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Metformin increases mammographic breast density: a randomized controlled trial

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ABSTRACT

Background: Mammographic breast density (MBD) is directly related to the risk of breast cancer. In vivo and in vitro studies have shown that metformin can reduce the proliferation and growth of breast cancer tissue. The aim of this study was to determine the effect of metformin on MBD in non-diabetic premenopausal women.

Methods: A double-blind, placebo-controlled study was performed in women who attended the Breast Clinic for opportunistic BC screening or mild breast symptoms. A total of 151 premenopausal women received 500 mg metformin tablets or a placebo made by the same company, twice a day for 6 months, . All the mammograms were evaluated by two expert radiologists. The changes in MBD were compared between the two groups.

Results: Final data were evaluated based on 67 and 84 women in the metformin and placebo groups, respectively. Based on results from ordinal logistic regression, the odds of achieving a higher density for the intervention group was approximately 2.33 (95% CI, 1.04 to 5.18) times that of the placebo group.

Conclusion: This clinical trial showed that consumption of metformin 500 mg twice daily for 6 months is associated with a higher mammographic breast density as compared to the placebo group. As metformin is used very commonly, we suggest that this medicine should be considered as a probable confounding factor when conducting studies about MBD.

IRCT: IRCT20100706004329N9

Keywords: metformin, breast density, mammography, confounding factor, weight.

INTRODUCTION:

Breast cancer (BC) is the most common female cancer in the world and is the most frequent cause of cancer death globally among women [1].

Mammographic breast density (MBD) is directly related to the risk of BC and an increase of 3-6% in MBD increases the relative risk of BC by 10% [2].This association has triggered many studies about the factors that can affect MBD. Some of the recognized influencing elements are endogenous, like age, menarche, and menopause [2]; while some others are exogenous modifiable factors, which are still under study. Among them, the use of some medications has got some interest, such as tamoxifen [3].

Metformin is being used as the first line treatment in diabetes [4]. However, it is interesting that some reliable evidence has also shown that metformin has anti-cancer effects through indirect and direct mechanisms [5].Treatment with metformin can not only reduce the risk and mortality of cancer, but can also improve the efficacy of cancer treatment in diabetics [6]. The incidence of cancer and mortality in diabetic patients taking metformin at doses ranging from 1500 to 2250 mg per day might be reduced by approximately 10 to 40 percent [7]. Also, in vivo and in vitro studies have shown that metformin can reduce the proliferation and growth of BC tissue [6].

Considering the association of MBD and BC, and the probable relation between metformin use and BC, a few studies have evaluated the effect of metformin on MBD in diabetic women; with diverse results. Moreover, the effect of this drug on MBD in people without diabetes is unknown. So, envisaging the safety of metformin and the uncommon occurrence of adverse effects accompanying its use [8], we conducted a study to determine the effect of this medication on MBD in non-diabetic premenopausal women.

Methods and Materials:

Study design and Participants

This study was approved by the Ethics Committee (Ethics Code: IR.TUMS.IKHC.REC.1399.307) of Tehran University of Medical Sciences (TUMS), and an informed consent was obtained from all participants. The protocol was registered in the Iranian Registry of Clinical Trials (IRCT) (Code: IRCT20100706004329N9; Date: 15/12/2020), and the study was held from December 2020 to June 2021. Participants were selected among women who attended the Breast Clinic for opportunistic BC screening or mild breast symptoms. The criteria for inclusion in the study consisted of female gender, age 40 years or above, non-diabetic premenopausal status, no mammography performed one year or more before the inclusion and willingness to participate in the study. Exclusion criteria consisted of any suspected malignancy, a glomerular filtration rate (GFR) < 90 ml/min/1.73 according to the Cockcroft-Gault Equation [GFR= ((140-age) × weight /Plasma creatinine ×72)× (0.85)] [9], history of BC in the patient or in her first-degree relatives, history of diabetes, severe hyperlipidemia, fatty liver grade 2 or higher, hypo or hyperthyroidism, ischemic heart disease or heart failure, asthma or epilepsy; pregnancy or intention to get pregnant within the next year, or a history of current or recent (within the recent two years) use of metformin or allergy to metformin. Probable unbearable side effects of metformin including abdominal pain, chest pain, diarrhea, dizziness, severe headache, hypoglycemic-like episodes, myalgia, nausea and vomiting, palpitation, or skin rashes were considered as withdrawal criteria from the study.

