Beyond diabetes mellitus: role of metformin in non-muscle invasive bladder cancer

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ABSTRACT

Introduction: Usage of metformin is associated with improved survival in lung, breast and prostate cancer, and metformin has been shown to inhibit cancer cell growth and proliferation in *in vitro* studies. Given the lack of clinical data on metformin use in patients with bladder cancer, we aimed to evaluate the role of metformin in their oncological outcomes.

Methods: Medication use from a prospectively maintained database of 122 patients with non-muscle invasive bladder cancer treated with intravesical Bacille Calmette-Guerin (BCG), who were recruited under a randomised, double-blinded, controlled clinical trial, was collected and analysed. Kaplan-Meier curves were used to assess overall survival (OS) and disease-specific survival (DSS).

Results: At a median follow-up duration of 102 (range 3–357) months, 53 (43.4%) patients experienced disease recurrence and 21 (17.2%) experienced disease progression. There was no significant difference in mortality between patients with diabetes mellitus and those without. There was significant difference in OS among patients without diabetes mellitus, patients with diabetes mellitus on metformin and patients with diabetes mellitus not on metformin (p = 0.033); patients with diabetes mellitus on metformin had the best prognosis. Metformin use was associated with significantly lower DSS (p = 0.042). Other oral hypoglycaemic agents, insulin or statins were not associated with disease recurrences or progression.

Conclusion: Metformin use was associated with improved oncological outcomes in patients with non-muscle invasive bladder cancer treated with intravesical BCG. Prospective studies with larger patient populations are needed to validate the role of metformin as potential therapy for bladder cancer.

Keywords: bladder carcinoma, diabetes mellitus, metformin, recurrences

INTRODUCTION

A recent report by the International Diabetes Federation (IDF) showed that Singapore has the second highest proportion of patients with diabetes mellitus among developed nations.[1] It was reported that 10.53% of the population in Singapore in the age range of 20–79 years were estimated to have the disease. Metformin, a biguanide, is one of the only two antidiabetic drugs on the World Health Organization list of essential medicines and one of the most commonly used drugs to treat non-insulin-dependent diabetes mellitus in Singapore. The drug's glucose-lowering and cardioprotective effect, and its relatively high benefit-risk ratio have made it a popular candidate of study among researchers looking to expand its uses.

Many studies have suggested association between metformin use and improved survival in patients with lung, breast and prostate cancer.[2] Mechanistically, metformin is hypothesised to exert its antitumour effect by various mechanisms, such as the liver kinase B1-dependent and AMP-activated protein kinase-dependent suppression of mammalian target of rapamycin (mTOR) pathways.[3,4] Reduced cell proliferation and expression of downstream mediators of the mTOR pathway with metformin has been demonstrated in bladder carcinoma cell line studies.[5] However, there is paucity of clinical data on the utility of metformin in patients with bladder cancer. Therefore, this study aimed to evaluate the role of metformin in the oncological outcomes of these patients.

METHODS

Information was collected from a prospectively maintained database of 122 patients with non-muscle invasive bladder cancer, who were recruited under a Good Clinical Practice standard, randomised, double-blinded, controlled clinical trial. Informed consent was obtained from all participants in the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research

committee, and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The original study received full domain-specific review board approval (Ref: DSRB 2006/00254).

All patients had undergone transurethral resection of bladder tumour between 1995 and 2013, and received postoperative intravesical Bacillus Calmette-Guerin (BCG) instillations. All patients received the full standard induction of six one-weekly BCG instillations and at least three booster doses, except for two patients who were unable to tolerate the full regime and underwent three and four instillations, respectively. Among these two patients, one was a patient with diabetes mellitus on metformin and the other was a patient without diabetes mellitus.

Inclusion criteria included patients with completely resected, histologically proven intermediate or high-risk non-muscle invasive urothelial carcinoma of the urinary bladder and Karnofsky score > 50. Exclusion criteria were immunodeficiency, transitional cell carcinoma of stage T2 or higher, transitional cell carcinoma of the upper urinary tract or active tuberculosis.

