Association between Metformin Use and Mortality after Cervical Cancer in Older Women with Diabetes

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Abstract

Background: To examine the association between metformin use and mortality in patients with diabetes and cervical cancer.

Methods: Using Ontario health databases, a retrospective, population-based cohort study was conducted in women with diabetes ≥ age 66 diagnosed with cervical cancer between 1997-2010. The association between metformin exposure and cervical cancer-specific mortality was examined using Fine-Gray regression models, with non-cancer death as a competing risk and cumulative metformin use as a time-varying exposure. The association with overall mortality was examined using Cox regression models.

Results: Among the 181 women with diabetes and cervical cancer, there were 129 deaths, including 61 cervical cancer-specific deaths. The median follow-up was 5.8 years (interquartile range 4.2-9.6 years) for surviving patients. Cumulative dose of metformin after cervical cancer diagnosis was independently associated with a decreased risk of cervical cancer-specific mortality and overall mortality in a dose-dependent fashion (HR 0.79, 95% CI 0.63-0.98; and HR 0.95, 95% CI 0.90-0.996 per each additional 365g of metformin use, respectively). There was no significant association between cumulative use of other anti-diabetic drugs and cervical cancer-specific mortality.

Conclusion: This study suggests an association between cumulative metformin use after cervical cancer diagnosis and lower cervical cancer-specific and overall mortality among older women with diabetes.

Impact: Cumulative dose of metformin use after cervical cancer diagnosis among older women with diabetes may be associated with a significant decrease in mortality. This finding has important implications if validated prospectively, as metformin is inexpensive and can be easily combined with standard treatment for cervical cancer.

Introduction

Metformin, an inexpensive, first-line oral drug treatment for type 2 diabetes, is thought to inhibit cancer growth by lowering circulating insulin level or directly activating the AMP-activated protein kinase (AMPK) pathway (1). Work from our group also discovered that metformin can enhance tumor response to radiation by inhibiting tumor cell oxygen consumption (i.e. improving tumor oxygenation) (2). Poor tumor oxygenation (hypoxia) has been independently associated with inferior survival in cervix cancer, and with increased metastasis and resistance to chemotherapy and radiotherapy (3, 4). Metformin has been found to be associated with a significant reduction in cancer-specific mortality in patients with diabetes (HR, 0.74; 95% CI, 0.62-0.88) on meta-analysis of observational studies, though limited to colon cancer on subgroup analyses (5). However, no study to date has investigated the association between metformin and cervical cancer-specific mortality. We therefore examined whether cumulative metformin exposure is associated with lower mortality among women with diabetes diagnosed with cervical cancer.

Materials and Methods

The institutional review board approved this population-based, retrospective cohort study, with waiver of informed consent.

Data Sources

All Ontario residents are covered under a universal health plan; those age ≥ 65 are also covered for prescription drugs. We used the following population-based health care databases, which are linkable through a unique, anonymized identifier: the Ontario Drug Benefit database (6), which contains all dispensed prescriptions for those age ≥ 65; the Ontario Cancer Registry

(OCR), a population-based cancer registry that is more than 95.0% complete (7); the Ontario Diabetes Database (8), a validated administrative data-derived registry of patients with diabetes; the Canadian Institute of Health Information (CIHI) Discharge Abstract Database (DAD) (9), which contains diagnostic and procedure information on all hospital admissions; the National Ambulatory Care Reporting System (NACRS), which captures information on emergency department visits; and the Ontario Health Insurance Plan (OHIP) claims database (10), which captures physician visits and procedures; the Registered Persons Data Base (RPDB) for demographics and vital status; and for cases diagnosed after year 2005 the Cancer Care Ontario's Activity Level Reporting, which captures detailed radiation and chemotherapy treatment information. The Ontario Cancer Data Linkage Program ('cd-link') is an initiative of the Ontario Institute for Cancer Research/Cancer Care Ontario Health Services Research Program, whereby risk-reduced coded data from the ICES Data Repository managed by the Institute for Clinical Evaluative Sciences is provided directly to Researchers with the protections of a comprehensive Data Use Agreement.