Sample size, Random Allocation and Blinding

Considering a 28% decrease in MBD reported by Bershtein et al [10] and a 52% vs. 48% rate of high vs. low MBD in Iranian women [11], a sample size of 73-81 women was envisaged as appropriate for a power of 80% and α= 0.05. Randomized assignment to metformin or placebo was conducted by a methodologist via the block randomization method using sealedenvelope.com in 6-piece blocks. None of the participants and the research medical team including the surgeons and the radiologists were aware of the group allocations.

Data Gathering, Procedures and Interventions

Questionnaires containing information about demographic characteristics, history of medical and surgical diseases, and family history of BC were completed by all participants. Weight, height, waist, and hip circumferences were measured by a trained staff. Blood samples were collected from all participants and complete blood counts, blood sugar level, liver, and renal function tests were assessed at the start of the intervention. Thereafter, a standard bilateral mediolateral-oblique and craniocaudal views mammography was performed for all participants. Participants in the intervention group received 500 mg metformin tablets (Osveh Pharmaceutical Company, Iran) twice a day for 6 months; and participants in the control group received identical placebo tablets made by the same company twice a day for 6 months. All women were reminded to use the medicines as required by short messages sent every 14 days. Participants were advised not to make major changes to their diet during the study. They were also asked to inform the research group if they changed their main dietary habits, or if they needed to take any new medications, supplements, or other therapies.

The serum levels of urea and creatinine were measured again at the end of the intervention. Then, 6 months after using the medicine or placebo, a standard bilateral four-view mammography was performed.

All the mammograms were evaluated by two expert radiologists. They recorded the MBD according to the Breast Imaging- Reporting and Data System (BIRAD) of the parenchymal mammographic classification system of the American College of Radiology (ACR) as almost entirely fatty (ACR a:), scattered fibroglandular densities (ACR b:), heterogeneously dense (ACR c:), and extremely dense (ACR d) [12]. In rare cases where their ACR rating was dissimilar, a third party who consisted of a radiologist expert in breast imaging in a sister hospital (Cancer Institute, TUMS) was asked to rate it. Finally, as the MBD change was our main outcome; this was compared between the two groups.

Statistical analysis

We summarized demographic and clinical data of the participants using descriptive statistics in each study group, mean (standard deviation; SD) for continuous variables and number (percentage) for categorical variables.

In our analysis, we determined the appropriate use of parametric or non-parametric statistical methods based on various assessments of the data. We compared means and medians, with significant differences indicating deviations from normality. The distribution was visualized through histograms, which allowed us to assess symmetry and skewness. Additionally, Quantile-Quantile (Q-Q) plots were generated to compare the data's distribution to a normal distribution, with deviations from the 45-degree line signifying non-normality. Finally, we conducted the Kolmogorov-Smirnov test to statistically test the normality hypothesis; a significant result ($p < 0.05$) confirmed that the data did not follow a normal distribution.

Ordinal regression model using the cumulative logit model was utilized to assess the effect of metformin on MBD, considering the baseline level of breast density as a covariate. Results were reported as Odds Ratio (OR) and 95% confidence interval (CI). This model yields a common OR for change from one breast density level to the next (higher) level.

The analysis was conducted using R version 4.3.1 (2023-06-16). All statistical analyses were conducted at a significance level of 0.05, and p-values less than 0.05 were considered statistically significant.

