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Survival Analysis of Diabetic Colorectal Cancer Patients on Metformin

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Abstract: Metformin, an antihyperglycemic drug, has been associated with antineoplastic effects and could potentially improve colorectal cancer prognosis. The association between metformin and colorectal cancer prognosis has shown conflicting results. This study aims to define the association between metformin and colorectal cancer prognosis in colorectal cancer patients in the Brunei population. The study was a retrospective cohort study that included colorectal cancer patients from The Brunei Cancer Center (TBCC) treated between July 2014 and July 2019. Kaplan-Meier and multivariate Cox proportional hazard regression models were used to analyze the data, construct survival curves, and adjust for comorbidities. Of a total of 112 diabetic patients, 79 patients (70.5%) were on metformin, and 33 patients (29.5%) were on other antihyperglycemic medications. An association between metformin use and lower incidence of stage IV colorectal cancer (p = 0.046) was observed, but no significant difference between the metformin group and the non-metformin group in terms of survival probability (log rank p = 0.13) was shown. Analysis using multivariate models showed that metformin reduces the hazard ratio by 31.2%, although this value is statistically insignificant (HR, 0.688; 95% CI 0.286 – 1.654; p = 0.403). Among the diabetic colorectal cancer patients, there was no association between survival and metformin therapy. However, the association between cancer progression and metformin use requires further investigation, and high-powered clinical trials are needed to support these findings.

Keywords: Colorectal cancer; Metformin; Diabetes Mellitus; Prognosis, Survival; SDG 3 Good health and well-being

1. Introduction

To date, cancer continues to be one of the major causes of mortality worldwide. It is expected to be the leading cause of mortality in the 21st century and will be a challenge that needs to be overcome to increase the life expectancy of the worldwide population. Within 2020, it was estimated that there were more than 1.9 million newly diagnosed colorectal cancer (CRC) cases and around 930,000 CRC-related deaths [1,2]. A projection of 3.2 million new CRC cases and 1.6 million CRC-related deaths has been predicted to occur by 2040 [3]. In the context of Brunei Darussalam, CRC carries a heavy burden on the population, attributing to 18.3% of cancer-related mortality [4].

Diabetes is associated with an increased risk of CRC [5–11]. This relationship has been highlighted as one of the potential comorbidities that should be considered and is one of the shared risk factors (old age, obesity, and inactivity) between the two diseases [12–14]. While this notion seems likely, a meta-analysis noted that there was still a positive association between CRC and diabetes, despite controlling for risk factors [15–17]. Thus, the study demonstrated that shared risk factors played little to no role in CRC incidence. This association is more likely because the hormonal and metabolic changes that occur in diabetes promote the formation of the microenvironment for tumor formation and progression, leading to a higher probability of developing cancer [15,18–20]. A meta-analysis of the relationship between diabetes and CRC has elucidated that diabetes further decreases the life expectancy of those with CRC by about five years, and overall survival is decreased by 18% [21].

Understanding the link between CRC and diabetes is important because metformin, the first-line oral drug given to type 2 diabetic patients, has been reported by several studies to improve the rates of survival as well as reduce the risk for CRC among diabetic patients [22–27]. Metformin is an oral hypoglycemic drug that falls into the biguanide family of drugs and is commonly used in obese type 2 diabetic patients due to its ability to cause weight loss[28]. While its mechanism of action has not been fully clarified, it is believed that metformin accumulates within mitochondria and inhibits complex I of the electron transport chain. This affects ATP production and causes an increase in the ADP: ATP and AMP: ATP ratio, which in turn leads to the inhibition of gluconeogenesis due to the inhibition of fructose-1,6-bisphosphatase. Moreover, Adenosine monophosphate-activated protein kinase (AMPK) is activated as a result of the increased ratios, and this further impairs the hepatic glucose production while also increasing the uptake of glucose into adipose and muscle cells through GLUT-4 channels [25,29–31].

In the context of cancer, not only has metformin been shown to improve the survival outcomes of colorectal cancer, but it has also been associated with the enhancement of other anticancer medications and chemotherapy [32,33]. These antineoplastic effects of metformin

are largely due to the inhibition of the mammalian target of the rapamycin complex 1 (mTORC1) pathway as well as the activation of the Liver Kinase B1 (LKB1)-AMPK pathways[34]. LKB1 has been identified as one of the kinases that phosphorylates and activates AMPK following energy stress, and it has been noted that during carcinogenesis, LKB1 is inactivated. The activation of this pathway is important, as it controls and inhibits the mTOR pathway, which comprises two distinct complexes, mTORC1 and mTORC2. mTORC1 is the more relevant complex as it regulates the translation of growth factors, including cyclin D1, hypoxia-inducible factor 1a, and c-myc [35]. Thus, the energy stress brought on by metformin causes the upregulation of the LKB1-AMPK pathway that, in turn, inhibits the mTORC1 complex. This inhibits processes, including cell growth, angiogenesis, and the progression of the cell cycle, which affects tumorigenesis [36].