Cohort Definition

We used the OCR to identify women age ≥ 66 diagnosed with incident cervical cancer (as their first cancer) in Ontario between July 1, 1997 and December 31, 2010 (n = 1415). The cohort was restricted to women age ≥ 66 to capture medication data and have a 1-year look-back, and limited to those diagnosed with diabetes on the same day as or prior to cervical cancer (n = 342) using the Ontario Diabetes Database. Finally, those who did not receive definitive treatment (surgery or radiotherapy consisting of external beam radiotherapy and brachytherapy) for their cervical cancer (n = 110), and those diagnosed with another (non-cervical) cancer were excluded (n = 51).

Outcomes

The primary outcome was cervical cancer-specific mortality, based on the validated cause-of-death variable from the OCR (11, 12). The secondary outcome was overall mortality, based on death records from RPDB.

Medication Exposure

All prescriptions for anti-diabetic medications were identified from the Ontario Drug Benefit database starting from the date of cancer diagnosis. These medications were categorized into metformin, other oral anti-diabetics (e.g. sulfonylureas, thiazolidinediones, meglitinides, gliptins, acarbose) and insulin. Given that previous studies (13, 14) and ours did not observe an association between metformin exposure prior to cancer diagnosis and cancer outcome, we focused on medication exposure after cervical cancer diagnosis. Exposure to anti-diabetic medications was defined in two ways. For primary analysis, the cumulative dose of metformin after cervical cancer diagnosis was calculated by multiplying the dose and quantity of tablets dispensed per prescription and summing the cumulative dose of each metformin prescription until the last follow-up date. For secondary analysis, the cumulative duration of metformin use was calculated by summing the durations of each metformin prescription between the date of cervical cancer diagnosis and last follow-up. Cumulative dose of metformin was the preferred method of measuring exposure because the dose of metformin taken per day varied widely; however, it was not possible to sum up the cumulative dose of other anti-diabetic medications (e.g. 1mg of glyburide not equivalent to 1mg of pioglitazone). A time-varying approach was used, whereby a subject's exposure classification was allowed to vary over time, depending on the prescriptions filled. If a medication was discontinued, the cumulative dose or duration of exposure remained the same as the value at the time the previous prescription expired.

Other Covariates

In addition to medication exposure, other factors potentially associated with overall and cervical cancer-specific mortality were evaluated: age; comorbidity based on the Johns Hopkins Adjusted Clinical Groups Case-Mix System weighted adjusted diagnostic groups (ADG) score (which has been shown to predict mortality in a population-based Ontario sample of patients with diabetes (15)); socioeconomic status; duration of diabetes at cervical cancer diagnosis; year of cervical cancer diagnosis (to adjust for temporal changes in management of cervical cancer and diabetes); histology (squamous cell carcinoma vs others); and treatment (surgery, radical radiation, or radical chemoradiation). Primary treatment was identified using billing, intervention and/or procedure codes for surgery, radiation and chemotherapy from OHIP, CIHI DAD and NACRS databases. Treatment information was also available from Cancer Care Ontario for cases diagnosed from 2006 onward.

Statistical Analysis

To avoid guarantee-time bias (16), medication exposures after cervical cancer diagnosis were modeled as time-varying covariates. The association between cumulative dose or duration of exposure to anti-diabetic medication and cervical cancer-specific mortality was assessed using a Fine and Gray competing-risk regression model (17), with death from other causes as competing risk. This approach takes into account the fact that women who died of other causes (competing events) will never die of cervical cancer, and hence provides more realistic estimates. The association with overall mortality was assessed by a Cox proportional hazard model.