Results:

One hundred fifty-one women were entered in the study, and according to their previous assignment in the groups, consisted of 67 women in the intervention and 84 in the placebo group (Figure1). Participants in the two groups were comparable in terms of age, BMI and gravidity (Table1).

After the intervention, the mean (± SD) body mass index (BMI) in the placebo and intervention groups were 27.81 (± 4.66) and 28.68 (± 5), respectively; there was

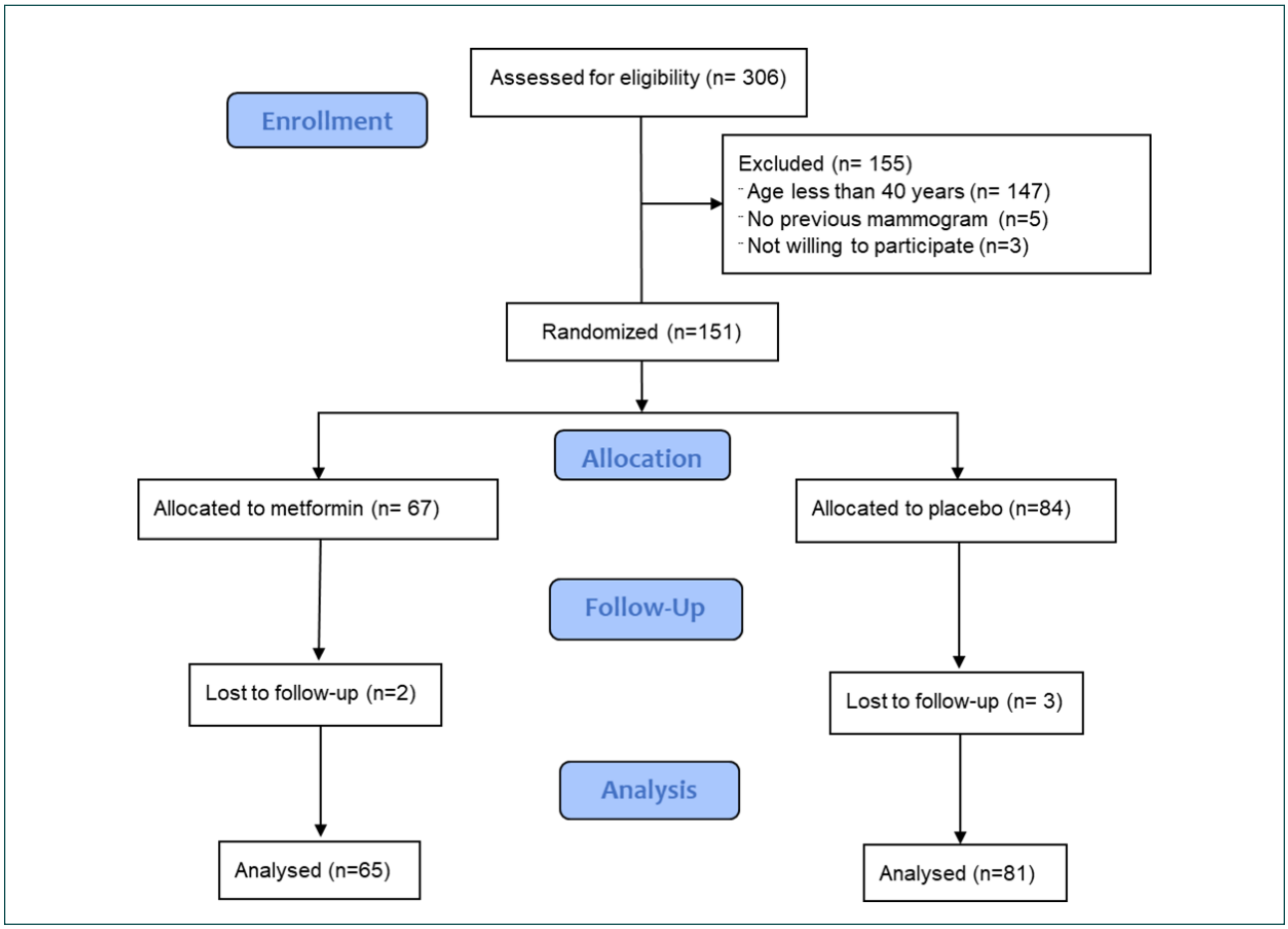

Figure 1. Summary of patients' flow

Table 1. Demographic characteristic of all participants

	Intervention Group	Placebo Group
Age*	48.80± 5.03	49.11± 5.65
BMI (before intervention)*	28.01± 4.71	27.50± 4.67
Gravidity*	2.72± 1.49	2.34± 1.22
History of breastfeeding**	8 (11.8)	6 (7.1)
History of infertility**	5 (7.4)	15 (17.9)
History of infertility treatment**	5 (7.4)	8 (9.5)

* Students T test: mean± standard deviation, **Chi- Squared test: number (percentage)

no statistically significant difference between the two groups (p-value= 0.5). Changes in BMI before and after the intervention were assessed in the two groups. There was no statistically significant difference between the two groups in this regard; the mean BMI change (± SD) was 0.017(± 1.80) in the intervention group and 0.27(± 0.88) in the placebo group (p=0.26).

There was a significant difference between the two groups in terms of baseline MBD (p=0.02) (Table 2). However, as the changes in MBD were the main outcome; we assessed and compared this issue between the two groups. Based on results from Ordinal Logistic Regression, the odds of achieving a higher density for the intervention group was approximately 2.33 (95% CI, 1.04 to 5.18) times that of the placebo group, Wald $\chi 2$ (1) = 4.251, p = .039; adjusted for the baseline level of MBD.

Discussion:

We performed a study to investigate the effect of metformin on mammographic breast density, and found that consumption of metformin (500 mg twice daily for 6 months) is associated with an increased MBD as compared to placebo.

Factors such as age at first birth, parity, age at menopause, and hormone therapy affect MBD [13, 14]. On the other hand, obesity, which increases the risk of BC during menopause, reduces MBD [15]. Many of these factors are dependent on estrogen levels.

