-approved cardioprotective agent against doxorubicin-induced cardiotoxicity. However, its use has been limited because of increased incidence of secondary malignancies and reduced anti-tumour efficacy of doxorubicin [18, 19]. Lifestyle interventions such as exercise and a healthy low-fat diet can lower the incidence of non-alcoholic fatty liver disease (NAFLD) [20].

The antidiabetic drug metformin has been shown to favourably reprogramme molecular and cellular pathways that arbitrate the clinical presentation of several diseases including cancer [21–23]. Such advantageous actions of metformin are largely attributed to the upregulation of adenosine monophosphate-activated protein kinase (AMPK) and consequent interruption of downstream inflammatory and antioxidative cascades [21, 22]. Oncology research has established antitumor benefits for metformin including its ability to reduce metastasis risk, facilitate chemotherapy efficacy, and improve patient survivability, and clinical and pathologic outcomes [24–29]. However, little or no information is available to date regarding whether metformin could protect or at least minimise the troublesome sequels of AC-T chemotherapy. In the current randomised clinical trial, we tested the hypothesis that the simultaneous administration of metformin rectifies the AEs caused by the neoadjuvant AC-T regimen in chemotherapy-naïve non-diabetic BC patients.

2 Materials and Methods

2.1 Study Design and Participants

This is a multicentre randomised controlled trial conducted at the Medical Research Institute and Alexandria Main University Hospital, Alexandria University, Egypt. Institutional ethical approvals by Alexandria University (IRB no. 00012098), and Damanhour University (IRB no. 919PP18) were obtained before commencement of the study. The study was conducted in compliance with the Declaration of Helsinki. All study participants provided a written informed consent. This study is a part of the METNEO study registered in ClinicalTrials.gov under registration number: NCT04170465 (https://clinicaltrials.gov/ct2/show/NCT04170465).

Patients were eligible if they were aged 18–65 years, candidates for neoadjuvant chemotherapy, received no prior chemotherapy, and had baseline left ventricular ejection fraction (LVEF) greater than 50%, normal liver functions, and Eastern Cooperative Oncology Group Performance Status (ECOG-PS) from zero to two. Patients with diabetes, history of anti-diabetic medication use, pregnancy, breastfeeding, metastatic or recurrent breast cancer, or increased risk of metformin-induced lactic acidosis, such as those with heart failure or estimated glomerular filtration rate (eGFR) ≤ 45 mL/min/1.73 m^2 were excluded from the study. Fasting blood glucose level test was done for patients with no history of diabetes to make sure they were not underdiagnosed and were eligible for participation in the study. Also, baseline LVEF%, renal and liver function, and complete blood count with differentials were determined.

Eligible patients were randomised in a 1:1 ratio to either metformin or control arms using blocked randomisation with random block sizes of two, four, and six. The randomisation scheme was generated by using the website "http://www.randomization.com". Allocation to either arm was concealed using sealed numbered envelopes by an independent third-party individual. The control arm received AC-T chemotherapy 16 cycles protocol (i.e., doxorubicin 60 mg/m^2 slow intravenous [IV] push over 10–15 min and cyclophosphamide 600 mg/m^2 IV infusion over 1 h every 21 days for a total of four cycles followed by paclitaxel 80 mg/m^2 IV infusion over 1 h every week for a total of 12 cycles). The metformin arm received the same chemotherapy protocol in addition to oral metformin 850 mg once daily for 1 week followed by 850 mg twice a day during the entire neoadjuvant treatment period and stopped metformin only 3–7 days before surgery. Metformin was slowly titrated and was prescribed with meals to reduce the incidence of associated gastrointestinal (GI) side effects. Moreover, vitamin B12 was supplemented to study participants when peripheral neuropathy symptoms develop in both metformin and control arms.

Patients were interviewed in person and data about birthdate, weight, height, ECOG-PS, menopausal status, history of gestation, pregnancy and abortion, breastfeeding, method of contraception, other comorbidities, and family history were collected.

2.2 Monitoring for Adverse Events

Complete blood picture with differentials was obtained, and patients were monitored for the incidence of haematological side effects (i.e., anaemia, thrombocytopenia, neutropenia, and febrile neutropenia) before each of the 16 cycles. Also, non-haematological side effects (i.e., fatigue, nausea, vomiting, diarrhoea, constipation, oral mucositis, and peripheral sensory neuropathy) were monitored after each chemotherapy session during the whole treatment period. At baseline, echocardiography and ultrasonography for abdomen and pelvis were done. Moreover, renal and liver function tests were assessed before each cycle.

Before the commencement of neoadjuvant therapy, patients were interviewed in person and patient education about the proper measures for preventing and self-management of oral mucositis was provided equally in both arms. After each chemotherapy session, each participant was assessed within the first week and again after 18–21 days from the session by an independent physician and the study investigator by telephone or face to face. Patients were assessed for the occurrence of any AE based on the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 5.0 [30], using an assessment form extracted from the NCI-CTCAE v.5 that contains the CTCAE terms for the studied AEs and their severity grades from 1 to 5 with a checkbox underneath each grade, and a key providing the definitions for the AEs and the corresponding severity grades (Electronic supplementary file 1). Moreover, echocardiography and ultrasonography were repeated after completing treatment, to assess the state of the patient before surgery. The haematological toxicities, cardiotoxicity, and hepatotoxicity were assessed by a blind investigator.