Several similar studies have been conducted with conflicting results. While most studies have demonstrated and concluded that metformin has clear impacts on the survival rates of diabetic colorectal cancer patients, few studies have shown no association between metformin use and colorectal cancer risk and survival [37–39]. However, these studies had their limitations. For example, in Kowall et al. [38], there was no significant association between risk and metformin therapy. However, lifestyle variables like smoking and physical activity were not adjusted for due to a lack of availability of such information, leading to potential confounding factors [38]. Thus, this study aims to provide supporting information and clarification regarding metformin's effect on survival outcomes when analyzed against confounding factors in type 2 diabetic colorectal cancer patients based in Brunei Darussalam.

In this study, we aim to compare survival outcomes between metformin use and colorectal cancer mortality using the Kaplan-Meier test and multivariate Cox proportional hazard regression models to eliminate potential confounding.

2. Materials and Methods

2.1. Study Design Population

The study was a retrospective cohort study, whereby data from patient records in The Brunei Cancer Center (TBCC) were collected and analyzed to determine the relationship between metformin therapy and mortality.

2.2. Population and Sample

The cases are comprised of colorectal cancer patients who presented to TBCC between July 2014 and July 2019. Information from all diabetic colorectal cancer patients was collected and included in this study. Patients without diabetes, patients with histologies other than colorectal adenocarcinoma, and patients with carcinoma in situ were excluded. All eligible cases were collected, and there were 480 colorectal cancer patients recorded before February 2020, of which 114 patients were diabetic. The records of these 114 colorectal cancer patients were used for analysis.

2.3. Data Collection

Data collection commenced after receiving approval from the joint committee of the Institute of Health Sciences Research Ethics Committee and the Ministry of Health Research Ethics Committee (Ethics reference number: UBD/PAPRSBIHSREC/2019/31). The data collected includes patient demographics and clinical findings (age and date of diagnosis, gender, race, smoking status, height, weight, BMI, stage of cancer, metformin usage, other specific treatments for diabetes and cancer, use of aspirin, HBA1c levels, presence of comorbidities, as well as overall survival status). The treatment and management of diabetes included the use of medications in the form of gliclazide, sitagliptin, tolbutamide, linagliptin, and acarbose, and the treatment of cancer was delineated through either surgery, chemotherapy, or radiotherapy. The survival outcome of patients was determined by the date of death as recorded in patient files at TBCC. The patient's survival status was last checked in February 2020.

A staging calculator (Integrated Cancer Research TNM Cancer Staging Calculator) was used to generate an overall TNM staging. Comorbidities were numericized using the Charlson Comorbidity Index (CCI), which takes into consideration factors such as age, HBA1c levels, history of heart failure, chronic kidney disease, as well as other medical conditions associated with mortality.

2.4. Data Analysis

Descriptive analyses were conducted for the collected socio-demographic and clinical data. Comparisons were made between the metformin and non-metformin user groups: Chi-squared test and Fisher's exact test for categorical data, and the independent Student's t-test for numerical data. Data analysis was done with a particular focus on estimating the effect of metformin usage on survival statistics. Kaplan-Meier analysis was used to determine whether metformin usage affects CRC patient survival and a logrank test was used to compare the generated survival curves. Multivariate Cox proportional hazard regression analysis was done to determine any factors associated with patient survival. The following factors were included: metformin usage, age at diagnosis, cancer stage, BMI, and CCL. Assumption checking for this model was done, including residuals and multicollinearity checking. All tests were two-sided and a *p*-value of less than 0.05 indicated significant findings. Data analysis was performed using the "RStudio Version 1.2.5033" software [40] with the following packages: survival, survminer, and ggplot2.

3. Results

3.1. Demographic and Clinical Characteristics

Initially, 114 diabetic colorectal cancer patients were identified. Two patients were excluded due to unavailable and insufficient information, leading to a total of 112 observations. Patient demographics and clinical characteristics based on their metformin status are summarised in Table 1.

