Metformin as an adjuvant therapy to an aromatase inhibitor in overweight and obese postmenopausal women with breast cancer: A pilot study

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Abstract

**Introduction:** Metformin may provide therapeutic benefits to patients with various types of malignancy. Through its insulin-sensitizing effect, metformin can also reduce weight in non-diabetic populations.

**Purpose:** Our research aimed at evaluating the effect of metformin as an adjuvant therapy to aromatase inhibitor (letrozole) on estradiol and other biomarkers serum levels in postmenopausal obese breast cancer women.

**Patients and methods:** This controlled study involved 45 postmenopausal breast cancer females who were assigned into three arms: the control arm (arm 1; n= 15 obese women) which received letrozole only (2.5 mg/day); the metformin arm (arm 2; n= 15 obese women) which provided a similar letrozole dosage in addition to metformin (2000 ± 500 mg/day); and the lean arm (arm 3; n= 15) which received letrozole. The intervention duration was 6 months. Blood samples were obtained at zero time and six months later for the measurement of serum estradiol, osteocalcin, insulin, leptin, lactate, and fasting blood glucose.

**Results:** After the intervention, the metformin arm had significantly lower osteocalcin and fasting blood glucose levels than both the control and the lean arms (p<0.0001, p<0.0001, respectively). The metformin and lean arms had significantly lower fasting insulin levels, homeostatic model assessment of insulin resistance (HOMA-IR), estradiol, and leptin concentrations than the control arm (p = 0.0064, p = 0.0020, p<0.0001, and p<0.0001, respectively). We observed non-significant variation in serum lactate levels among the three study arms.

**Conclusion:** Metformin might represent a promising adjuvant therapy to letrozole in postmenopausal women with breast cancer.

**ClinicalTrials.gov Identifier:** NCT05053841/Registered September 23, 2021 - Retrospectively

**Introduction**

Breast cancer is the most prevalent type of non-cutaneous cancer in women. About two-thirds of estrogen and/or progesterone receptors are expressed in about two-thirds of breast tumors. As a result, blocking estrogen signaling in cancer cells is essential in the treatment of breast cancer [1].

In premenopausal females, most of the estrogen is synthesized by the ovaries. On the other hand, following menopause, the major estrogen's source is the extra ovarian adipose tissue where the androstenedione and testosterone are converted to estrone and estradiol [2, 3].

Obesity is considered a risk factor for cancer and cancer-related mortality [4]. Obesity is usually associated with poor prognosis particularly in postmenopausal breast cancer females. Despite the real mechanisms that aren't completely understood, obesity is accompanied by elevated estradiol levels, a recognized postmenopausal breast cancer risk factor [5]. Moreover, the inflammatory signaling reported within the breast tissue of obese females extensively promotes signaling of estrogen, principally by
changing the aromatase enzyme expression which in turn increases estrogen production and induces
tumor formation and progression [6].

Endocrine therapy options available for the management of breast cancer include selective estrogen-
receptor modulators (Tamoxifen), a selective estrogen-receptor degrader (Fulvestrant) as well as
aromatase inhibitors (AIs). Aromatase inhibitors hinder the aromatase enzyme from converting
androgens to estrogens, lowering estrogen concentrations. The AIs of the third generation include the
steroidal irreversible inhibitor ( exemestane) and the non-steroidal reversible inhibitors (letrozole and
anastrozole) [7]. AIs are given nowadays at the same dose regardless of body weight or body surface
area [8]. However, there is evidence for the differential effects of anastrozole in overweight or obese
versus normal-weight postmenopausal women with breast cancer [9].

Metformin, an oral antidiabetic medication, is considered a cornerstone for the management of type 2
diabetes and polycystic ovary syndrome [10, 11]. Furthermore, some studies showed that patients with a
variety of cancers may get therapeutic benefits from metformin [12]. Metformin's anti-cancer properties
could be linked to its negative effects on metabolism [13, 14].