2.3 Statistical Analysis

All statistical analyses were performed using Stata version 14.2. For interval variables, Shapiro–Wilk test was used to inspect data normality, Levene's test was used to check equality of variances between arms, followed by the two-sided two-sample independent *t* test with equal or unequal variances, which was used as appropriate to test for differences between treatment arms. Pearson's Chi-square, or Fisher's exact tests were used as appropriate for categorical variables. To detect the difference in the severity grades of different AEs (i.e., peripheral neuropathy, oral mucositis, fatigue, gastrointestinal, and haematological adverse effects) between the two arms at each time point, the Mann–Whitney *U* test was used, and data were expressed as frequencies and percentages of occurrence of different severity grades. Then ordered logistic regression was performed to detect the effect size of treatment per cycle, and the odds ratio (OR) with its 95% confidence intervals (CI) was reported. On the other hand, the paired *t* test was used to compare pre-treatment to post-treatment values of LVEF% in each arm, and data were expressed as means ± SD, then the multiple linear regression was performed to detect the effect size of metformin and baseline LVEF% on post-treatment LVEF% values, and the β-coefficient with its 95% CIs was reported. Binary logistic regression was used to estimate the effect size of metformin treatment and other parameters on fatty liver development, and the OR with its 95% CIs was reported. Two-sided *p* values of < 0.05 were considered significant. The sample size was estimated based on a previous study [31], in which 60 patients (i.e., 30 per arm) were needed to detect a change in the incidence of peripheral neuropathy using Chi-square test with an alpha error of 5%, a beta error of 10%, and a 20% drop-out rate. As indicated above, peripheral neuropathy is one of the most common toxicities encountered in BC patients treated with AC-T chemotherapy [32, 33].

3 Results

3.1 Patients' Demographics and Tumour Characteristics

Between November 2019 and February 2021, 663 patients with biopsy-proven BC were screened for eligibility to participate in the study. Of these, 586 patients did not meet the inclusion criteria and 7 patients refused to participate. Seventy patients met the eligibility criteria and were randomised to either metformin or control arms in a 1:1 ratio. All patients were assessed for study outcomes. The CONSORT diagram summarises the study flow (Fig. 1).

Patient and tumour characteristics are summarised in Table 1. Patients in both metformin and control arms showed similar baseline characteristics. Most females were aged < 40 years and were premenopausal (68.57% and 71.43%, respectively). Most patients were obese, representing 67.14% of participants. Most patients (67.14%) had no family history of BC. Moreover, the luminal molecular subtype (65.71%) and clinical prognostic stage of IIIB (44.29%) were more prevalent among patients.

3.2 Adverse Events

The frequency and proportion of AEs during the whole treatment period were summarised in Table 2. No AE-related deaths (i.e., grade 5) were observed during the entire study. The most frequently occurring AEs observed were anaemia, fatigue, neutropenia, peripheral sensory neuropathy, oral mucositis, nausea, and diarrhoea (Table 2). Most AEs were of grades 1 and 2. The metformin arm showed a lower incidence and severity of fatigue, oral mucositis, and peripheral neuropathy (Electronic supplementary file 2, Fig. 1). On the other hand, no significant differences were observed between the two arms in diarrhoea, constipation, nausea, vomiting, anaemia, thrombocytopenia, neutropenia, and febrile neutropenia.

3.2.1 Effect of Metformin on Peripheral Neuropathy

Peripheral neuropathy is a common AE encountered with paclitaxel therapy. As shown in Fig. 2, the incidence of peripheral neuropathy was minimal and approximately similar in the 2 arms during the 4 AC cycles. Discrepancies started to appear after the first paclitaxel cycle. An intriguing finding of this study was that metformin significantly reduced the odds of peripheral neuropathy during paclitaxel cycles 4 to 12, respectively, as illustrated in Table 3 (see Electronic supplementary file 3 for additional information).

3.2.2 Effect of Metformin on Oral Mucositis

The incidence and severity of oral mucositis was lower in the metformin arm compared to the control arm throughout the whole treatment period as shown in Fig. 3. During the doxorubicin-cyclophosphamide cycles, metformin significantly reduced the odds of oral mucositis (Table 3). Moreover, patients in the control arm experienced oral mucositis with higher frequencies and grades of severity compared to

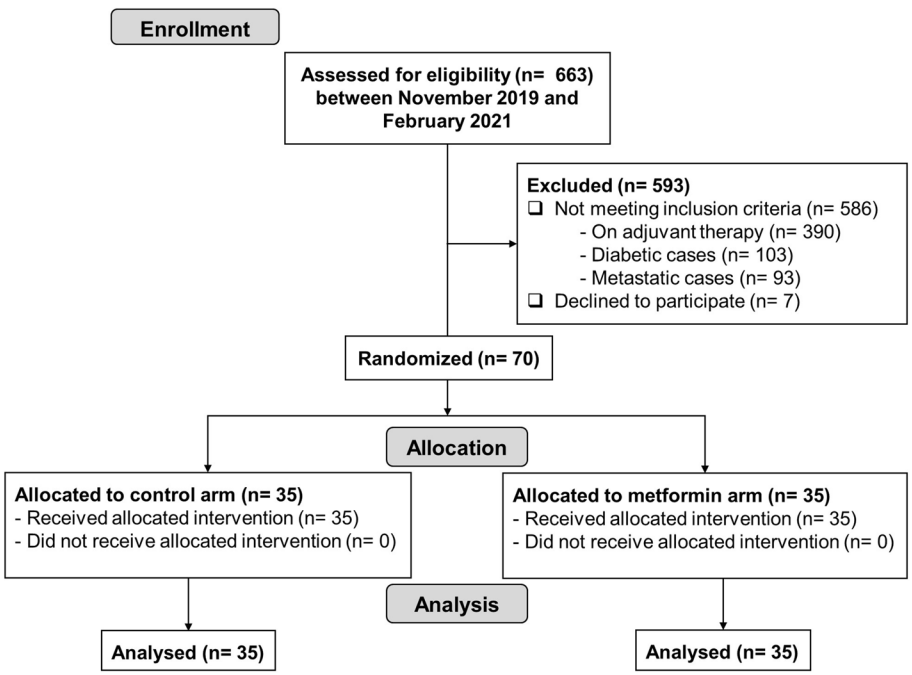

Fig. 1 The Consolidated Standards of Reporting Trials (CONSORT) diagram

Table 1 Baseline characteristics of breast cancer patients in control and metformin arms

