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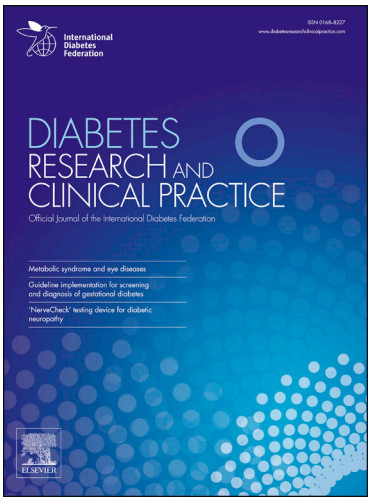

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Gad Rennert, Hedy S. Rennert, Naomi Gronich, Mila Pinchev, Stephen B Gruber

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Use of metformin and risk of breast and colorectal cancer

Gad Rennert, MD, PhD[1,2], Hedy S. Rennert, MPH[1], Naomi Gronich, MD[1], Mila Pinchev, MD[1], Stephen B Gruber[3]

[1]Department of Community Medicine and Epidemiology, Carmel Medical Center and B. Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

[2]Office of Chief Physician, Clalit Health Services Headquarters, Tel Aviv, Israel

[3]USC Norris Comprehensive Cancer Center, USC, Los Angeles, USA

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Corresponding author:
Gad Rennert, M.D., Ph.D.,
Department of Community Medicine and Epidemiology,
Carmel Medical Center, 7 Michal St., Haifa 34362, Israel;
e-mail: rennert@ technion.ac.il
Tel; +972-4-825 0474, Fax: +972-4-834 4358

Abstract

Background

Diabetes has been associated with increased risk of cancer, including breast cancer and colorectal cancer. Metformin, an oral hypoglycemic drug, but not other anti-diabetic drugs, has been associated with reduced risk of breast and of colon cancers in some, but not in other, studies.

Methods

Data from two large-scale, population-based, case-control studies of breast and colorectal cancers etiology, conducted in Northern Israel since 1998 were analyzed to evaluate the association between regular use (>3 times) of metformin prior to diagnosis and risk of developing cancer. The multivariate analyses for both cancer sites included age, family history of breast/colorectal cancer, history of diabetes, sports participation, fruits/vegetables consumption, aspirin and statins use, and for breast cancer, also included use of oral contraceptives and postmenopausal hormones and number of pregnancies. Use of metformin and diabetes status were determined based on valid electronic medical records of the participants.

Results

Metformin use prior to diagnosis of cancer was associated with a decrease in risk of both breast cancer (OR=0.821, 0.726-0.928, p=0.002) and colorectal cancer (OR=0.754, 0.623-0.912, p=0.004). An inverse association was not identified with use of other anti-diabetic medications. Diabetes was found to be associated with risk of colorectal cancer (OR=1.204, 1.014-1.431, p=0.034) but not of breast cancer. No dose response by years of use of metformin was found.

Conclusion

These analyses of large population-based studies provide evidence of a strong inverse association of metformin with breast and, even more so, with colorectal cancer risk.

Background

Diabetes has been shown to be associated with a mildly increased risk of breast cancer diagnosis (1,2), increased risk of recurrence (3) and elevated risk of developing triple negative tumors (4). Similarly, diabetes has been associated with a mildly increased risk of colorectal cancer (5,6) and with reduced survival of CRC patients (7).

Metformin is an oral hypoglycemic drug in widespread clinical use for treating type 2 diabetes mellitus. It acts through stimulation of fatty acid oxidation, glucose uptake and non-oxidative metabolism, which lead to decreased blood insulin levels (8). Metformin does not cause hypoglycemia in non- diabetics and has also been used safely in polycystic ovary syndrome and chronic active hepatitis. The leading hypothesis linking the mechanism of action of metformin as a chemopreventive agent involves increased activity of the AMP-dependent protein kinase (AMPK) (9,10) which is a nutrient sensor that inhibits tumorigenesis by targeting tumor metabolism and inhibiting mammalian Target Of Rapamycin (mTOR)-associated oncogenic signaling pathway. mTOR coordinates nutrient availability and energy metabolism in response to growth factors. Metformin can negatively affect growth of human tumors even in the presence of activating mutation in PIK3CA, another regulator of cell metabolism that converges on the mTOR pathway. Dose-dependent AMPK phosphorylation (10,11), apoptosis or necrosis (12), and a cytotoxic effect at clinically achievable concentrations (13) were demonstrated following exposure to metformin. Metformin has been shown to induce S phase arrest and apoptosis in triple negative (basal-like) breast cancer cells, while inhibiting cell proliferation (G1 arrest) without the induction of apoptosis in luminal A, B, and Her2 positive breast cancer cell types in vitro and in vivo. (14,15).