The predominantly Malay population (79.5%) comprised 79 diabetic patients on metformin (70.5%) and 33 patients who were on antihyperglycemic medications other than metformin (29.5%). The mean age of patients was 61 years old, with the youngest patient being 29 years old and the oldest being 85 years old. The group of patients who were on metformin therapy was younger than the group of patients who were on other therapies when comparing the mean age of both groups. The chi-squared test revealed a statistically significant difference between metformin use and cancer stage (*p*-value = 0.046), with a lower proportion of stage IV patients among metformin users when compared with non-metformin users.

Table 1. Demographics of diabetic colorectal cancer patients by metformin treatment status.

Variable	**Total Population n (%)**	**Metformin n (%)**	**Non-Metformin n (%)**	***p*-value**
Age at diagnosis	61.0 (10.6) †	60.0 (11.0) †	63.6 (9.1) †	0.101
Age at death	64 (11.3) †	62.9 (11.6) †	65.7 (11.0) †	0.491
Sex				0.852
Male	60 (53.6%)	41 (51.9%)	19 (57.6%)	
Female	52 (46.4%)	38 (48.1%)	14 (42.4%)	
Race				0.243
Malay	89 (79.5%)	60 (75.9%)	29 (87.9%)	
Chinese	18 (16.1%)	15 (19%)	3 (9.1%)	
Others	5 (4.4%)	4 (5.1%)	1 (3.0%)	
Stage				**0.046***
1	13 (10.9%)	7 (9.0%)	6 (15.6%)	
2	35 (31.7%)	24 (30.8%)	11 (34.4%)	
3	44 (39.1%)	37 (46.2%)	7 (21.9%)	
4	20 (18.2%)	11 (14.1%)	9 (28.1%)	
Smoking Status				0.057
Smoker	11 (10.3%)	5 (6.7%)	6 (18.8%)	
Non-Smoker	88 (82.2%)	66 (88.0%)	22 (68.8%)	
Ex-Smoker	8 (7.5%)	4 (5.3%)	4 (12.5%)	
Body Mass Index	25.1 (4.8) †	25.5 (5.2) †	24.3 (3.8) †	0.258
HbA1C	7.3% (1.9%) †	7.2% (1.8%) †	7.4% (2.1%) †	0.566
Cancer treatments				
Chemotherapy, Yes	87 (79.1%)	63 (80.8%)	24 (72.7%)	0.499
Radiotherapy, Yes	21 (18.9%)	13 (15.4%)	8 (25.0%)	0.298
Surgery, Yes	63 (56.8%)	46 (59.0%)	17 (51.5%)	0.623

† mean (Standard Deviation), * $p < 0.05$

3.2. Use of Metformin and Survival

Among the 112 patients, 80 patients (71.4%) were still alive as of the time of the last follow-up, while 32 patients (28.6%) were reported to be deceased. The group on metformin therapy showed better survival statistics compared to the group without metformin. In the metformin group, there were 60 patients alive (75.9%) and 19 deaths (24.1%), while the non-metformin group had 20 patients still alive (60.6%) and 13 deaths (39.4%). Figure 1 shows the survival curves of these two groups. Although not statistically significant (*p*-value = 0.13), visual inspection shows that the group not on metformin therapy was associated with a lower chance of survival compared to the group on metformin therapy.