	Control arm ($n = 35$) *N* (%)	Metformin arm ($n = 35$) *N* (%)	*p* value
Age on admission (years)[a]			0.303
< 40	26 (74.29%)	22 (62.86%)	
≥ 40	9 (25.71%)	13 (37.14%)	
Menopausal status[a]			1.00
Pre	25 (71.43%)	25 (71.43%)	
Post	10 (28.57%)	10 (28.57%)	
Body mass index (kg/m^2)[c]			0.9244
Mean ± SD (range)	32.10 ± 5.52 (20.61- 44.51)	31.98 ± 4.59 (22.76- 42.24)	
Breast feeding[a]	26 (74.29%)	29 (82.86%)	0.382
Contraception method history[b]			0.947
Hormonal	10 (28.57%)	10 (28.57%)	
Non-hormonal (IUD)	12 (34.29%)	13 (37.14%)	
Both	7 (20.00%)	8 (22.86%)	
Nothing	6 (17.14%)	4 (11.43%)	
Family history of breast cancer[a]	10 (28.57%)	13 (37.14%)	0.445
Family history of malignancy[a]	16 (45.71%)	21 (60.00%)	0.231
Medical history			
Hypertension[a]	12 (34.29%)	6 (17.14%)	0.101
IBD[b]	0 (0.00%)	1 (2.86%)	1.000
Rheumatoid arthritis[b]	0 (0.00%)	2 (5.71%)	0.493
Hepatitis C[b]	2 (5.71%)	3 (8.57%)	1.000
Fatty liver[a]	8 (22.86%)	11 (31.43%)	0.420
ECOG-PS[b]			0.086
Zero	28 (80.00%)	20 (57.14%)	
One	6 (17.14%)	14 (40.00%)	
Two	1 (2.86%)	1 (2.86%)	
Tumour histologic type[b]			1.000
IDC	34 (97.14%)	33 (94.29%)	
IMC	1 (2.86%)	2 (5.71%)	
Clinical prognostic stage[b]			0.347
IA	1 (2.86%)	0 (0.00%)	
IIA	3 (8.57%)	4 (11.43%)	
IIIA	1 (2.86%)	5 (14.29%)	
IB	7 (20.00%)	3 (8.57%)	
IIB	1 (2.86%)	2 (5.71%)	
IIIB	17 (48.57%)	14 (40.00%)	
IC	0 (0.00%)	0 (0.00%)	
IIC	0 (0.00%)	0 (0.00%)	
IIIC	4 (11.43%)	3 (8.57%)	
N/A	1 (2.86%)	4 (11.43%)	
Molecular subtype[b]			0.572
Luminal	23 (65.71%)	23 (65.71%)	
HER2-enriched	2 (5.71%)	5 (14.29%)	
Triple negative	9 (25.71%)	7 (20.00%)	
N/A	1 (2.86%)	0 (0.00%)	

ECOG-PS Eastern cooperative oncology group performance status, *HER2* human epidermal growth factor receptor-2, *IBD* inflammatory bowel disease, *IDC* invasive ductal carcinoma, *IMC* invasive mammary carcinoma, *IUD* intrauterine device, *N/A* not available

[a]Pearson's Chi^2 test

[b]Fisher's exact test

[c]Two-sided two-sample *t* test with equal variances

Table 2 Cumulative incidence of adverse events encountered during the 16 cycles (4AC+ 12T) neoadjuvant treatment period, graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0

	Control arm ($N = 35$)				Metformin arm ($N = 35$)			
	Grade 1 *n*/35 (%)	Grade 2 *n*/35 (%)	Grade 3 *n*/35 (%)	Grade 4 *n*/35 (%)	Grade 1 *n*/35 (%)	Grade 2 *n*/35 (%)	Grade 3 *n*/35 (%)	Grade 4 *n*/35 (%)
Fatigue	30 (85.71)	26 (74.29)	4 (11.43)	–	28 (80.00)	24 (68.57)	3 (8.57)	–
Gastrointestinal								
Nausea	19 (54.29)	13 (37.14)	0 (0.00)	–	23 (65.71)	18 (51.43)	0 (0.00)	–
Vomiting	14 (40.00)	5 (14.29)	0 (0.00)	0 (0.00)	26 (74.29)	5 (14.29)	0 (0.00)	0 (0.00)
Diarrhoea	14 (40.00)	9 (25.71)	3 (8.57)	0 (0.00)	23 (65.71)	10 (28.57)	1 (2.86)	0 (0.00)
Constipation	20 (57.14)	5 (14.29)	0 (0.00)	0 (0.00)	23 (65.71)	4 (11.43)	0 (0.00)	0 (0.00)
Oral mucositis	16 (45.71)	11 (31.43)	16 (45.71)	0 (0.00)	25 (71.43)	8 (22.86)	4 (11.43)	0 (0.00)
Peripheral sensory neuropathy	27 (77.14)	22 (62.86)	1 (2.86)	0 (0.00)	25 (71.43)	14 (40.00)	0 (0.00)	0 (0.00)
Haematological								
Anaemia	32 (91.43)	13 (37.14)	0 (0.00)	0 (0.00)	35 (100.00)	18 (51.43)	0 (0.00)	0 (0.00)
Thrombocytopenia	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Neutropenia	19 (54.29)	16 (45.71)	6 (17.14)	1 (2.86)	25 (71.43)	17 (48.57)	6 (17.14)	0 (0.00)
Febrile neutropenia	–	–	3 (8.57)	0 (0.00)	–	–	1 (2.86)	0 (0.00)

those in the metformin arm during the following 12 paclitaxel cycles, where the likelihood of oral mucositis in the metformin arm was significantly reduced (Table 3) (see Electronic supplementary file 3 for additional information).

