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Metformin Efficacy on Proliferation Indices of Tumoral Cells in Non-Diabetic Patients with Invasive Breast Cancer Referring to the Cancer Institute of Iran

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ABSTRACT

Background: Because of the decreasing effect of metformin on insulin resistance, it has been suggested as an anti-obesity and anti-cancer drug. So, we aimed to study the effect of metformin therapy on tumor cell proliferation in non-diabetic breast cancer patients.

Methods: We conducted a prospective clinical trial and studied the effect of metformin therapy on the level of Ki67 as a measure of tumor cell proliferation. Our primary endpoint was to evaluate the changes in Ki67. The intervention group consisted of 25 non-diabetic breast cancer patients with no indication for neoadjuvant chemotherapy. They were followed up from the time of biopsy to operation. Metformin (1500 mg/day) was prescribed in the intervention group from the date of diagnosis until the surgery (2.8 weeks). Controls were 20 early breast cancer patients who had been followed up with no prescription from biopsy until operation.

Results: We could not find any statistically significant difference between the two groups regarding baseline clinical or tumor characteristics such as age, stage, grade, estrogen receptor, HER2 status or time, and type of surgery. However, the immunohistochemistry (IHS) study showed a decrease in median Ki67 from 35.14 to 29.6 in the intervention group (P-value= 0.02). While an increase from 24.5 to 30.6 was detected in the control group (P-value= 0.02). Both of these changes were statistically significant. Although mild gastrointestinal symptoms were seen in approximately 50% of cases, generally, patients tolerated metformin well. There was a correlation between the score of HOMA, a metabolic factor, and the changes in KI67.

Conclusion: Metformin prescription in a short period of time between biopsy and definitive surgery leads to the inhibition of breast cancer cell growth. We found a relationship between metformin anti-proliferative effect and glucose and insulin metabolism.

Keywords: Metformin, Tumor Cell Proliferation, Breast Neoplasms

INTRODUCTION:

Today, breast cancer is the second cause of cancer death among women worldwide [1,2,3]. While obesity is associated with an increased risk of breast cancer, several reports suggested that type II diabetes mellitus is also a major predisposing factor for breast cancer [4]. Metformin is an effective drug that has been used for the treatment of diabetes mellitus since half a century ago. Because of the decreasing effect of metformin on insulin resistance, it has been suggested as an anti-obesity and anti-cancer drug [5]. In addition, evidence suggests a direct effect of metformin on cell proliferation control in breast cancer [6].

According to retrospective studies, the prognosis of diabetic breast cancer patients can be significantly improved by metformin use [7,8]. Nevertheless, tumor growth-promoting effect of insulin and complications of advanced diabetes could bias the results of the retrospective studies. Few studies investigated the biological effect of metformin on cell growth and differentiation [9] and reported a beneficial effect of metformin in breast cancer treatment [10]. We conducted this study to investigate the role of metformin by measuring Ki-67 changes, which is a cell proliferative index [7,8,9]. The main objective of the present study was to evaluate how metformin use can change the Ki-67 percentage from the time of biopsy to the time of surgery.

METHODS:

We conducted a non-randomized clinical trial from September 2014 to February 2015 at the Cancer Institute of Iran. Cases were 25 non-diabetic breast cancer patients with a confirmed pathologic diagnosis, physically fit, suited for taking metformin, with a tumor restricted to local breast tissue with or without the involvement of regional lymph nodes, and with no plan for preoperative chemotherapy. The exclusion criteria included a history of diabetes mellitus, prior treatment with metformin due to any cause, evidence of distant metastasis, and a history of other cancers or chronic diseases. Patients with abnormal results of laboratory tests such as a threefold increase in liver enzymes, creatinine level higher than 1.5 in men and 1.4 in women, and creatinine clearances less than %60 were also excluded. The ethics committee of the Tehran University of Medical Sciences approved this study (Cod:92-03-51-24050). All of the participants were recruited voluntarily and signed a written informed consent after asking their potential questions and being convinced to participate in the study.

We first measured the percentage of the Ki67 on biopsy samples from the tumor tissue, and CRP, ESR, glucose, and insulin in the blood of the patients. The Ki67 were measured in formalin-fixed paraffin-embedded blocks of breast tumors using immunohistochemistry (IHC) staining.