All patients were generally followed up in accordance with the European Association of Urology guidelines for non-muscle invasive bladder cancer. Follow-up comprised history, physical examination, urinary cytology and cystoscopies.

Metformin use, clinical data and survival data were collected from the hospital's computerised health records system. The primary exposure definition was use of metformin, defined as receiving at least one prescription between cohort entry and the last date of follow-up. For the purposes of secondary analysis, we also collected the duration and doses of metformin use.

Characteristics of patients with and without diabetes mellitus were compared using chi-square test for categorical variables and t-test for continuous variables. Current knowledge

from literature reviews and causal diagrams were used to further analyse for potential

confounders, such as cardiovascular risk factors, other oral hypoglycaemic agents and statins.

Kaplan-Meier survival curves were employed to compare disease-specific survival (DSS) and

overall survival (OS) for patients with and without diabetes mellitus. Data collected was

analysed using the PASW Statistics version 18.0 (SPSS Inc, Chicago, IL, USA).

RESULTS

The study population comprised 122 patients with non-muscle invasive bladder cancer. Mean

age of patients was 64.7 ± 11.1 years and most (82.8%) were men. The percentage of Chinese

(81.1%) patients with bladder cancer was proportionately higher, similar to the ethnic

composition of the Singapore population, while Malay (9.8%) and Indian (7.4%) patients

accounted for a minority. 22 (18.0%) patients had diabetes mellitus, among whom 13 patients

were on metformin. Demographics of our study population are shown in Table I.

Table I. Characteristics of patients with non-muscle invasive bladder cancer.

Variable	No. (%)				p-value
	Total (n = 122)	**Patients without diabetes mellitus (n = 100)**	**Patients with diabetes mellitus**		
			On metformin (n = 9)	**Not on metformin (n = 13)**	
Proportion of patients	122 (100.0)	100 (82.0)	9 (7.4)	13 (10.7)	
Gender					0.345
Male	101 (82.8)	81 (81.0)	9 (100.0)	11 (84.6)	
Female	21 (17.2)	19 (19.0)	0 (0)	2 (15.4)	
Age (yr)[†]	64.7 ± 11.1	64.7 ± 11.4	67.3 ± 8.4	62.8 ± 11.4	0.654
Median (IQR)	65.0 (58.8–74.0)	65.0 (58.3–74.0)	70.0 (60.5–74.5)	63.0 (56.0–73.0)	
Ethnicity					0.237
Chinese	99 (81.1)	82 (82.0)	9 (100.0)	8 (61.5)	
Malay	12 (9.8)	10 (10.0)	0 (0)	2 (15.4)	
Indian	9 (7.4)	6 (6.0)	0 (0)	3 (23.1)	
Other	2 (1.6)	2 (2.0)	0 (0)	0 (0)	
Pathological staging					0.817
pTa	38 (31.1)	32 (32.0)	2 (22.2)	4 (30.8)	
pTis	18 (14.8)	13 (13.0)	1 (11.1)	4 (30.8)	
pT1	66 (54.1)	55 (55.0)	6 (66.7)	5 (38.5)	

					0.307
Concomitant carcinoma *in situ*					
No	105 (86.1)	88 (88.0)	6 (66.7)	11 (84.6)	
Yes	17 (13.9)	12 (12.0)	3 (33.3)	2 (15.4)	
Pathological grading					0.952
G1	16 (13.1)	14 (14.0)	1 (11.1)	1 (7.7)	
G2	35 (28.7)	29 (29.0)	2 (22.2)	4 (30.8)	
G3	71 (58.2)	57 (57.0)	6 (66.7)	8 (61.5)	
Smoking status					0.190
No	55 (45.1)	45 (45.0)	2 (22.2)	8 (61.5)	
Yes	67 (54.9)	55 (55.0)	7 (77.8)	5 (38.5)	
Comorbidity					
Hypertension	46	33	5	8	0.070
Hyperlipidaemia	42	27	6	9	0.001[*]
Ischaemic heart disease	17	12	2	3	0.420
Cerebrovascular disease	2	2	0	0	0.800
Karnofsky score	90.1 ± 9.2	90.0 ± 9.3	87.7 ± 10.9	92.7 ± 6.5	0.477