3.2.3 Effect of Metformin on Fatigue

The incidence of fatigue in all study participants was higher during the first 4 AC cycles, than with paclitaxel cycles. Nevertheless, patients in the metformin arm experienced less fatigue during the whole treatment period (Fig. 4). The difference in fatigue severity grades between arms during the 4 AC cycles did not reach statistical significance. However, during the following paclitaxel cycles there was a significant reduction in the odds of fatigue observed in metformin arm in cycles 1–5, and 8–10 (Table 3) (see Electronic supplementary file 3 for additional information).

3.2.4 Effect of Metformin on AC-T-Induced Cardiotoxicity

Echocardiography test was done for all patients before the initiation of therapy, and again after the last chemotherapy cycle, right before surgery. At baseline, there was no significant difference in the LVEF% between either arm (mean ± SD [95% CI]: 66.69 ± 4.57% [65.12– 68.25] vs 64.87 ± 4.84% [63.21–66.53] in the control and metformin arms, respectively [$p = 0.1115$]). However, post-treatment LVEF% differed significantly between arms (62.2 ± 5.22% [60.41– 63.99] vs 65.94 ± 3.44% [64.76–67.12] in control and metformin arms, respectively [$p = 0.0007$]).

The LVEF% of control arm, as expected with chemotherapy, tended to decrease and this reduction was found to be significant when a paired *t* test was performed ($p = 0.0004$). On the other hand, the mean LVEF% did not significantly change in metformin arm ($p = 0.2667$) (Electronic supplementary file 2, Fig. 2). Interestingly, metformin could preserve the cardiac function and protect against AC-T-induced cardiotoxicity, with a predicted increase of 3.88 units in LVEF% ($\beta = 3.878 \pm 1.082$ [1.719– 6.037], $p = 0.001$) with metformin ingestion compared to control patients. Moreover, baseline LVEF% value had no impact on post-treatment values ($\beta = 0.074 \pm 0.114$ [− 0.154 to 0.303], $p = 0.518$).

3.2.5 Effect of Metformin on Hepatotoxicity

All patients were evaluated by ultrasonography for fatty liver at baseline and after chemotherapy. The frequency of fatty liver in the control arm at baseline was 22.86% ($n = 8$), compared to 31.43% ($n = 11$) in the metformin arm. No significant difference was found at baseline ($p = 0.420$). After completion of therapy, ultrasonography showed a significant reduction ($p < 0.0001$) in the frequency of fatty liver in the metformin arm ($n = 6$, 17.14%) compared to the control arm ($n = 21$, 60.00%), which was approximately tripled (Electronic supplementary file 4, Fig. 3a and Table 2). In univariate analysis, only metformin ingestion, patients' age, pre-existing fatty liver disease, and obesity (BMI > 30 kg/m^2), had significant influence ($p < 0.05$) on development of chemotherapy-induced fatty liver. However, when multivariate analysis was performed, patients with history of fatty liver had approximately 6 times the likelihood for developing AC-T-induced fatty liver (OR: 5.67 [1.19–26.98], $p = 0.029$), and those taking metformin had 92% protection from fatty liver development (adjusted