Patients in the intervention group were allocated to the metformin therapy (500 mg Glucophage —a brand name for metformin—three times daily) for approximately three weeks. Drug side-effects were classified according to the NCI criteria. Notably, we re-measured Ki67 in tissue samples removed during their operation. We also measured CRP/ESR/Glucose/Insulin in the blood again before the surgery. Patients underwent surgery at the best affordable schedule of the surgery ward. There was no delay in operation due to the usage of metformin. In addition, based on the study plan, patients stopped using metformin if any adverse events occurred during the study.

We selected a control group (n=20) from non-diabetic early breast cancer patients who were admitted at the Cancer Institute for surgery. The control group did not receive metformin or any other drugs between biopsy sampling and surgery. They were checked for Ki67 in

the biopsy sample and tumor tissues collected during the surgery.

The main goal of this study was to compare changes in the Ki67 between the intervention and control groups after three weeks. Minor outcomes were a comparison of CRP, ESR, Glucose, Insulin, and HOMA (an index of insulin resistance) score in blood samples of the intervention group before and after metformin treatment.

Statistical analysis

All analyses were performed using STATA 11 software. Chi-square was used for determining the difference between the proportion of the categorical variables, and the t-test was used to assess differences between the means in intervention and control groups. A paired t-test was used to compare the mean values in the intervention group before and after the treatment.

RESULTS:

This study consisted of 45 breast cancer patients, including 25 patients in the intervention group (mean age 47.7, SD 12.3), and 20 patients into the control group (mean age 47.7, SD 7.7). The clinical and pathologic characteristics of the two groups did not show statistically significant differences (**Table 1**).

The intervention group received metformin for a median duration of 2.8 weeks. Control and intervention groups were mostly diagnosed as stage II. In both groups, about 70% of the patients underwent a lumpectomy, and 30% underwent a total mastectomy. We found that 80% of patients were estrogen receptor-positive (ER+), 80% were progesterone receptor-positive (PR+), 24% were positive for HER2, and 56% had positive P53 mutations.

As expected, the range of Ki67 in the intervention group showed a statistically significant decrease from 35.14 to 29.6. However, the mean of Ki67 level significantly increased from 24.5 to 30.6 in the control group. The difference between the two groups was statistically significant (**Table 2**). In addition, insulin level and HOMA score significantly decreased in the intervention group (**Table 3**). We found no serious side effects of metformin in this study except mild nausea (52%) and vomiting (44%) (**Table 4**).

DISCUSSION

In a non-randomized clinical trial, we studied the role of metformin usage before definite surgery on early breast cancer outcomes and found a significant decrease in Ki67 from 35.1 during the biopsy to 29.6 during the surgery in the intervention group. The Ki67 level increased in the control group.

Hadad et al. conducted a similar study in the Cancer Institute of Iran and found similar results[11]. In the pilot phase, they recruited eight non-diabetic patients with breast cancer and prescribed 500 mg metformin for one week, followed by 1000 mg metformin daily before surgery. Afterward, they randomly allocated 47 patients into the placebo and metformin group. Although there was a downward trend in the Ki67 in both groups, they showed a significant decrease in Ki67 only in the intervention group[11]. Nirauala et al. reported the correlation between metformin use and the decline in Ki67 [12]. The same results were reported in a large clinical trial with 200 breast cancer patients. They found a significant decrease in Ki67 among patients with insulin resistance. However, metformin was stopped three days before the surgery. Therefore, they could only show the effect of short-time use of metformin, not to the exact time of the surgery. Due to these findings and based on Cazzaniga et al.[13], metformin impact is mostly related to the level of metabolic indexes of a breast cancer patient. Because the HOMA score was high in our study, our findings seem to be in line with the results of previous studies[14,15], which had shown the efficacy of metformin in patients with high insulin levels and HOMA scores. We found a relationship between these indexes of insulin resistance and a decrease in Ki67 as a marker of

Table 1. Demographic variables of breast cancer patients who received metformin (intervention group) compared to the control group