Many studies have investigated the function of metformin in various tumor sites and several association studies have been reported. The data, however, are conflicting. Metformin was shown to suppress breast cancer growth in vivo (16-20) and in vitro in both ER-positive and ER-negative cell lines, but the effect was stronger in ER positive cells.

Metformin use was further associated in population studies with reduced incidence of cancers in various sites such as the breast (21,22), colon (6,23-25) and other (26,27); an association which was not seen with use of other anti-hypoglycemic drugs. However, others report no evidence of reduced risk (28-30). Therapeutically, metformin has also been tested for treatment of breast cancer and several morphological and molecular changes have been demonstrated (31-35). Survival benefits in diabetic Metformin users have been reported for colorectal cancer (36). Other studies showed lower formation rate of adenomas (37,38) and lower colonic adenoma recurrence rates (39-41)

Given the strong mechanistic rationale for a possible risk reducing effect of metformin, and the somewhat inconsistent results of association studies of metformin use and cancer incidence, we further evaluated this association in two large-scale, methodologically robust population-based case-control studies.

Methods

Participants

Participants in this analysis came from two population-based case-control studies of consecutively diagnosed patients with breast and colorectal cancer. Patients who lived in a geographically defined area of northern Israel at time of diagnosis were eligible to

participate and healthy controls were matched by age (+/- 2 years), gender, ethnicity (Jewish/Arab) and residence (location of primary care clinic) and randomly chosen from the list of eligible. The Molecular Epidemiology of Colorectal Cancer (MECC) Study and the Breast Cancer in Northern Israel Study (BCINIS) are population-based, case–control studies of incident colorectal and breast cancers in northern Israel with recruitment starting at 1998 for MECC (42) and 2000 (43) for BCINIS. Participants provided written informed consent at the time of enrollment and were interviewed to obtain information about their personal and family history of cancer, reproductive history, medical history, medication use and health habits including a food-frequency questionnaire. Venous blood samples were drawn and paraffin embedded tumor blocks of tumor tissue were sought for all cancer cases. Included in this analysis are all cancer cases and controls who were Clalit Health Services (CHS) insurees, as these are the participants for whom computerized full prescription data were unselectively available. CHS is the largest health care provider in Israel and during the study years covered approximately 70 percent of the older population (persons 50 years of age or older). Health care coverage in Israel is mandatory and is provided by four groups akin to not-for-profit health maintenance organizations. Thus, all study participants (including those excluded due to lack of access to their EMRs) had a similar health insurance plan and similar access to health services, including prevention, cancer screening and oncologic treatments. The institutional review board at Carmel Medical Center, Haifa, approved all procedures for both studies and the IRB of USC, Los Angeles, USA additionally approved all procedures for the MECC study.

Due to differences in the dynamics and clinical behavior of diabetes between ethnic groups in Israel, this analysis is limited to Jewish cases and controls from both studies.

Exposure data

The use of Metformin was determined on the basis of CHS pharmacy records that were available for all CHS members MECC and BCINIS studies participants. These records included all filled prescription from the year 1998 to current and could therefore be separated into medications used before and after cancer diagnosis. Detailed prescription information enabled us to study the influence of the length of use and the number of prescriptions filled (44-46). Because of the very low co-payment required when filling prescriptions within the Clalit Health Services pharmacy system and its consignation pharmacies, it is unlikely that prescribed anti-diabetes medications were purchased in private, non-CHS, pharmacies. For this study, Metformin users are defined as any study participant who filled at least three prescriptions for the drug before the index cancer diagnosis (or time of interview for healthy control). The use of other diabetes related medications was similarly evaluated.

Other exposures included data on family history of cancer, reproductive practices (use of oral contraceptives and of hormone replacement therapy, number of children), leisure-time physical activity , diagnosis of diabetes, use of aspirin/non-steroidal anti-inflammatory(NSAIDs) and statins, and nutritional habits as reflected in a semi-quantitative food-frequency questionnaire (47).