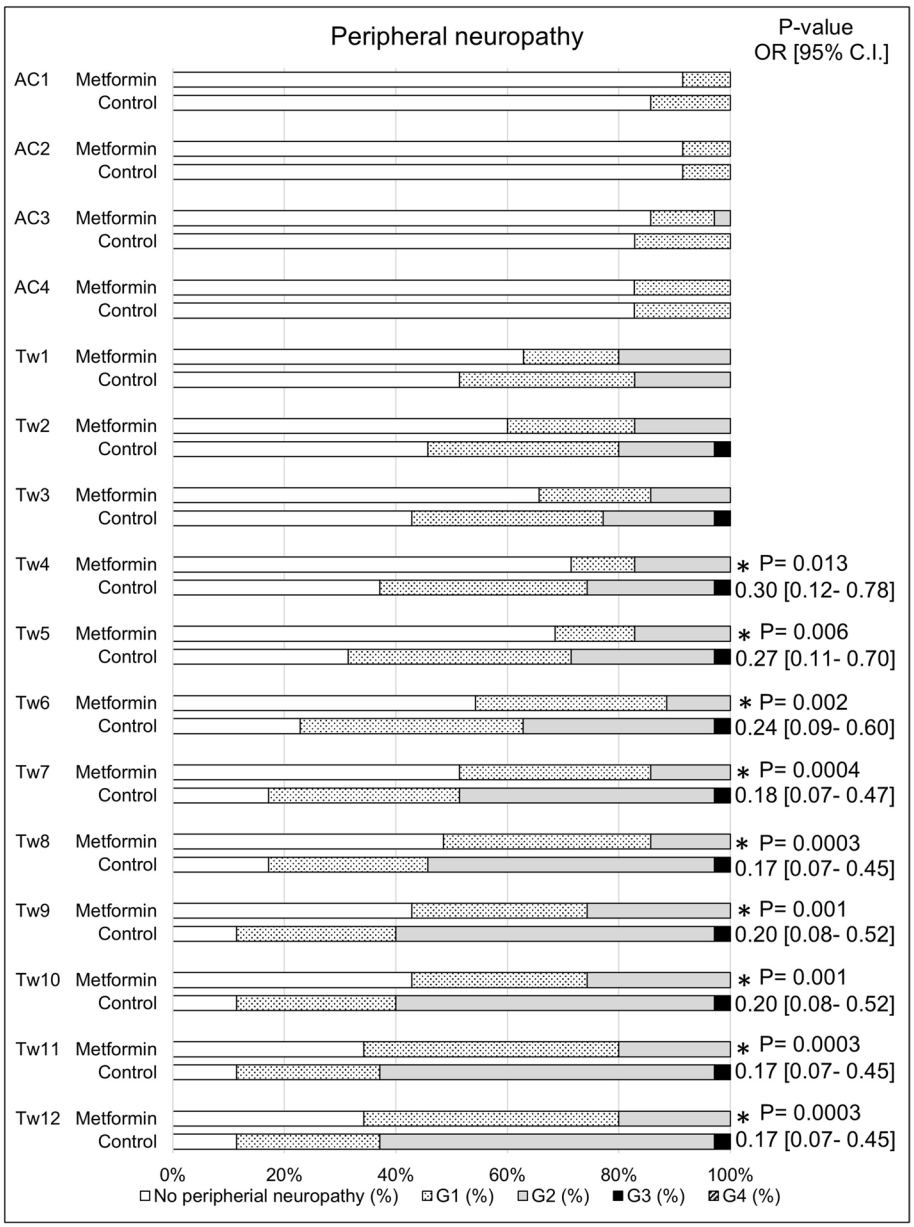

Fig. 2 Incidence of different Common Terminology Criteria for Adverse Events (CTCAE) grades of peripheral neuropathy by cycle between metformin and control arms experienced during neoadjuvant therapy. Data are expressed as percentages of frequencies of incident events of 35 observations in each arm. *AC* adriamycin-cyclophosphamide cycle, *CI* confidence interval, *G* grade of severity, *OR* odds ratio, *Tw* paclitaxel weekly cycle. **p* value < 0.05 versus control values

OR: 0.08 [0.02–0.33], $p < 0.0001$) (Electronic supplementary file 4, Table 3).

Moreover, when limiting the analysis on patients with no pre-existing fatty liver in control ($n = 27$) and metformin ($n = 24$) arms, 14 patients (51.85%) in the control arm developed fatty liver after neoadjuvant treatment, compared to only 2 patients (8.33%) in the metformin arm ($p = 0.001$) (Electronic supplementary file 4, Fig. 3b and Table 2). In univariate analysis, the effect of metformin on the development of fatty liver was the only significant factor (OR: 0.08 [0.02–0.43], $p = 0.003$).

3.2.6 Effect of Metformin on Other Gastrointestinal AEs

No significant differences were observed in the incidence or severity of diarrhoea, constipation, nausea, and vomiting between either arm. Diarrhoea tended to increase in the metformin arm; however, this increase was not significant. Also, vomiting tends to non-significantly increase

Table 3 The percentages of reduction in odds of peripheral neuropathy, oral mucositis, and fatigue in the metformin arm during the 16 cycles (4AC+ 12T) neoadjuvant treatment period

	Peripheral neuropathy (*p* value)	Oral mucositis (*p* value)	Fatigue (*p* value)
AC1	NS[a]	75% (0.003)	NS[a]
AC2	NS[a]	67% (0.022)	NS[a]
AC3	NS[a]	61% (0.047)	NS[a]
AC4	NS[a]	67% (0.021)	NS[a]
Tw1	NS[a]	88% (0.0002)	74% (0.005)
Tw2	NS[a]	87% (0.001)	70% (0.010)
Tw3	NS[a]	90% (0.001)	69% (0.014)
Tw4	70% (0.013)	87% (0.0003)	75% (0.005)
Tw5	73% (0.006)	85% (0.001)	75% (0.005)
Tw6	76% (0.002)	68% (0.027)	NS[a]
Tw7	82% (0.0004)	82% (0.001)	NS[a]
Tw8	83% (0.0003)	75% (0.012)	66% (0.027)
Tw9	80% (0.001)	79% (0.004)	64% (0.034)
Tw10	80% (0.001)	77% (0.007)	64% (0.034)
Tw11	83% (0.0003)	73% (0.014)	NS[a]
Tw12	83% (0.0003)	65% (0.041)	NS[a]

AC adriamycin-cyclophosphamide cycle, *NS* non-significant, *Tw* paclitaxel weekly cycle

[a]There was no significant difference between metformin and control arms in the incidence and severity of peripheral neuropathy, oral mucositis, and fatigue in those cycles, and the values are presented in Electronic supplementary file 3

in the metformin arm during AC cycles II, III, and IV (Electronic supplementary file 5).

3.2.7 Effect of Metformin on Haematological AEs

In our study population, haematological AEs such as anaemia, neutropenia, febrile neutropenia, and thrombocytopenia were similarly demonstrated in both arms (Electronic supplementary file 6). No thrombocytopenia was observed in participants. Grade I and II anaemia was the most common haematological toxicity, followed by grades I and II neutropenia. While febrile neutropenia was a rare event (Table 2).

4 Discussion

Chemotherapy-induced toxicities remain a major problem in the management of BC. In the present study, we investigated whether metformin would protect against these toxicities in non-diabetic BC patients receiving neoadjuvant AC-T chemotherapy. This is the first randomised controlled trial to endorse a protective effect of metformin on PIPN, oral mucositis, fatigue, cardiotoxicity, and hepatotoxicity induced by AC-T chemotherapy regimen.