In this context, the purpose of this study was to see how metformin, as an adjuvant therapy to aromatase
inhibitors (letrozole), affected serum levels of estradiol and other biomarkers in postmenopausal obese
breast cancer females.

**Patients And Methods**

**Study design, patients' population, and treatment allocation**

The study design was prospective open-labeled pilot study. Our study involved postmenopausal women
with breast cancer who were recruited from the Clinical Oncology Department, Faculty of Medicine,
Menoufia University, Menoufia, Egypt. Eligible females were recruited in the study. The study included
postmenopausal breast cancer women who were assigned to hormonal therapy with aromatase
inhibitors (postmenopausal is defined as age 55 years old or more and one year or more of amenorrhea
or age less than 55 years old and one year or more of amenorrhea, with an estradiol level less than 20
pg/mL [15]), overweight women (BMI ≥ 25 kg/m² and less than 30 kg/m²), obese women (BMI ≥ 30
kg/m²) and non-obese women (BMI between 18 kg/m² and 25 kg/m²). Diabetic women, women with
metabolic syndrome, women with a last menstrual cycle less than one year ago, patients with any
disorder that increases the risk of acidosis (heart failure, renal failure, COPD), and women who were
-treated with luteinizing hormone-releasing hormone agonists (LHRH) were all excluded. Tanta University
National Research Ethics Committee approved this study (Approval code: 34653/4/21). This study was
carried out under the ethical standards outlined in the Declaration of Helsinki of 1964 and its
modifications, or equivalent ethical standards. The study was registered at [www.ClinicalTrials.gov](http://www.ClinicalTrials.gov) with
ID: NCT05053841. All participants gave their informed consent.
Patients in the control group (group 1; n = 15) were obese women with breast cancer who received letrozole 2.5 mg/day for six months. Patients in the metformin group (group 2; n = 15) were obese women who received the same letrozole dose as in the control group plus metformin 2000 ± 500 mg/day for six months. The metformin dosage was slowly titrated upward, beginning with 500 mg/day through the first week and then titrated up by 500 mg every week till achieving the final dose which was determined according to the BMI of each participant [16]. women with BMI < 30 kg/m² were given 1500 mg/day, women with BMI ≥ 30 kg/m² and < 35 kg/m² were given 2000 mg/day, and women with BMI ≥ 35 kg/m² were given 2500 mg/day of metformin. Patients in the lean group (group 3: n = 15) received the same dose of letrozole as the control group. The study duration was 6 months. All patients were recommended to decrease their carbohydrates intake in the evening, but no one of them followed a concomitant diet plan.

**Demographic, clinical, and anthropometric data**

Following enrollment, clinical and demographic data were collected such as age, stage of the disease, and receptor status. Additionally, all participants were submitted to anthropometric measurements including weight and height with subsequent calculation of body mass index [BMI = weight (kg)/height² (m)] at baseline and six months after the assigned treatment.

**Blood sample collection**

At baseline and six months after the intervention, blood samples were drawn after overnight fasting from the antecubital vein into plain venipuncture tubes. Blood samples were allowed to coagulate and then centrifuged for 10 minutes at 3000 rpm. The serum samples were distributed to be used for the detection of biological markers (fasting blood glucose, fasting insulin, estradiol, osteocalcin, leptin, and lactate levels).

**Biochemical analysis**

Fasting blood glucose levels was determined by glucose oxidase technique (Spinreact, Spain; Catalogue No.: MD41011). Fasting insulin level (FPI) was assayed using an enzyme-linked immunosorbent assay (ELISA) kit (Diagnostic Automation/Cortez Diagnostics, Inc., USA: Catalogue No.: 1606-15). The HOMA-IR index was used to estimate insulin resistance (IR), which is defined as fasting insulin level (IU/ml) times fasting blood glucose (mg/dl) divided by 405 [17]. An enzyme-linked immunosorbent test kit was used to measure serum estradiol (DRG International, Inc., USA; Catalogue No.: EIA-4399). Serum human osteocalcin was determined using an enzyme-linked immune-sorbsent assay kit (Epitope Diagnostics Inc., San Diego, USA; Catalogue No.: KT 809). Serum leptin was determined using an enzyme-linked immune-sorbsent assay kit (Diagnostic Biochem Canada Inc., Ontario, Canada, Catalogue No.: CAN-L-4260). Serum lactate was determined by the colorimetric method (Spinreact, Spain; Catalogue No: 1001330).