Statistical analysis

Although the data come from matched case-control studies, a non-matched analysis was performed. It may be noted that there are more controls than cases in this analysis from the BCINIS study. Analysis limited to case-control pairs provided the exact same point estimates and we chose to show the full available series to increase the power of the findings. Non-conditional, backward stepwise logistic regression was used to estimate the effect of metformin use on the risk of developing breast and colorectal cancers. The full model included use of Metformin as the independent variable and a line of potential effect

modifiers: age, history of diabetes, use of other anti-diabetes drugs, physical activity (sports participation), consumption of vegetables, fruits, use of aspirin and statins, first degree family history of breast cancer or colorectal cancer (per the relevant study question), and ever use of oral contraceptives, hormone replacement therapy and number of pregnancies for the breast cancer risk analysis only. Statistical analyses were performed using SPSS (v23).

Results

This report includes information collected from 18,527 Jewish participants of the BCINIS and MECC, population-based, case-control studies in Northern Israel, who met the eligibility criteria for analysis. Diabetes, at the time of cancer diagnosis, was reported by 19.4% of the study participants (18.1% of the breast cancer study, 21.1% of the colorectal cancer study). Any anti-diabetes medications were taken for any length of time before diagnosis of cancer by 20.8% (19.0% of the breast cancer study, 22.9% of the colorectal cancer study) and metformin specifically, alone or in combination, was taken for more than three months by 14.5% of the participants (13.4% of the breast cancer study, 15.8% of the colorectal cancer study), resulting in 2,680 ever users of metformin before cancer diagnosis (Table 1). In univariate analyses, in addition to the association with metformin, significant associations with colorectal cancer risk were found for consumption of vegetables (5 or more servings a day), fruits (3 or more servings per day), and use of daily aspirin or any statins (Table 1). However, univariate significant associations with breast cancer risk were found only for sports participation, any use of statins, and any use of oral contraceptive, but not for any hormone replacement therapy and number of pregnancies (Table 1). Metformin use was significantly negatively associated with risk of colorectal cancer (OR=0.857, 95%CI 0.762-0.964) and of breast cancer (OR=0.848, 95%CI 0.755-0.952) in a univariate analysis.

Multivariate analysis

We included in a multivariate model variables which were formerly reported to possibly relevant to the risk of one or both tumors. These include age at diagnosis of cancer, prior diagnosis of diabetes, daily use of (mostly low dose) aspirin, any use of statins, sports participation (marker of physical activity), vegetable consumption (5+ portions per day), fruit consumption (3+ portions a day) and history of a first degree relative with CRC.All of the

above variables were found to be significant. Any use of metformin before diagnosis of cancer was found to be inversely associated with colorectal cancer risk, when statins were kept in the model as well or other variables (OR=0.84, 0.75-0.94, p=0.02) as well as when daily aspirin was kept in the model instead of statins (**0.754, 0.623-0.912**, **p=0.004**) (Table 2).

In a similar multivariate model employed in the breast cancer study, Metformin use for at least three months before the diagnosis of breast cancer was negatively associated with breast cancer risk **(0.821, 0.726-0.928, p=0.002)** in the final model which included age at diagnisis), family history of first degree relative where we introducesbreast cancer, sports participon, number of pregnancies and oral contraceptives use (Table 3). First degree family history was the single strongest risk factor of these cancers when a model (tables 2, 3).

The mean duration of Metformin use among BCINIS controls was 60.9 months and 54.4 months, with medians of 51 and 41 months of use correspondingly. The mean daily number of 850mg pills was 1.8 pills a day.

When evaluating duration of use of metformin prior to diagnosis, significant inverse associations with breast cancer risk were noted only in those using the drug for over 4 years (0.83 (0.716-0.97). For colorectal cancer risk the association is more complicated with significant inverse associations identified even within the first year of use OR=0.84 (0.70-1.00), but with also a close-to-significance association after 4 years (Table 4).

A significant trend for risk reduction with increasing dose of metformin was noted for both breast and colorectal cancers. However, when evaluating every quintile of use separately, only users of the upper quartile of metformin cumulative dose demonstrated significant risk

reduction; OR=0.80 (0.65-0.99) for breast cancer, OR=0.75 (0.59-0.95) for colorectal cancer (Table 5).