An intriguing finding of our study is the ability of metformin to protect against peripheral neuropathy induced by the AC-T regimen. The paclitaxel component of the AC-T regimen is often blamed for the evoked neuropathy response, possibly due to the disruption of the assembly of neuron microtubules, and subsequent impairment in axonal transport and neuronal functions [34]. Considering the debilitating effects of peripheral neuropathy on sensory and motor functions [35], the improvement of PIPN by metformin is expected to warrant a better post-treatment quality of life for BC patients. Remarkably, the anti-neuropathic action of metformin is unlikely to be linked to its antidiabetic effect and is mostly reported in preclinical studies [36–40]. Metformin displays an anti-neuroinflammatory effect in spinal cord-injured rats comparable to that of minocycline, the tetracycline antibiotic with potent neuroprotective properties [41]. In another in vivo study, metformin reverses the structural brain plasticity that accompany neuropathic pain [42]. In one clinical study undertaken in patients with stage III colorectal cancer, metformin was found to reduce peripheral neuropathy induced by oxaliplatin [43]. The present study is therefore the first randomised controlled study to report on the protective effect of metformin against peripheral neuropathy caused by AC-T in non-diabetic BC patients.

Another fascinating finding of our study is that the metformin arm had significantly lower incidence of and less severe oral mucositis during the entire neoadjuvant treatment period. Oral mucositis is a well-known AE of chemotherapy, in which the generation of reactive oxygen species (ROS) produces DNA damage and subsequent basal epithelial cell death in the oral mucosa [44]. The protective effect of metformin against oral mucositis caused by AC-T chemotherapy regimen, has not been investigated in preclinical or clinical settings. That said, a promising therapeutic potential for metformin in mucosal protection and regeneration has been demonstrated only in preclinical studies such as: (i) metformin administration before indomethacin inhibits the development of gastric ulcer in rats [45], (ii) metformin heals indomethacin-induced gastric ulcer in rats in a way comparable to the proton pump inhibitor omeprazole [46], (iii) topical hydrogel preparation of metformin hydrochloride effectively promotes wound healing and re-epithelisation [47], (iv) metformin off-sets radiotherapy-induced mucositis [48], and metformin protects against 5-fluorouracil-induced oral mucositis in mice via reducing endoplasmic reticulum stress [49]. The current clinical study, therefore, is the first to authenticate the protective effect of metformin against AC-T-induced oral mucositis in BC patients.

Adriamycin treatment induces skeletal muscle loss and consequent fatigue and decreased quality of life [50, 51].