**Assessment of patients' adherence and drug safety**

Every month, patients were closely monitored to determine their compliance and record any adverse reactions to the studied drugs. Metformin's safety was evaluated by asking the patients about any
Results

During the current study, 75 postmenopausal women with breast cancer were screened for eligibility, 22 women were excluded, and 53 women were allocated into three groups. During the study course, 8 women in the three study groups were dropped out secondary to missed data, death, non-adherence, change of regimen, and their preliminary data were not included in the final analysis. Therefore, only 45 females’ patients completed the study (15 patients in each group). The flow chart of the study participants is displayed in Fig. (1).

Demographic, clinical, and anthropometric data

At baseline, all groups were statistically similar regarding age, height, stage of cancer, and receptor status. As expected and according to the selection criteria, there was a statistically significant variation among the lean arm and both the control and metformin arms concerning body weight and BMI (p < 0.001). However, body weight and BMI showed non-significant variations between the control and metformin arms at baseline. Baseline demographic, clinical, and anthropometric data of the study participants are demonstrated in Table (1).

Six months following therapy, the lean arm had significantly lower mean body weight and BMI than the metformin and the control arms. [60.5 kg versus 84.93 kg and 86.97 kg; p < 0.0001 & p < 0.0001 respectively (for body weight) and 24.14 kg/m² versus 34.2 kg/m² and 35.8 kg/m²; p < 0.0001 & p < 0.0001, respectively (for BMI)]. There was non-significant decrease neither in mean body weight nor in observed side effects during the treatment course including abdominal pain, loss of appetite, diarrhea, nausea, vomiting, and others. Each woman in the three groups was asked to return the empty tablet strips at the end of each month to ensure her adherence. The adherence was estimated using the medication refill rate (Percentage of drug coverage = number of days in the period covered/number of days in the period).

Sample size

Although the sample size in this study is small, it exceeds the suggested sample size for a pilot study (12 per group). [18].

Statistical analysis

The ANOVA test was performed to calculate the mean values of parametric data among the three groups, followed by the post-hoc Tukey’s test. Paired Student’s t-test was used to determine whether there was a significant difference between each group at baseline and 6 months after the treatment course. The Chi-Square analysis was applied to compare categorical data between the three groups and to examine the reported adverse effects. Values were presented as mean ± standard deviation (SD) for quantitative variables and percent for qualitative variables. Data were coded and inserted using Version 7.0 of GraphPad Prism (GraphPad Software, San Diego, CA, USA). All the P-values were two-tailed, and a p-value < 0.05 was regarded as significant.
BMI between metformin and control groups (84.93 kg versus 86.97 kg; \( p = 0.8788 \) for body weight and 34.2 kg/m\(^2\) versus 35.8 kg/m\(^2\); \( p = 0.6480 \) for BMI). Changes in body weight and BMI among the three study groups are depicted in Fig. (2).

**Effect of intervention on glycemic parameters**

Fasting blood glucose levels revealed non-significant variations between the control and metformin arms at baseline. However, the previously mentioned groups and the lean group had a statistically significant difference (91.2 mg/dl and 93 mg/dl versus 83.67 mg/dl; \( p = 0.0266 \) and \( p = 0.0049 \), respectively). Six months after therapy, both the metformin and lean arms had significantly lower mean fasting blood glucose levels than the control arm (79.27 mg/dl and 85.8 mg/dl versus 91.87 mg/dl; \( p < 0.0001 \) and \( p = 0.0373 \), respectively). Furthermore, the metformin arm showed significantly lower fasting blood glucose levels as compared to the lean arm (79.27 mg/dl versus 85.8 mg/dl; \( p = 0.0232 \)). At baseline, both the control and metformin arms showed non-significant variations in fasting insulin levels. However, both arms had a significant elevation in fasting insulin levels in comparison to the lean arm. (14.13 µIU/mL and 14.15 µIU/mL versus 10.24 µIU/mL; \( p = 0.0415 \) and \( p = 0.0401 \), respectively). Six months after the intervention, both the metformin and the lean groups revealed a significant decrease in the mean level of fasting insulin in comparison to the control group (8.991 µIU/mL and 8.95 µIU/mL versus 12.1 µIU/mL; \( p = 0.0157 \) and \( p = 0.0142 \), respectively). Moreover, there was no significant variation in the mean value of fasting insulin between the metformin and lean arms (8.991 µIU/mL versus 8.95 µIU/mL; \( p = 0.9992 \)).