Data presented as mean ± standard deviation. †p < 0.05 was statistically significant. DM: diabetes mellitus; IQR: interquartile range

There was no significant difference between the three groups of patients in terms of demographics, smoking status, and pathological grading and staging of disease. A higher proportion of patients with diabetes mellitus (59.1%) had hypertension when compared to those without diabetes mellitus (33.0%). This was likely due to the association of diabetes mellitus with a patient's cardiovascular health status. Similarly, patients with diabetes mellitus (68.2%) were significantly more likely than patients without diabetes mellitus (27.0%) to have a history of hyperlipidaemia (p = 0.001). There was no significant difference between the three groups in terms of mean Karnofsky score or other comorbidities.

Patient outcomes included OS, DSS, recurrence-free survival and progression survival (Table II). Mean follow-up period was 102 (range 3–357) months. During this period, 53 (43.4%) patients experienced recurrences. Among patients with disease recurrences and progression, mean time to recurrence was 127 months and mean progression time was 179 months. The 15-year OS was 66.4% in the overall patient population, 65.0% for patients without diabetes mellitus, 44.4% for patients with diabetes mellitus on metformin and 92.3%

for patients with diabetes mellitus not on metformin. DSS was 85.2% in the overall patient population, 85.0% for patients without diabetes mellitus, 100.0% for patients with diabetes mellitus on metformin and 66.7% for patients with diabetes mellitus not on metformin.

Table II. Outcomes of patients with non-muscle invasive bladder cancer.

Variable	No. (%)			
	Total (n = 122)	Patients without diabetes mellitus (n = 100)	Patients with diabetes mellitus	
			On metformin (n = 9)	Not on metformin (n = 13)
Disease recurrence				
No	69 (56.6)	56 (56.0)	5 (55.6)	8 (61.5)
Yes	53 (43.4)	44 (44.0)	4 (44.4)	5 (38.5)
Disease progression				
No	101 (82.8)	81 (81.0)	8 (88.9)	12 (92.3)
Yes	21 (17.2)	19 (19.0)	1 (11.1)	1 (7.7)
Cancer-specific death				
No	104 (85.2)	85 (85.0)	6 (66.7)	13 (100.0)
Yes	18 (14.8)	15 (15.0)	3 (33.3)	0 (0)
All-cause death				
No	81 (66.4)	65 (65.0)	4 (44.4)	12 (92.3)
Yes	41 (33.6)	35 (35.0)	5 (55.6)	1 (7.7)

Figs. 1 and 2 present the findings of survival analyses. There was no significant difference in OS or DSS between patients with diabetes mellitus and patients without diabetes mellitus at 15 years. Although one would normally expect patients with diabetes mellitus to have poorer cardiovascular health when compared to patients without diabetes mellitus, it is possible that the close monitoring of our patients with diabetes mellitus and bladder carcinoma as well as the concurrent treatment of their coexisting diseases at a tertiary centre had prolonged life expectancy, such that there was no difference in survival between the various patient groups at the 15-year follow-up. The use of metformin was not associated with a reduced rate of bladder cancer (p = 0.45).

A secondary analysis, performed for the purposes of exploring the effects of duration of exposure, found that there were significant differences in DSS after exposure to metformin for 15 years. There was significant difference in DSS (p = 0.042) between patients without diabetes mellitus, patients with diabetes mellitus on metformin and patients with diabetes mellitus not on metformin; patients with diabetes mellitus treated with metformin had the best prognosis (DSS 100.0% at 15 years; Fig. 3). Meanwhile, patients without diabetes mellitus had DSS of 84.4% and patients with diabetes mellitus not on metformin had DSS of 54.7%. There was also a significant difference with regard to the OS of all three patient groups (p = 0.033); patients with diabetes mellitus on metformin had the best prognosis. The doses of metformin ranged from 250 mg twice daily to 850 mg thrice daily. There were no significant changes after adjusting for potential confounders, such as metformin dose, age, and pathological grading and staging of disease. The effects of hypertension, hyperlipidaemia and statins on recurrence and progression were also evaluated. There was no significant difference in DSS and OS of patients with and without the potential confounders.