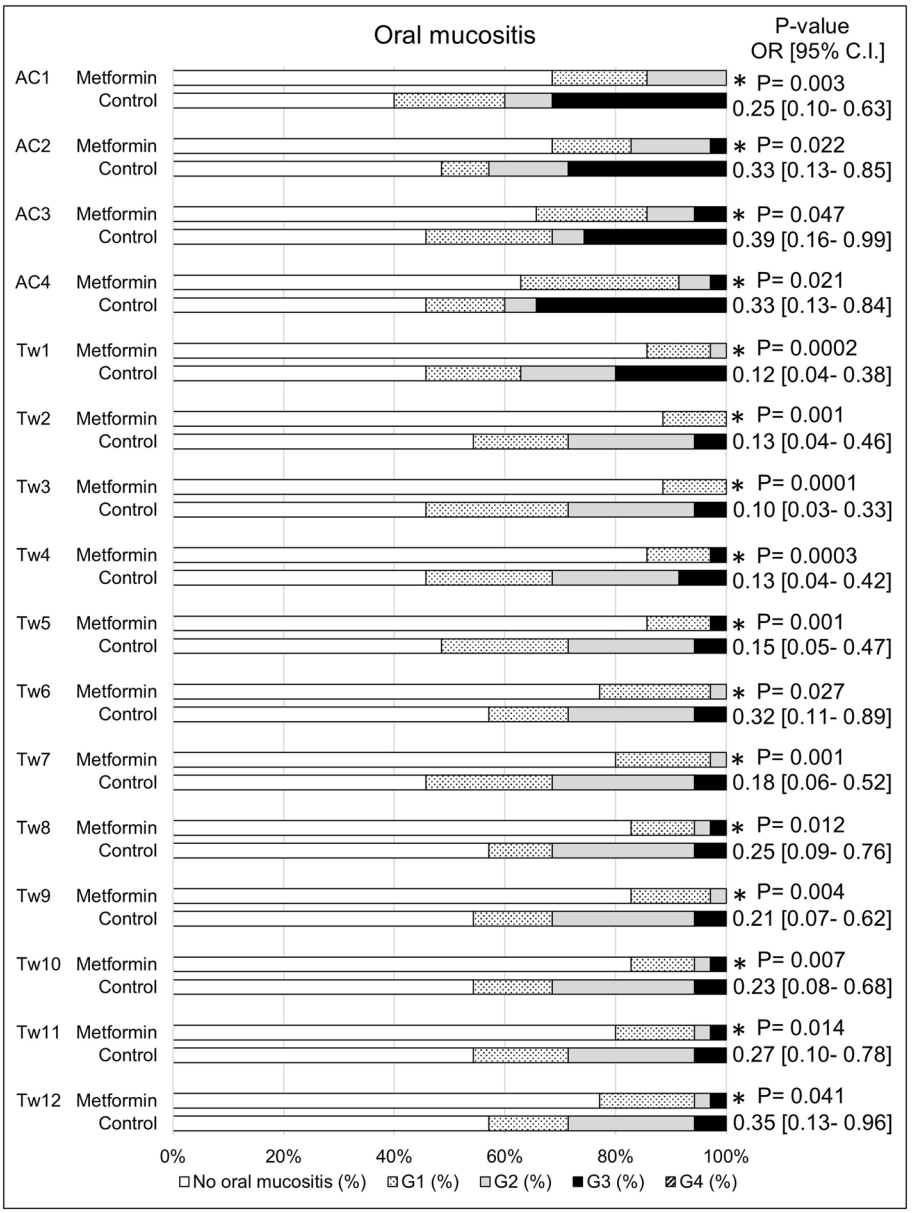

Fig. 3 Incidence of different Common Terminology Criteria for Adverse Events (CTCAE) grades of oral mucositis by cycle between metformin and control arms experienced during neoadjuvant therapy. Data are expressed as percentages of frequencies of incident events of 35 observations in each arm. *AC* adriamycin-cyclophosphamide cycle, *CI* confidence interval, *G* grade of severity, *OR* odds ratio, *Tw* paclitaxel weekly cycle. **p* value < 0.05 versus control values

Several studies demonstrated no additional benefit for adding metformin to adriamycin therapy in terms of skeletal muscle protection [52–54]. This observation was in line with our findings, where we found no privileged effect for metformin on fatigue during the four adriamycin-cyclophosphamide (AC) chemotherapy cycles. By contrast, during paclitaxel chemotherapy sessions we found that the simultaneous treatment with metformin clearly diminished in the incidence and severity of fatigue. While no data are available in the literature regarding the effect of metformin on AC-T regimen-related fatigue, a recent clinical study conducted on patients with stage IV non-small cell lung cancer receiving immune-checkpoint inhibitors revealed significantly less fatigue upon co-administration of metformin [55]. As fatigue has traditionally been associated with paclitaxel therapy [56, 57], the current data are the first to highlight that metformin conceivably reduces the heightened risk of fatigue incited by paclitaxel in non-diabetic BC patients.

Despite the high efficacy of adriamycin and related anthracyclines as anticancer agents in solid tumours, their cumulative and dose-dependent cardiotoxicity remains one of the most devastating consequences when used alone [58], or combined with cyclophosphamide plus paclitaxel [59]. The cardiotoxic influences of adriamycin such as myocardial morphological damage, cardiomyopathy, and congestive heart failure could necessitate cardiac transplantation or have fatal consequences [58]. The overproduction of ROS has been largely blamed for the cardiotoxicity of adriamycin, but other proposed mechanisms include mitochondrial dysfunction, disruption of Ca^{2+} homeostasis, and upregulated

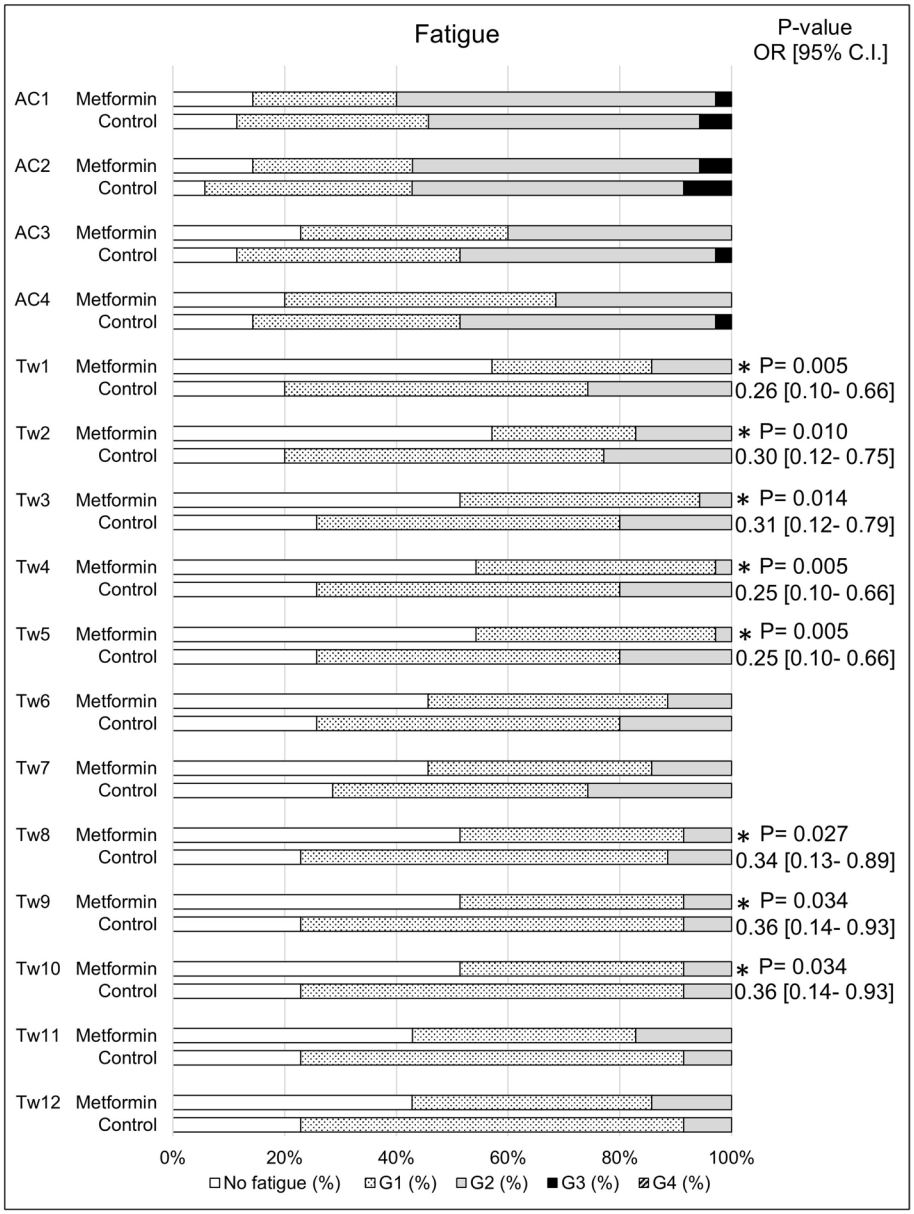

Fig. 4 Incidence of different Common Terminology Criteria for Adverse Events (CTCAE) grades of fatigue by cycle between metformin and control arms experienced during neoadjuvant therapy. Data are expressed as percentages of frequencies of incident events of 35 observations in each arm. *AC* adriamycin-cyclophosphamide cycle, *CI* confidence interval, *G* grade of severity, *OR* odds ratio, *Tw* paclitaxel weekly cycle. *p value < 0.05 versus control values