Before initiation of any treatment, both the control and metformin arms revealed non-significant variation in HOMA-IR values. However, the control and metformin arms had significantly higher HOMA-IR values than the lean arm. (3.153 and 3.227 versus 2.12; \( p = 0.0118 \) and \( p = 0.0067 \), respectively). Six months after the therapy, both the metformin and lean groups had significantly lower mean HOMA-IR values than the control group (1.777 and 1.893 versus 2.687, \( p = 0.0032 \) and \( p = 0.0109 \), respectively). Additionally, a non-significant decrease in the mean value of HOMA-IR was found between metformin and the lean group (1.777 versus 1.893, \( p = 0.8967 \)).

Glycemic parameters for the three study groups at the start and 6 months following intervention are presented in Table (2).

**Impact of intervention on estradiol level**

At baseline, there was no statistically significant variation in estradiol serum levels among the control and metformin groups. However, both control and metformin groups showed significantly higher estradiol levels as compared to the lean group (7.806 pg/mL and 8.056 pg/mL versus 4.726 pg/mL; \( p = 0.0163 \) and \( p = 0.0088 \), respectively). Following the therapy, the mean estradiol level was statistically significantly lower in both metformin and lean groups in comparison to the control group (2.14 pg/mL and 1.519 pg/mL versus 3.381 pg/mL; \( p = 0.0071 \) and \( p < 0.0001 \), respectively). On the other hand, the difference in estradiol levels between the metformin and lean groups was not statistically significant. (2.14 pg/mL vs. 1.519 pg/mL, \( p = 0.2541 \)). The changes in the serum level of estradiol in the three study groups are postulated in Table (3).

**Effect of intervention on serum osteocalcin level**
Before initiation of any treatment, osteocalcin levels revealed non-significant variation between the three study groups. Six months after treatment, the metformin arm had significantly lower mean osteocalcin serum concentrations than the control and lean arms (8.33 ng/mL versus 16.45 ng/mL and 14.49 ng/mL, p < 0.0001 and p < 0.0001, respectively). Table (3) shows the changes in osteocalcin serum levels in the three study groups.

**Effect of intervention on serum leptin level**

At baseline, both the control and metformin arms showed statistically similar leptin levels. However, both arms showed significantly higher leptin levels as compared to the lean group (44.58 ng/mL and 56.09 ng/mL versus 16.2 ng/mL, p < 0.0001 & p < 0.0001, respectively). After the intervention, both the metformin and lean arms had significantly lower serum leptin levels than the control arm. (24.26 ng/mL and 17.72 ng/mL versus 51.6 ng/mL, p < 0.0001 and p < 0.0001, respectively). There was a non-significant variation in serum leptin levels among the metformin group and the lean group (24.26 ng/mL versus 17.72 ng/mL, p = 0.3499). Table (3) demonstrates the differences in leptin serum levels in the three study groups.

**Effect of intervention on serum lactate level**

At baseline and six months after the intervention, serum lactate levels showed no statistically significant variation among the three studied groups, as shown in Table (3).

**Drug safety and tolerability**

All the reported side effects were grade 1 and 2 in nature, and no serious adverse events were observed in the study arms. Lactate levels didn't change significantly in all study groups. The reported adverse effects included hot flushes and gastrointestinal tract related side effects. The metformin arm had a non-significantly greater incidence of side events than the other two arms (p > 0.05). The reported adverse effects are illustrated in Table (4).