Studies have shown the evidence of cancer reduction with metformin. However, it is not clear whether this effect is due to the direct anti-cancer effect of this drug, or secondary to the adjustments of blood sugar and insulin levels [4]. Metformin has several biological effects that can have anti-cancer effects, including direct anti-proliferative effects mediated by activating adenosine monophosphate kinase (AMPK); or through indirect mechanisms, such as lowering circulating insulin levels and improving glycemic hemostasis [16].

Metformin, in addition to its probable anti-carcinogenic effects, can reduce circulating androgen and estrogen levels. Therefore, it is expectable that metformin, by reducing endogenous estrogen levels, could reduce MBD [17].To the best of our knowledge, there are few studies regarding the association between MBD and consumption of metformin.

Bershtein et al [10] evaluated the changes in MBD in 25 postmenopausal women randomly receiving 1-1.5 g metformin (n = 14) or 400-600 mg N-acetyl cysteine for a period of around 10 months. MBD was measured before and after treatment. Out of14 women, MBD decreased in 4 women (28.5%), and in 7 patients the dense areas decreased in comparison to non-dense areas. The absence of a control group and the small sample size suggests caution in the interpretation of that study.

Tapia et al [18] carried out a placebo-controlled trial to evaluate the effect of 1700 mg daily metformin for one

Table 2. The distribution of mammographic breast density before and after the intervention in the two groups

Mammographic Breast Density	ACR1	ACR2	ACR3	ACR4	P-value
Before the intervention					
Placebo Group	1(1.2)	16(19)	47(56)	20(23.4)	0.02
Intervention Group	5(7.5)	8(11.9)	47(70.1)	7(10.4)	
After the intervention					
Placebo Group	3(3.70)	10(12.30)	60(74.10)	8(9.90)	0.5
Intervention Group	2(3.10)	4(6.2)	50(76.90)	9(13.80)	

ACR= American College of Radiology

year on MBD in 76 obese or overweight premenopausal women affected by metabolic syndrome; and compared them with 83 controls. They saw no effect on MBD, but a reduced waist circumference and waist-to-hip ratio.