DISCUSSION

One of the proposed mechanisms for the antitumour properties of metformin involves the inhibition of the phosphatidylinositol 3-kinase (PI3k)/protein kinase B (Akt)-mTOR pathway. Recent data suggest that the PI3k-Akt-mTOR signalling pathway plays a central role in controlling bladder cancer cell growth.[6] This is further corroborated by studies that show that 55% of human bladder tumours had increased expression of phosphorylated AKT.[7] *In vitro*, mTOR inhibition has been shown to inhibit the growth of bladder cancer cells in a dose-dependent manner.[8] In addition, the combination of metformin and other mTOR inhibitors have indicated a synergistic effect on the inhibition of cell proliferation in other cell lines.[9,10]

The antiproliferative actions of metformin are hypothesised to manifest via the inhibition of the activation of AKT1, mTOR and insulin-like growth factor 1 receptor among other downstream signalling mediators. Further evidence for a potential role of metformin in the PI3k-Akt-mTOR pathway was also presented by Wang and Wu,[11] who found that treating bladder cancer cell lines T24 and BIU-87 with metformin or cisplatin resulted in reduced cell proliferation and downregulation of phosphorylated-mTOR on Western blot arrays. Zhang et al reported similar findings of inhibited proliferation and colony formation in the cell lines 5637 and T24 with metformin and demonstrated inhibition of tumour growth in a xenograft model, with daily dosing of metformin.[5]

The results of our study are concordant with the *in vitro* and clinical data on the association between metformin use and bladder cancer recurrence risk. Multiple studies have shown that patients with diabetes mellitus not on metformin have increased incidence of bladder cancer and, particularly, cancers with poorer oncological outcomes.[12,13] Our findings follow the general trend that has been demonstrated in these previous studies. Metformin is a cheap and popular drug commonly prescribed by Singapore physicians for patients with diabetes mellitus. Given the high prevalence of exposure, it was ideal to study the effect of metformin within our population. Additionally, we considered the effect of duration of exposure to metformin, which has not commonly been studied in previous studies.

However, our study was not without limitations. As it was a retrospective analysis of prospectively collected data, potential confounders, such as smoking status and compliance to medication, could not be recorded or analysed. However, we performed extensive evaluation of the effects of potential confounders, such as cardiovascular risk factors (e.g. hypertension and hyperlipidaemia) and the use of statins, which revealed that there were no associations with the DSS and OS of our patient population. Selection bias was another inherent issue. Our patients with diabetes mellitus on metformin were restricted to those who received treatment

at a tertiary cancer hospital, and may not have been reflective of the general population with diabetes mellitus. The small sample size of patients with diabetes mellitus on metformin was also another limitation.

However, it should be noted that most studies reflecting such associations are retrospective in nature and hence prone to such methodological limitations, which should be taken into account when interpreting our results. Notably, a cohort of 87,600 patients based study in the US[14] found no reduced incidence of bladder carcinoma with metformin use. Data from randomised controlled trials by Colmers et al[15] and Lewis et al[16] also did not reflect any additional protective benefit of metformin over rosiglitazone. However, the number of bladder cancer patients in both these earlier studies (n < 10) was modest, at best, and caution should be urged when arriving at a conclusion from the data. Furthermore, the performance of active comparator studies could indicate that there remains the likelihood that both the medications of metformin and rosiglitazone have absolute risk reduction of bladder malignancy risk but may not be above the other.

A meta-analysis by Hu et al[17] found that metformin intake could improve the prognosis of patients with bladder cancer. This was concordant with our data, but the data in the meta-analysis was recruited from retrospective cohort studies and potential confounders, such as gender, age and smoking status, were not considered.