**Discussion**

Estrogen has a key function in the pathogenesis of breast cancer [19]. AIs prevent the conversion of androgens into estrogens via blocking the aromatase enzyme resulting in a low level of estradiol [20]. Activity of aromatase enzyme is predominant in adipose tissue and this leads to the assumption that, aromatase activity could be elevated in obese females. In this context, the clinical activity of aromatase inhibitors in those females may be declined [21]. Metformin is a “star” drug used for type 2 diabetes mellitus whereas growing evidence demonstrated that, it may be a promising chemotherapeutic agent [22]. It may also have a weight-loss effect on the non-diabetic population through enhancing insulin sensitivity. however, the primary mechanisms have to be identified [14]. Our research goal was to investigate the effect of adding metformin as an adjuvant therapy to letrozole, a well-known aromatase inhibitor, in postmenopausal obese females with breast cancer.
In this study, metformin reduced body weight and BMI significantly six months following therapy compared to the baseline. However, there was a non-significant decrease in mean body weight between the metformin arm and the control arm. These results seem consistent with the results reported by Tapia et al 2021 [23].

Basic research advocates that long-term exposure to high insulin levels promotes breast cancer progression. Either directly through insulin receptor isoform A and insulin-like growth factor 1 (IGF-1) receptor activation or indirectly through alternations in circulating estrogen levels [24]. In the Women's Health Initiative Observational Study, hyperinsulinemia was an independently associated risk factor for postmenopausal breast tumors [25]. Our study showed that, metformin significantly decreased serum insulin level, glucose level, and HOMA-IR six months after treatment in comparison to the control arm. These results are similar to those reported by Meyerhardt et al. 2020 [26]. Metformin reduces hyperinsulinemia and hyperglycemia by enhancing hepatic and peripheral insulin sensitivity. It inhibits gluconeogenesis and glucose synthesis in the liver while increasing glucose utilization in muscles and adipose tissues [27]. The effect of metformin on glucose level could be mediated through activation of AMP-activated protein kinase (AMPK) as a consequence of inhibiting mitochondrial respiratory chain complex I8 and reducing glycerol and lactate conversion into glucose [28]. Several studies revealed that the intestine involved in the metformin blood-glucose-lowering effect. This favorable effect may be attributed to the changes in glucose uptake and anaerobic metabolism of enterocytes and the increase in the synthesis of the incretin hormone namely glucagon-like peptide-1 (GLP-1) [29].

Regarding the results obtained with estradiol, metformin produced a significant decrease in the estradiol levels when compared to the control arm. This effect could be partly explained by the reduction in insulin levels. Insulin inhibits the hepatic biosynthesis of sex-hormone-binding globulin (SHBG). As a result the bioavailability of estradiol can be increased [30].This effect may be also a result of direct suppression of aromatase activity by metformin [31, 32]. Our result appears to be consistent with the result reported by Campagnoli et al. 2013 [33].

The result obtained with osteocalcin six months after treatment revealed that, metformin significantly reduced osteocalcin level than both the control and the lean groups. Our findings are compatible with the data published by Roomi et al. 2019 [34]. There are conflicting reports about the effect of metformin on osteocalcin level. Hegazy et al. in 2015 reported that, metformin produced a slight and non-significant decrease in the osteocalcin level after 12 weeks of treatment and the authors concluded that, metformin is neither osteogenic nor anti-osteoporotic[35]. Molinuevo et al. 2009 reported that, metformin administration produced an increase in osteocalcin expression [36]. These conflicting results could be attributed to variations in study protocols, durations, and the implicated doses of metformin.