Variables	Intervention Numbers (%)	Control Numbers (%)	P-value
Grade tumor			0.1
G1	2(8)	8(40)	
G2	18(72)	8(40)	
G3	5(20)	4(20)	
Tumor size			0.09
1	7(28)	5(25)	
2	17(68)	15(75)	
3	1(4)	----	
Lymph metastasis			0.3
Yes	13(48)	12(60)	
No	12(52)	8(40)	
Metastasis position			0.5
Yes	0	0	
No	20(100)	25(100)	
Surgery method			0.9
MRM	5(25)	9(36)	
BC	15(75)	16(64)	
Lymphadenectomy			0.88
Dissection	6(30)	9(36)	
No dissection	14(70)	16(64)	0.7
Estrogen receptor			
Positive	15(75)	20(83.3)	
Negative	5(25)	4(16.7)	0.3
Progesterone receptor			
Positive	16(80)	20(83.3)	
Negative	4(20)	4(16.7)	0.09
HER2 receptor			
Positive	4(20)	6(24)	
Negative	16(80)	19(76)	0.4
P53 receptor			
Positive	10(50)	11(45.8)	
Negative	10(50)	13(54.2)	0.3

Table 2. Changes in Ki67 since tumor biopsy until surgery between the control and treatment groups

	P-value	Mean Changes before and after the treatment	Ki67 of the Surgery specimen Mean (SD)	Ki67 of the first Biopsy Mean (SD)
Control group	0.02	-6.1	30.6(7.2)	24.5(13.12)
Intervention group	0.02	5.54	29.6(18.5)	35.14(22.4)
Significant Difference between Control and Intervention Group	0.01	11.6		

Table 3. Changes in Fasting Glucose, Serum insulin, CRP, and HOMA Score before and after metformin treatment

	P-value	Mean after metformin treatment	Mean before metformin treatment
Fasting Blood sugar (FBS)	0.2	92.7(21.4)	92.48(24.9)
Fasting Serum insulin	0.04	11.75(4.9)	14.3(7.6)
CRP	0.1	2.4(1.1)	3.2(2.1)
HOMA score	0.07	2.8(1.25)	3.4(1.8)

Table 4. Side effects of metformin treatment among breast cancer patient

Side effect	Never No. (%)	low	high	Very high
Nausea	12 (48%)	13(52)	0	0
Vomiting	14 (56%)	11(44%)	0	0
Constipation	21(48%)	4(52%)	0	0
Diarrhea	20(80%)	5(20%)	0	0
Dry mouth	25(100%)	0(0%)	0	0

tumor cell proliferation level.

A comparison of Ki67 between biopsy and surgery raises potential concern, as Romero et al. believed that time period significantly reduces Ki67 measured between biopsy and surgery samples [16]. However, the decrease in the Ki67 was very high in our study, and we found a significant increase between biopsy and surgery in the control group. Therefore, the limited sample size and the above concern cannot be true and cannot confound the results we acquired through the presented study.

Based on our findings, metformin exposure was associated with a significant reduction in insulin level and insulin resistance, as average fasting blood sugar level on biopsy was 14.3, which changed to 11.75 after treatment and also as HOMA score significantly decreased from 3.4 to 2.8. In the same way, in three recent trials, a remarkable decline in insulin markers and insulin resistance was shown in diabetic patients with breast cancer. The decrease in insulin level and insulin resistance indicates an indirect impact of metformin on the decline of tumoral cell proliferation rate. In the mentioned study, the extraction of hepatic sugar decreases and caused increased insulin levels. Because insulin-like growth factor receptors are present in several cells, a high level of insulin may lead to tumoral cell proliferation and growth. The insulin-dependent growth-promoting pathway mediates this effect.

Metformin has additional direct methods to deal with tumorogenesis. Activating AMPK causes downregulation of tumor pathway and, consequently, a decline in carcinoma and metastasis in breast cancer patients. Also, the P53 level has shown to one of the main controlling proteins of cancer proliferation [17]. Unfortunately, we could not measure factors related to this pathway in our study.

In the present study, while 50% of the patients showed minor gastrointestinal side-effects, metformin generally was well tolerated by the patients. We also did not find major detrimental effects of the drug on the lifestyle of the patients.

In conclusion, we found that metformin usage has a significant impact on controlling the proliferation of breast cancer cells in a limited period of time. We believe that the prescription of metformin can be an appropriate means to improve the treatment outcome of breast cancer patients. However, our results should be confirmed by larger randomized clinical trials and a comprehensive assessment of different treatment outcomes at the molecular and clinical levels.

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