In two cross-sectional studies [5, 19], metformin use in diabetics was associated with a decrease in MBD; nevertheless after BMI adjustment, the density was not related to the consumption of metformin. In another case-control study [20], there was no difference in MBD between the postmenopausal women that received metformin and those without treatment.

The cohort study of Danish women above the age of 50 showed that breast density in diabetic women decreased by consumption of oral anti-diabetic agents or diet, and that there was an association between high breast density and insulin use [15]. However, that study showed a similar effect for oral anti-diabetic drugs and diet modification .

Eslami et al [21] investigated 712 women by asking them if they used aspirin or metformin, and evaluated the relation with their MBDs. Although they found a significantly indirect association with MBD, this became insignificant after correction for confounding factors. They propose more studies to evaluate the subject further.

The comparison of the findings of these studies with our results is difficult due to differences in study design and study population. However, our study has been specifically designed to assess the effects of metformin and includes a placebo group, which makes it much more reliable for investigation of the association of metformin use and MBD; and shows that use of metformin can increase MBD.

Significant weight loss was observed in previous studies with metformin doses above 2000 mg per day. Zafar [22] also reported that taking metformin for 6 months was not associated with weight loss and showed that weight loss was dependent on the dose of the medicine. A dose of 2500 mg was associated with more weight loss than 1500 mg. Also, Sharma et al. showed that metformin consumption (500 mg twice daily for 3 months) leads to a weight loss of 0.08 in overweight, obese, and normal-weight women with polycystic ovarian syndrome [23]. However, use of 1000 mg metformin for six months did not induce weight loss in our study, so MBD increases in the intervention group was not related to weight loss. Despite the lack of changes in weight or BMI, we think that as metformin decreases body fat mass [24, 25], it has caused reductions in the fatty content of the breasts in our participants. This would justify the increments in MBD caused by metformin use in the intervention group.

Metformin is a relatively safe and well-tolerated medicine. Its most frequently observed side effects are mild gastrointestinal complaints. Some serious adverse effects have also been reported, but these are very rare; like lactic acidosis (9 per 100000) or hepatotoxicity [26, 27]. No side effects were observed in our study. Also, the medicine was well-tolerated by all users and all participants in the intervention group adhered completely to the prescription.

The randomized and blinded design of our study provides generalizable results. However, we must note that some unknown confounding factors might have affected our results. For example, we have previously shown that endometriosis lowers MBD in Iranian women [28], and that the frequent use of cosmetics can increase MDB in the Iranian population [29]. These factors have not been considered in the study, while they might have affected the results. Our study had some limitations. We did not evaluate the blood levels of insulin, blood sugar, androgen, and estrogen in our study, while these could modulate the effects of metformin on MBD. Also, the effects of the intervention on breast exam are not reported in this study.

As metformin is used very commonly, the result of our study should be taken into account when designing studies about MBD. Researchers must pay attention that the use of metformin could affect MBD and can interfere with their study results; consequently, this factor should be considered as a probable confounding factor in researches on MBD.

Conclusions:

This clinical trial study showed that consumption of metformin (500 mg) twice daily for 6 months is associated with higher mammographic breast density as compared to the use of a placebo. Therefore, considering the frequency of use of metformin in non-diabetics, it is necessary to contemplate metformin consumption as a potential confounding factor in clinical studies about MBD.

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Abbreviations:

ACR: American College of Radiology
AMPK: Activating adenosine monophosphate kinase
BC :Breast cancer
BIRADS: Breast Imaging- Reporting and Data System
CI :Confidence interval
MBD: Mammographic breast density
OR :Odds Ratio

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