A High level of leptin has been linked to both breast tumers aggressiveness and poor prognosis [37]. Leptin was also reported to enhance aromatase expression in MCF7 cell lines and consequently promotes the synthesis of estrogen and increases the risk for breast cancer [38]. Our study showed that metformin made a significant decrease in serum leptin levels as compared to the control arm. However,
there was a non-significant variation in mean leptin level among the metformin and lean arms. These results are compatible with the results described by Annie et al. 2020 and Kargulewicz et al. 2016 [39, 40]. Although leptin concentration is strictly related to body fat mass, the reduction in leptin levels cannot be completely explained by the weight-reducing effect of metformin since metformin was reported to reduce leptin concentration even in normal-weight subjects [41]. The results obtained with a previously reported \textit{in-vitro} study demonstrated that metformin suppresses mitogen activated protein kinase (MAPK) activity in adipocytes and consequently can reduce leptin levels [42]. The impact of metformin on leptin concentration may be due to the modulation of the hypothalamic leptin receptor gene (ObRb) [43].

The levels of lactate didn't change significantly in all groups during the study. This result indicates that, metformin didn't cause lactic acidosis in those patients' populations. Lactic acidosis is a rare event that takes place with the accumulation of metformin. The risk of lactic acidosis is usually augmented in the elder population and patients with heart, kidney and hepatic diseases [44]. Before running the current study, we excluded all women with conditions that predispose to the development of lactic acidosis including women with heart failure, renal failure, and COPD. The gastrointestinal side effects reported with the current study included diarrhea, nausea/vomiting, and heartburn. The incidence of these gastrointestinal side effects was non-significantly higher in the metformin group than in the other two groups. However, these side effects developed at the early period of the treatment and were mild, temporary, and disappeared with continuous use of medications. These gastrointestinal side effects were counteracted by taking the study medications after a meal.

**Conclusion**

This present research advocates that metformin might represent a promising adjuvant therapy to an aromatase inhibitor for postmenopausal women with breast cancer, secondary to its favorable effect on estradiol level, insulin level, HOMA-IR, and leptin level. However, further studies are still necessary.

**Study limitations** The present study's limitations concern its relatively small sample size and its design as a pilot study.

**Declarations**

**Acknowledgment** We are so grateful to our participants without them this work could be never accomplished, wishing them full health and rapid recovery. We are so appreciative to the physicians at Menoufia Oncology Institute, Menoufia, Egypt for their assistance and recommendations.

**Authors' contribution** Osama M. Ibrahim, Aya A. El-attar, and Tarek M. Mostafa reviewed the literature and created the study design. Eligibility evaluation, diagnosis, and recruitment of research participants were performed by Suzan A. Alhassanin. Aya A. El-attar and Enas S. Essa collected the clinical data and laboratory samples. Sample analysis was accomplished by Aya A. El-attar and Enas S. Essa. Aya A. El-
attar and Tarek M. Mostafa performed the statistical analysis. All authors wrote, revised, and approved the manuscript's final version.

**Funding**  This current study was self-funded, not funded by any organization, agency, or pharmaceutical company.

**Data availability**  All data and materials are transparent, support published claims, and adhere to field standards transparency.

**Compliance with ethical standards**

**Conflict of interest**  There are no conflicts of interest to disclose for any of the authors.

**Ethical consent**  All the study participants provided their informed consent. Since there is no personal data, publication consent is not requested.

**Ethical approval**  Tanta University's National Research Ethics Committee gave its approval to this study (Approval code: 34653/4/2). The study was consistent with the Helsinki Declaration's ethical principles and its later modifications in 1964.