apoptosis [60]. Previous preclinical studies have revealed a cardioprotective action for metformin against adriamycin cardiotoxicity [61, 62]. Such an advantageous effect of metformin was mimicked in a single clinical study against radiation-induced cardiotoxicity in diabetic BC patients [63]. Importantly, the current clinical study is the first to establish an effective cardioprotective action for metformin in non-diabetic BC women receiving AC-T neoadjuvant chemotherapy. This view is validated by the observation that the significant drop in the LVEF% caused by AC-T regimen in the control arm was nullified after concurrent administration of metformin.

Non-alcoholic fatty liver disease has always been linked to obesity, insulin resistance, and type 2 diabetes mellitus [64]. Clinical studies have indicated a beneficial action for metformin on NAFLD in diabetic and nondiabetic patients and the effect of metformin surpassed the improvement produced by lifestyle interventions, the only available interventional option for NAFLD [20, 65, 66]. The risk of developing fatty liver during chemotherapy in BC patients has not been properly studied and knowledge on the exact molecular mechanisms for AC-T protocol-induced fatty liver is scarce [2, 67]. Izadpanahi et al, showed that AC-T chemotherapy leads to the development of fatty liver in more than half of BC patients [2], through a mechanism that mainly involves mitochondrial dysfunction [68]. In the present study, 31% of all participants who had no fatty liver at baseline, developed fatty liver after AC-T administration. More importantly, the decreased proportion of fatty liver in the metformin arm points to a protective effect for metformin against

chemotherapy-induced hepatotoxicity. It is worth noting that the recruitment of nondiabetic patients and the equal distribution of obese individuals in the two arms of the study ($p = 0.799$) made comparisons between both arms reliable.

The present study data revealed that signs of GI toxicity induced by AC-T regimen, such as diarrhoea and constipation, were still manifest in the metformin arm. This is consistent with earlier reports in which metformin failed to relieve episodes of diarrhoea caused by chemotherapeutic drugs such as 5-fluorouracil and irinotecan [69, 70]. Notwithstanding, a protective effect of metformin against diarrhoea provoked by abdominal radiotherapy has been recently reported [71]. Such discrepancy in metformin effects might relate to the specific anticancer therapy employed, chemotherapy versus radiotherapy. In fact, differences in the magnitude/nature of intestinal lesions induced by the two therapies and predisposing cellular mechanisms have been described [72, 73]. Additionally, a protective effect for metformin against 5-fluorouracil-induced diarrhoea has been reported [74], but this study was conducted in mice and employed a shorter period of therapy with metformin and 5-fluorouracil. It should be remembered that in addition to the specific anticancer intervention utilised, other factors may contribute to gastrointestinal upsets in cancer patients such as dietary habits, exercise, infection, anxiety and psychological distress [75]. In the present investigation, we attempted to limit the impact of these distractors and reduce the incidence of gastrointestinal anomalies by gradual escalation of the metformin dose and randomly assigning patients to the two arms of the study.

4.1 Limitations

This study was an open-label randomised controlled study. However, the implementation of allocation concealment and the blind outcome assessment for haematological toxicity, cardiotoxicity and hepatotoxicity may have reduced bias in the estimated effect of treatment. Also, results may have been reinforced by measuring specific confirmatory biomarkers for AC-T-induced toxicities.

5 Conclusion

The data suggest a multitude of beneficial effects for metformin when co-administered with AC-T chemotherapy in BC patients. Metformin reduces the incidence and severity of serious toxicities typically associated with AC-T use, namely PIPN, oral mucositis, fatigue, cardiotoxicity, and hepatotoxicity. This contrasts with haematological and gastrointestinal toxicities of the AC-T regimen that are preserved following metformin administration. Undertaking the study in a population of non-diabetic patients argues against the dependence of the metformin effects on the diabetic state. Moreover, the random assignment of patients to the control and metformin regimens eliminates the influence of confounders that could have affected the outcome of the study. Notably, further large-scale double-blind randomised controlled studies are needed to reinforce the protective effect of metformin against the harmful actions caused by AC-T chemotherapy or likely other chemotherapeutic protocols.

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Declarations

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Conflicts of interest Manar A. Serageldin, Amira B. Kassem, Yasser El-Kerm, Maged W. Helmy, Mahmoud M. El-Mas, and Noha A. El-Bassiouny declare no conflict of interest.

Availability of data and material All data generated or analysed during this study are included in this published article and its supplementary information files.

Ethical approval This study was performed in line with the principles of the Declaration of Helsinki and its later amendments. Institutional ethical approvals were obtained from Alexandria University (IRB no. 00012098), and Damanhour University (IRB no. 919PP18) before the commencement of the study.

Consent to participate Written informed consent was obtained from all participants before participation in this study.

Consent to publish Not applicable.

Code availability Not applicable.

Authors' contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Manar A. Serageldin. The first draft of the manuscript was written by Manar A. Serageldin and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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