In conclusion, our study supported the association of improved DSS and OS in patients with diabetes mellitus on metformin with non-muscle invasive bladder carcinoma treated with intravesical BCG. As existing studies in the literature have indicated conflicting results, prospective studies specifically designed to address the matter should aim to validate the role of metformin as potential therapy for non-muscle invasive bladder carcinoma.

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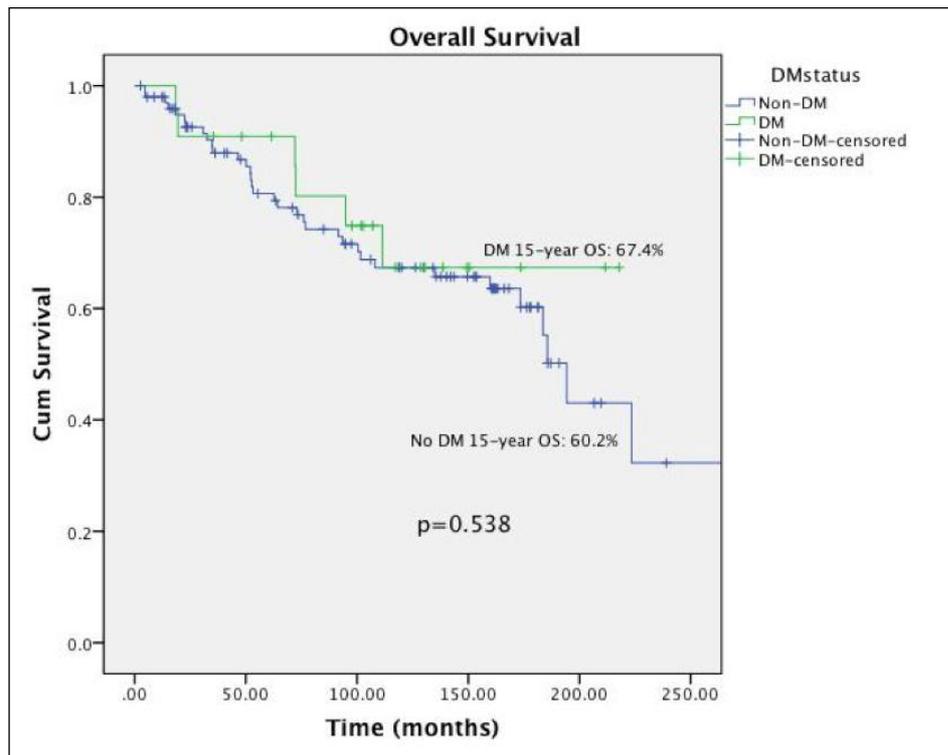
FIGURES

Fig. 1 Overall survival of patients with non-muscle invasive bladder cancer with and without diabetes mellitus. DM: diabetes mellitus; non-DM: without diabetes mellitus
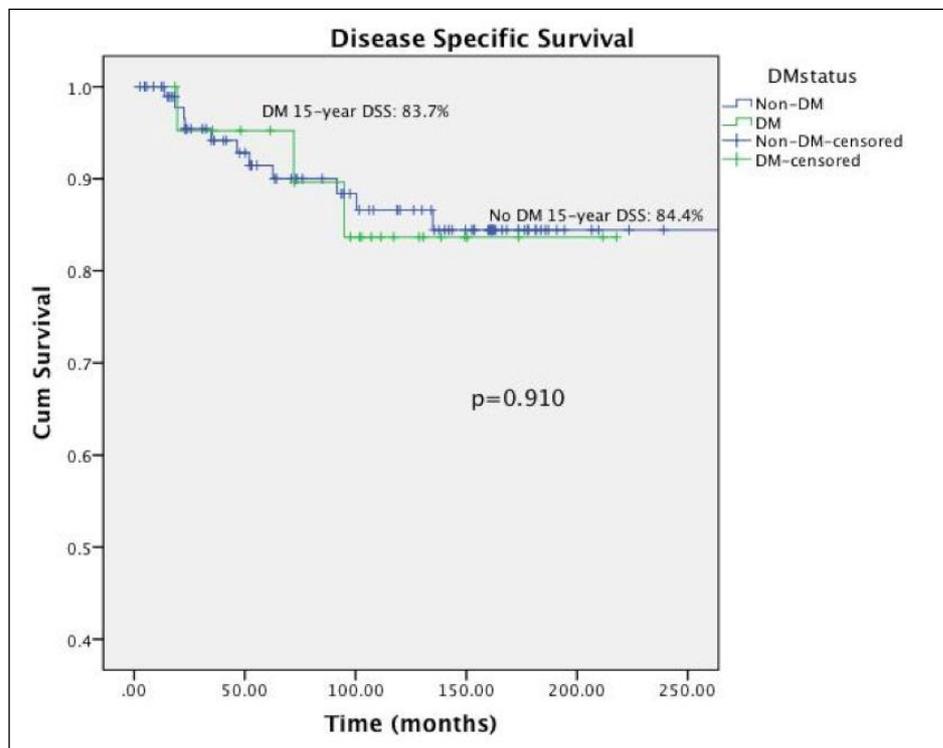