**References**


**Tables**

**Table (1):** Baseline demographic, anthropometric, and clinical data of the study participants
<table>
<thead>
<tr>
<th>Variables</th>
<th>Lean group (n=15)</th>
<th>Control group (n=15)</th>
<th>Metformin group (n=15)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>51 ± 6.74</td>
<td>53.33 ± 7.68</td>
<td>53.67 ± 5.49</td>
<td>0.499</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>59.73 ± 3.24</td>
<td>87.13 ± 12.99</td>
<td>91.87 ± 17.57</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>157.7 ± 2.46</td>
<td>155.9 ± 3.54</td>
<td>157.9 ± 7.04</td>
<td>0.462</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.01 ± 1.2</td>
<td>35.86 ± 5.35</td>
<td>37.02 ± 7.71</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Breast cancer stage</td>
<td></td>
<td></td>
<td></td>
<td>0.554</td>
</tr>
<tr>
<td>Stage II A</td>
<td>3 (20%)</td>
<td>1 (6.667%)</td>
<td>4 (26.667%)</td>
<td></td>
</tr>
<tr>
<td>Stage II B</td>
<td>2 (13.333%)</td>
<td>3 (20%)</td>
<td>3 (20%)</td>
<td></td>
</tr>
<tr>
<td>Stage III A</td>
<td>5 (33.333%)</td>
<td>6 (40%)</td>
<td>5 (33.333%)</td>
<td></td>
</tr>
<tr>
<td>Stage III B</td>
<td>2 (13.333%)</td>
<td>1 (6.667%)</td>
<td>3 (20%)</td>
<td></td>
</tr>
<tr>
<td>Stage III C</td>
<td>3 (20%)</td>
<td>4 (26.667%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Receptor status</td>
<td></td>
<td></td>
<td></td>
<td>0.8979</td>
</tr>
<tr>
<td>ER-positive</td>
<td>15 (100%)</td>
<td>14 (93.33%)</td>
<td>14 (93.33%)</td>
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</tr>
<tr>
<td>PR-positive</td>
<td>14 (93.33%)</td>
<td>15 (100%)</td>
<td>14 (93.33%)</td>
<td></td>
</tr>
<tr>
<td>HERT2-positive</td>
<td>2 (13.33%)</td>
<td>1 (6.67%)</td>
<td>3 (20%)</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ±SD, number, and percentage

BMI body mass index; ER estrogen receptor; PR progesterone receptor and HER2 human epidermal growth factor receptor 2.

Chi-Square test was used for categorical data and ANOVA test was applied for continuous data.

*Significant difference (p<0.05)

**Table (2):** Glycemic parameters at baseline and six months after intervention for the three study groups
<table>
<thead>
<tr>
<th>Variables</th>
<th>Lean group (n=15)</th>
<th>Control group (n=15)</th>
<th>Metformin group (n=15)</th>
<th>*p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FBG (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline</td>
<td>83.67± 8.347</td>
<td>91.2± 5.697</td>
<td>93± 8.569</td>
<td>0.0041*</td>
</tr>
<tr>
<td>After six months</td>
<td>85.8 ± 5.506</td>
<td>91.87± 7.06</td>
<td>79.27 ± 6.829</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>0.3891</td>
<td>0.7690</td>
<td>0.0015**</td>
<td></td>
</tr>
<tr>
<td><strong>Insulin (µIU/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline</td>
<td>10.24± 2.791</td>
<td>14.13± 4.722</td>
<td>14.15± 4.901</td>
<td>0.0213*</td>
</tr>
<tr>
<td>After six months</td>
<td>8.95 ± 1.846</td>
<td>12.1± 3.306</td>
<td>8.991± 3.375</td>
<td>0.0064*</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>0.0925</td>
<td>0.1605</td>
<td>0.0003**</td>
<td></td>
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<tr>
<td><strong>HOMA-IR</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline</td>
<td>2.12± 0.6281</td>
<td>3.153± 0.9841</td>
<td>3.227± 1.13</td>
<td>0.0034*</td>
</tr>
<tr>
<td>After six months</td>
<td>1.893± 0.425</td>
<td>2.687± 0.9226</td>
<td>1.777± 0.703</td>
<td>0.0020*</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>0.2148</td>
<td>0.1469</td>
<td>&lt;0.0001**</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD

FBG: fasting blood glucose, HOMA-IR: homeostatic model assessment of insulin resistance

*p-value (comparison of post-treatment data among the three study groups using ANOVA test).

**p-value (comparison of post-treatment data versus baseline data within the same group using paired t-test).

**Figures**
Figure 1

Flow chart of the study participants
Figure 2

Changes in body weight (a) and BMI (b) among the three groups throughout the treatment course