Discussion

Metformin was found to be mildly inversely associated with the risk of both colorectal cancer and breast cancer in our large, population-based, case-control studies. The association was evident in both univariate models and in multivariate models adjusting for age, diabetes status, family history of the relevant tumor and other recognized behavioral risk factors. These results are in line with those of some, but not all, other reports. The diversity in results of the various published studies could be a function of the ability to control for many other known potential confounders. Our breast and colorectal cancer studies in Northern Israel include vast amounts of data on a wide array of putative risk modifiers, collected by face to face interviews, using validated questionnaires. For this analysis we have also used highly validated EMRs for information on both diabetes status and the use of hypoglycemic agents, including metformin. Information bias is one of the key pitfalls of observational studies. The use of highly validated (45-47) prescription-filling information (rather than self-report which is bias prone) in this study minimizes misclassification of metformin exposure. Furthermore, control for effects of other known/established risk factors, combined with the large size of the involved studies and the large number of metformin users, increases the probability of the currently reported estimates to represent an unbiased effect. When evaluating the various risk factors included in the multivariate models, all were found to function in the expected direction based on former studies. Having a diagnosis of diabetes was reported to be associated with elevated risk of breast cancer (1,2) and colorectal cancer risk (5-7). Family history of both breast cancer (48) and colorectal cancer (49) are well documented risk factors of the corresponding tumors. Physical activity and consumption of vegetables and fruits have long been associated with risk reduction in both tumor sites (50-53). Hormonal risk factors have recently been reported to be of lower relevance to the risk of breast cancer, potentially

pointing at changes in the nature of these factors (lower hormonal concentrations in oral contraception pills, less variability in number of children). Statins and aspirin which have been shown to be associated with reduced risk of colorectal cancer (43,54) and in some publications also of breast cancer (55,56), did not remain significant predictors in the final breast cancer model, but either aspirin or statins did remain significant in the colon risk models. It is possible that the lack of a major effect in the current analyses is due to the concentration on diabetic patients. Such patients are routinely prescribed these two medications (ref) and therefore lack of variation precludes their effect from being demonstrated. In our studies 37.1% of diabetics receive both aspirin and statins vs 12.9% of the non-diabetics. (MECC 33.4/14.3, BCINIS 40.7/11.8).

The inverse association of the studied cancers with metformin use, shown already after short periods of use, raises the possibility that the leading effect is the control of diabetes rather than the pharmacological action of this specific agent. In addition, the relatively weak evidence of a dose-response effect, observed only with cumulative dose, is consistent with this hypothesis since cumulative dose is more likely to be associated with glucose control than usual dose.

A major question related to repurposing drugs for new indications is whether or not we should bring the drug of interest to a new randomized clinical trial before recommending it for use. Opinions split on this matter. Some argue that is required (57) while other agree that not only is it not feasible but it is also not necessary (58).

In summary, we have further demonstrated a potential negative association between use of metformin before diagnosis of breast and colon cancers. The association is modest, and not as strong as the protective association with use of aspirin; especially in the colorectal model and most significant in long term users and users of higher doses.

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Conflict of interest statement

None of the authors have any relevant conflict of interest to report

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Table 1

Demography and risk factors distribution of Jewish participants in the Metformin-use and breast (BCINIS Study) and colorectal (MECC) cancer analysis, Israel

	BCINIS			**MECC**		
	Cases	**Controls**	**P-Value**	**Cases**	**Controls**	**P-Value**
n	**4577**	**5587**		**4209**	**4154**	
Gender - Males (%)	0	0		2193 (52.2%)	2176 (524%)	0.85
Age at diagnosis (mean + SD)	62.2 (12.83)	62.5 (12.79)	0.384	70.9 (11.50)	71.7 (11.40)	**0.000**
1st degree family history of BC/CRC	838 (18.6%)	631 (11.2%)	**0.000**	561 (13.5%)	368 (8.9%)	**0.000**
Aspirin use (daily)	1189 (26.5%)	1535 (27.2%)	0.382	1315 (32.0%)	1724 (41.6%)	**0.000**
Statins use (any)	1593 (35.3%)	2075 (36.9%)	0.105	1161 (27.6%)	1341 (32.3%)	**0.000**
Vegetable consumption (5+ servings/day)	2920 (65.2%)	3760 (66.8%)	0.095	2265 (56.3%)	2579 (62.2%)	**0.000**
Fruit consumption (3+ servings/day)	1653 (36.9%)	2077 (36.9%)	0.995	1335 (33.1%)	1651 (39.9%)	**0.000**
Number of pregnancies (mean + SD)	4.2 (2.80)	4.4 (2.76)	**0.000**	NA	NA	NA
Oral Contraceptives (any)	1249 (28.3%)	1766 (31.6%)	**0.000**	NA	NA	NA
HRT (any)	877 (19.4%)	1140 (20.2%)	0.303	NA	NA	NA
Sports participation	2014 (44.9%)	2739 (48.6%)	**0.000**	1283 (31.2%)	1849 (44.5%)	**0.000**
Diabetes Mellitus before cancer diagnosis	774 (17.1%)	1066 (18.9%)	**0.020**	873 (20.7%)	890 (21.4%)	0.44
Use of Metformin >3 months before cancer diagnosis	558 (12.3%)	803 (14.2%)	**0.005**	621 (14.8%)	698 (16.8%)	**0.01**