Fig. 2 Disease-specific survival of patients with non-muscle invasive bladder cancer with and without diabetes mellitus. DM: diabetes mellitus; non-DM: without diabetes mellitus
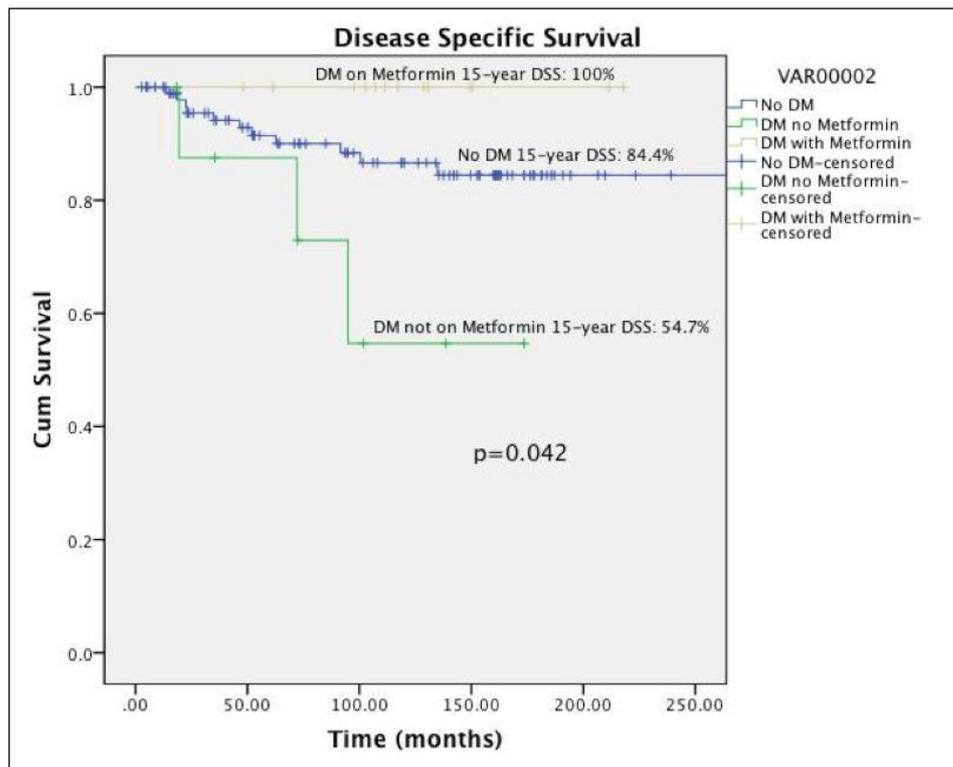
Fig. 3 Disease-specific survival of patients with non-muscle invasive bladder cancer without diabetes mellitus, with diabetes mellitus on metformin and with diabetes mellitus not on metformin. DM: diabetes mellitus