A multivariable clinical model was first built by considering the potential factors (described above) using a backward elimination method, retaining significant covariates with $P < 0.05$. Cumulative medication exposure was then added to the clinical model. For all models, the

assumption of proportional (subdistribution) hazards was examined using Schoenfeld residuals. Model results were reported using adjusted hazard ratios (HRs) with 95% confidence intervals. All *P* values were 2-sided with a significance level of < 0.05. R version 3.1.0 was used for all analyses.

Sensitivity Analyses

To explore the robustness of our results, we restricted the cohort to those treated with definitive radiation ± concurrent chemotherapy (i.e. excluded those treated with definitive surgery) and repeated the analyses. We could not perform sensitivity analysis on those treated with surgery because of the limited number of events (8) in that group.

Results

During the study period, 181 women with diabetes ≥ age 66 were diagnosed with and received definitive treatment for cervical cancer in Ontario (Table 1). Median follow-up time of all women was 3.9 years (interquartile range [IQR], 1.5-7.2 years), and 5.8 years (IQR 4.2-9.6 years) for those still alive. During the follow-up period, 110 women (60.8%) were prescribed metformin, 129 died (71.3%) and 61 (33.7%) died of cervical cancer. Metformin users were exposed to metformin for a median of 25.9 months (IQR 9.5-57.0 months) after cervical cancer diagnosis. There were no significant differences in baseline patient or tumor characteristics between metformin users and nonusers (Table 1), except for a trend towards younger age in metformin users (Fisher's exact test $p = 0.07$).

Cervical-Cancer Specific Mortality

Year of cervical cancer diagnosis, histology and treatment formed the baseline multivariable clinical model for cervical-cancer specific mortality (Table 2). Cumulative dose of

metformin after cervical cancer diagnosis was then added to the baseline multivariable clinical model and found to be independently associated with a significant decreased risk of cervical cancer-specific mortality in a dose-dependent fashion (HR 0.79 per each additional 365g of metformin use, 95% CI 0.63-0.98, P = 0.03) (Table 2). The cumulative duration of metformin use, although not significant, exhibited an effect in the same direction (HR = 0.79 per each additional year of metformin use after cervical cancer diagnosis, 95% CI 0.57-1.09, P = 0.15). Increasing cumulative use of other anti-diabetic medications was not associated with cervical cancer-specific mortality (HR 0.88 per each additional year of other oral anti-diabetic medication use, 95% CI 0.68-1.13, P = 0.33; and HR 1.06 per each additional year of insulin use, 95% CI 0.74-1.51, P = 0.77).

Overall Mortality

On multivariable analysis, cumulative dose of metformin after cervical cancer diagnosis was independently associated with a significant decreased risk of overall mortality in a dose-dependent fashion (HR 0.95 per each additional 365g of metformin use, 95% CI 0.90-0.996, P = 0.03) (Table 3). When cumulative duration of metformin use was added to the model instead of cumulative dose, the HR was 0.93 per each additional year of metformin use after cervical cancer diagnosis (95% CI 0.86-1.02, P = 0.11).

Sensitivity Analyses

When the cohort was limited to the 124 women treated with definitive (chemo)radiation, cumulative dose of metformin remained independently associated with a lower risk of cervical cancer-specific mortality (HR 0.76 per each additional 365g of metformin use, 95% CI 0.60-0.97, P = 0.03), and lower risk of overall mortality (HR 0.91 per each additional 365g of metformin use, 95% CI 0.85-0.99, P = 0.02). The HR for the association between each additional

year of metformin use and cervical cancer-specific mortality was 0.77 (95% CI 0.55-1.1, P = 0.15), and 0.86 (95% CI 0.76-0.98, P = 0.02) for overall mortality.

Discussion

This population-based study suggests that cumulative dose of metformin use after cervical cancer diagnosis among older women with diabetes is associated with significant decrease in cervical cancer-specific and overall mortality. For every additional 365g of metformin (i.e. equivalent to taking 1g of metformin daily x 365 days), there is a 21.4% decrease of cervical cancer-specific mortality. This is the first study to our knowledge to investigate a potential benefit of metformin therapy for cervical cancer.