Table 2

Multivariate analysis of Metformin-use and risk of colorectal cancer in Jews in the Molecular Epidemiology of Colorectal Cancer (MECC) Study, Northern Israel

	Full and Final Model	p-value
Metformin Use*	**0.754 (0.623-0.912)**	**0.004**
Age at diagnosis	0.991 (0.987-0.995)	0.000
Diabetes Mellitus before cancer diagnosis	1.204 (1.014-1.431)	0.034
1st degree family history of CRC	1.575 (1.365-1.818)	0.000
Aspirin (daily)	0.692 (0.628-0.763)	0.000
*Statins instead of aspirin***	*0.882 (0.7990.974)*	*0.013*
Sports participation	0.580 (0.527-0.637)	0.000
Vegetable consumption (5+ servings/day)	0.856 (0.779-0.940)	0.001
Fruit consumption (3+ servings/day)	0.817 (0.742-0.900)	0.000

***>3 months before cancer diagnosis**

***When statins replace aspirin in the model. When both are in simultaneously, only aspirin remains statistically significant.*

Table 3

Multivariate analysis of Metformin-use and risk of breast cancer in Jews in the Breast Cancer In Northern Israel Study (BCINIS)

	Full Model	p-value	Minimal model	p-value
Metformin use*	0.847 (0.693-1.035)	0.105	**0.821 (0.726-0.928)**	**0.002**
Age at diagnosis	0.996 (0.992-1.000)	0.031	0.996 (0.992-0.999)	0.018
Sports participation	0.872 (0.802-0.949)	0.001	0.870 (0.802-0.944)	0.001
1st degree family history of breast cancer	1.823 (1.627-2.042)	0.000	1.821 (1.626-2.040)	0.000
Oral contraceptive	0.814 (0.738-0.897)	0.000	0.815 (0.739-0.898)	0.000
No. pregnancies	0.977 (0.962-0.991)	0.002	0.976 (0.962-0.991)	0.002
Statins	0.976 (0.888-1.072)	0.609		
Aspirin	1.011 (0.913-1.120)	0.826		
Vegetable consumption (5+ servings/day)	0.954 (0.874-1.041)	0.292		
Fruit consumption (3+ servings/day)	1.018 (0.935-1.136)	0.635		
HRT (Y/N)	1.025 (0.925-1.136)	.0635		
Diabetes Mellitus before cancer diagnosis	0.972 (0.813-1.162)	0.758		

***>3 prescriptions prior to cancer diagnosis**

Table 4 Duration and metformin use and risk of Breast and Colorectal Cancer

Duration of use of Metformin and BC risk			Duration of use of Metformin and CRC risk		
<= 12 months	0.921 (0.770-1.101)	0.364	<= 12 months	0.837 (0.699-1.001)	0.052
13-24 months	0.965 (0.721-1.291)	0.810	13-24 months	0.855 (0.649-1.126)	0.265
25-36 months	0.951 (0.689-1.313)	0.760	25-36 months	0.915 (0.675-1.240)	0.566
37-48 months	0.782 (0.547-1.119)	0.179	37-48 months	0.935 (0.685-1.277)	0.674
49-60 Months	0.827 (0.706-0.968)	0.018	49-60 months	0.850 (0.716-1.010)	0.064

Table 5 Cumulative doses of metformin and risk of Breast and Colorectal Cancer

BCINIS

Metformin cumulative dose	OR (95% CI)	
Reference	1.00	
Quartile I	0.831 (0.663-1.040)	
Quartile II	0.964 (.769-1.209)	
Quartile III	0.832 (0.668-1.036)	
Quartile IV	0.800 (0.647-0.988)	p for trend = 0.007

MECC

Metformin cumulative dose	OR (95% CI)	
Reference	1.00	
Quartile I	0.812 (0.655-1.007)	
Quartile II	0.952 (.769-1.179)	
Quartile III	0.916 (0.732-1.147)	
Quartile IV	0.749 (0.592-0.947)	p for trend = 0.012