There are many potential biologic mechanisms underlying the effects of metformin in cervical cancer. Metformin has been shown to inhibit cervical cancer growth *in vitro* via various pathways, such as the Wnt/β-catenin/DVL3 and LKB1/AMPK/mTOR signaling pathways (18-20). Radiotherapy is the primary modality of treatment for locally advanced cervical cancer, and metformin has been shown to radio-sensitize lung cancer cells *in vivo* (21). Finally, our group has shown that metformin can decrease tumor hypoxia and improve tumor response to radiotherapy *in vivo*. When we restricted the analysis to those treated with definitive radiotherapy, cumulative dose of metformin remained associated with significant decrease in cervical cancer-specific and overall mortality. Given that a significant proportion of locally advanced cervical cancer are hypoxic, we are conducting a phase II trial randomizing women with locally advanced cervical cancer to standard chemoradiation with or without metformin. A recent study showed that tumor cells harboring mitochondrial DNA mutation are more sensitive

to the anti-cancer effect of phenformin (a metformin analogue) (22); 38-60% of cervical cancer harbor mitochondrial DNA mutation (23, 24).

Several studies have evaluated the effect of metformin therapy on other cancers among patients with diabetes; many did not account for non-cancer death as a competing risk. We performed competing risk analysis for the cervical cancer-specific mortality endpoint, because the risk of death due to causes other than cancer is high in older women with diabetes. Indeed, there were slightly more non-cervical cancer deaths than cervical cancer deaths in this cohort. Interestingly, when we analyzed the cervical cancer-specific mortality endpoint using Cox regression (i.e. ignoring non-cervical cancer death as a competing risk), both the HR and *P* values became smaller: HR 0.72 per each additional 365g of metformin use after cervical cancer diagnosis, 95% CI 0.57-0.90, $P = 0.004$; and HR 0.69 per each additional year of metformin use, 95% CI 0.5-0.95, $P = 0.02$. There remained no significant association between other anti-diabetic medication exposure and cervical cancer-specific mortality. In a previous population-based study on prostate cancer, metformin was also the only anti-diabetic drug associated with decreased cancer-specific and overall mortality, when considering all drug exposures as time-varying covariates on Cox regression analysis (13).

We modeled medication exposures after cervical cancer diagnosis as time-varying covariates to avoid guarantee-time bias (16), and also as cumulative exposure to evaluate dose-response effect. Guarantee-time bias has been a concern in prior studies that dichotomized metformin exposure as a never/ever fixed variable based on baseline exposure or exposure that occurred during the follow-up period (25). Patients who die early in the follow-up period have less opportunity to be exposed to metformin; those who live longer have more opportunity to be exposed to metformin. Using the fixed approached, patients who did not use metformin at the

time of cohort entry but started using it later during the follow-up period would be misclassified as "exposed" to metformin during the time between cohort entry and the first metformin prescription (25). Thus, the potential benefits of metformin are exaggerated with a fixed (time-independent) approach. Our time-varying medication exposures were modeled as cumulative exposures after cervical cancer diagnosis, allowing for comparison not just between users and nonusers, but also between users who had different doses of cumulative exposure. We observed a dose-dependent effect. While this finding does not prove causality, it strengthens the evidence. The cumulative approach should also help mitigate the healthy-user effect and other indication biases (13, 26). The cumulative duration of metformin use was not significantly associated with cervical cancer-specific mortality (but exhibited an effect in the same direction as cumulative dose of metformin) likely due to the variation in daily metformin dose across patients (e.g. 0.5g, 1g, 1.5g, 2g or 2.5g per day).

As with other population-based retrospective studies, our study has some limitations. First, it is an observational study where metformin use was not randomly assigned. While we tried to minimize potential biases, we cannot correct for all sources of bias, especially those that cannot be measured. As we did not use a cohort of women with incident diabetes, the possibility of selection bias based on differing diabetes severity exists. However, we found no significant association between the duration of diabetes at the time of cervical cancer diagnosis (surrogate for severity of diabetes) and cervical cancer-specific mortality. Adding the duration of diabetes to the multivariable model did not change the HR for cumulative metformin dose. Second, exposures or outcomes may have been misclassified. Where possible, variables available in >1 database were cross-referenced. For example, treatment information derived from OHIP, CIHI DAD and NACRS databases were verified against that from Cancer Care Ontario (reported by

cancer centers) for cases treated since 2006, and found to be 100% concordant. Third, the Ontario health databases lacks variables such as performance status, severity of diabetes, smoking, laboratory data and staging information for cases diagnosed prior to 2006. Despite maximal efforts, it was not possible to obtain staging information for cases diagnosed prior to 2006 from the original charts across Ontario due to privacy reasons and data use agreement with the Institute for Clinical Evaluative Sciences. While stage was obtained for 46 patients, there was insufficient power and follow-up time to evaluate the effects of metformin on cancer mortality in these patients alone. When we included stage in the multivariable model for all patients (with an unknown category), the HR for the association between cumulative metformin dose and cervical cancer-specific mortality remained the same (HR 0.79 per each additional 365g of metformin use, 95% CI 0.64-0.99, $P = 0.04$). We also included in the multivariable model the covariate treatment, which is related to stage (early stage cervical cancer treated with surgery whereas locally advanced cases treated with radiation ± concurrent chemotherapy). While we could not perform sensitivity analysis on those treated surgically (expected to all have stage I disease) because of the limited number of events, 5 of the 19 patients (26%) who did not take metformin died from cervical cancer, whereas 3 of the 38 patients (8%) who took metformin died from cervical cancer. In the meta-analysis by Lega et al. (5), stage did not appear to have an effect on the risk estimate (HR 0.73, 95% CI 0.64-0.83 for all studies; HR 0.66; 95% CI 0.52–0.83 when only including those that adjusted for cancer stage). Fourth, the cohort is relatively modest in sample size. Finally, our cohort was limited to those ≥ age 66 because the Ontario Drug Benefit database only captures prescriptions in that age group. Further studies are needed to confirm the effect of metformin on mortality in younger women with cervical cancer, and also in non-

diabetic women. Metformin has shown anticancer efficacy in non-diabetic patients with other types of cancer (27).

Despite these limitations, the strengths of this study include its novelty as it is the first study on metformin use in cervical cancer, population-based sampling, methodology (competing risk and time-varying analyses) and demonstration of a dose-dependent effect. Our finding of a significant association between metformin use and cervical cancer-specific mortality can have important implications if it is validated prospectively. Metformin is inexpensive, non-toxic and accessible in low- and middle-income countries, where the majority of cervical cancers are diagnosed. For women who cannot tolerate concurrent chemotherapy with definitive radiation, concurrent use of metformin may improve disease outcome.

In summary, our study is the first to report that cumulative metformin use after cervical cancer diagnosis may be associated with decreased cervical cancer-specific and overall mortality among older women with diabetes. The potential of metformin to improve cervical cancer outcome deserves further interventional studies in depth.

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Table 1. Patient and tumor characteristics

	Total n = 181	Metformin users n = 110	Metformin non-users n = 71
		No (%)	
Age at cervical cancer diagnosis, years			
66-70	76 (42)	53 (48)	23 (32)
71-75	38 (21)	23 (21)	15 (21)
≥ 76	67 (37)	34 (31)	33 (46)
Time between diabetes and cervical cancer diagnosis, years [a]	5.9 ± 4.4	6.2 ± 4.6	5.4 ± 4.1
Weighted comorbitidy score [a,b]	24 ± 13	23 ± 13	26 ± 13
Socioeconomic status [c]			
1 (lowest)	64 (36)	34 (31)	30 (42)
2	31 (17)	19 (17)	12 (17)
3	42 (23)	27 (25)	15 (21)
4	25 (14)	20 (18)	5 (7)
5 (highest)	18 (10)	9 (8)	9 (13)
Missing	1	1	0
Rural	19 (11)	11 (10)	8 (11)
Histology			
Squamous cell carcinoma	131 (72)	78 (71)	53 (75)
Others	50 (28)	32 (29)	18 (25)
Treatment			
Definitive Surgery	57 (31)	38 (35)	19 (27)

Radical Radiation	85 (47)	45 (41)	40 (56)
Radical Chemoradiation	39 (22)	27 (25)	12 (17)
Medications after cervical cancer diagnosis			
Metformin	110 (61)	110 (100)	0 (0)
Other oral anti-diabetics	94 (52)	76 (69)	18 (25)
Insulin	46 (25)	29 (26)	17 (24)
Cervical cancer-specific death	61 (34)	27 (25)	34 (48)
Overall mortality	129 (71)	73 (66)	56 (79)

[a] Mean (standard deviation)

[b] Comorbidity scores were estimated using the Johns Hopkins Adjusted Clinical Groups (ACG) Case-Mix System, with a specific weight to each adjusted diagnostic group.

[c] Based on the Registered Persons Data Base neighborhood median income quintile

Table 2. Time-Dependent Multivariate Model for Cervical Cancer-Specific Mortality

	HR	HR 95% CI	*P*
Clinical Model			
Year of cervical cancer diagnosis	0.95	0.90-1.0	0.04
Histology (vs other)			
Squamous Cell Carcinoma	0.43	0.23-0.80	0.008
Treatment (vs surgery)			
Radiation	5.38	2.40-12.08	< 0.001
Chemoradiation	3.58	1.33-9.59	0.01
Clinical Model + Metformin			
Year of cervical cancer diagnosis	0.95	0.90-1.0	0.07
Histology (vs other)			
Squamous Cell Carcinoma	0.35	0.19-0.67	0.002
Treatment (vs surgery)			
Radiation	6.22	2.70-14.33	< 0.001
Chemoradiation	4.14	1.50-11.44	0.006
Cumulative Metformin Dose [a]	0.79	0.63-0.98	0.03

[a] Per each additional 365g of metformin use

Abbreviations: HR, hazard ratio; CI, confidence interval

Table 3. Time-Dependent Multivariate Model for Overall Mortality

	HR	HR 95% CI	*P*
Clinical Model			
Age (vs 66-70)			
71-75	1.86	1.14-3.02	0.012
≥ 76	2.01	1.30-3.11	0.002
Histology (vs other)			
Squamous Cell Carcinoma	0.58	0.39-0.88	0.01
Treatment (vs surgery)			
Radiation	2.69	1.71-4.23	< 0.001
Chemoradiation	1.74	0.97-3.10	0.06
Comorbidity Score [a,b]	1.02	1.01-1.04	0.003
Clinical Model + Metformin			
Age (vs 66-70)			
71-75	1.90	1.18-3.08	0.009
≥ 76	2.02	1.30-3.11	0.002
Histology (vs other)			
Squamous Cell Carcinoma	0.52	0.35-0.78	0.002
Treatment (vs surgery)			
Radiation	3.01	1.89-4.79	< 0.001
Chemoradiation	1.94	1.12-3.36	0.02
Comorbidity Score [a, b]	1.02	1.01-1.03	0.002
Cumulative Metformin Dose [c]	0.95	0.90-0.996	0.03

[a] Comorbidity scores were estimated using the Johns Hopkins Adjusted Clinical Groups (ACG) Case-Mix System, with a specific weight to each adjusted diagnostic group.

[b] Per 1 unit increase

[c] Per each additional 365g of metformin use

Abbreviations: HR, hazard ratio; CI, confidence interval