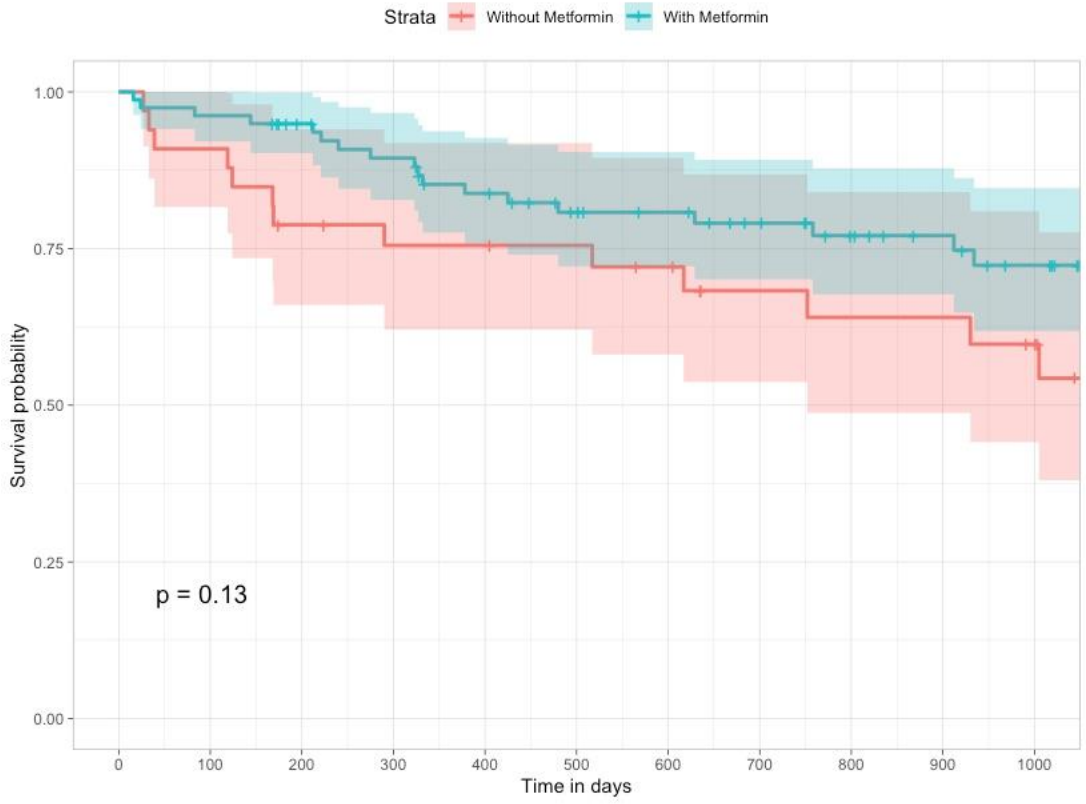

Figure 1. Overall Survival according to metformin use.

The findings of the multivariate Cox proportional hazards regression model are summarised in Table 2. Multivariate regression analysis revealed no statistically significant differences, though we observed a negative association between metformin usage and survival (adjusted HR, 0.688; 95% CI 0.286 – 1.654; $p = 0.403$)

Table 2. Showing adjusted hazard ratios after considering the comorbidities.

Variable	**Regression Coefficient**	**Hazard Ratio (95% CI)**	***p*-value**
Metformin Used	- 0.374	0.688 (0.286, 1.653)	0.403
Age at diagnosis	0.006	1.007 (0.994, 0.959)	0.791
Stage			
1	0.000	1.000	
2	- 1.115	0.328 (0.045, 2.407)	0.273
3	0.786	2.196 (0.472, 10.205)	0.316
4	1.509	4.522 (0.607, 33.714)	0.141
BMI	- 0.253	0.776 (0.467, 1.292)	0.330
Charlson Comorbidity Index	- 0.007	0.993 (0.717, 1.374)	0.964

4. Discussion

Overall, the findings of this study showed no significant association between metformin and all-cause mortality in diabetic colorectal cancer patients, even after adjusting for confounding factors using multivariate analysis. While the survival curves show that metformin patients have a higher survival probability compared to those not on metformin, this association is not statistically significant. These findings are similar to the findings of a study conducted by McMenamin et al. [39] which boasted several strengths, including large sample size, completeness of data allowing more detailed analyses as well as adjustments to prevent immortal time bias [39]. Other studies have also shown that metformin is not associated with colorectal cancer risk and has no impact on disease-free and progression-free survival [41,42].

While there was no significant association between metformin use and mortality in diabetic colorectal cancer patients, there are multiple aspects of this study that are worth noting. From Table 1, in terms of cancer stage, the group on metformin therapy appears to be suffering from more advanced stages of cancer (60.3%) compared to the group not on metformin therapy (50.0%). Despite this, the survival curves still show that metformin increases the probability of survival in these patients. The negative association observed in our study between metformin usage and survival is in line with the study by Paulus et al. [5] where they found a significant reduction in the hazard ratio by 13.0%[5]. The fact that this study was a population study and did not do sampling should also be a factor that should not be overlooked.

One significant finding, however, was the association between metformin use and the reduced incidence of metastatic colorectal cancer. This may point towards the notion that metformin therapy helps to improve prognosis by inhibiting the progression of cancer to a metastatic state, contrary to the idea that metformin therapy has direct impacts on colorectal

cancer mortality. Kang et al. [43] researched the anti-metastatic effects of metformin through repression of IL-6 induced epithelial mesenchymal transition (EMT), demonstrating this notion. IL-6 is a cytokine that is vital in mediating inflammation and immune responses, as well as mediating the tumor-promoting effects of inflammation-related conditions by inducing EMT. EMT, in turn, promotes the migration and invasion of cancer cells and initiates metastasis. The study found that through genomic data analysis, there is reduced IL-6 signaling epithelial mesenchymal transitioning [43].

Despite the limitations, there were certain merits in our study that are worth mentioning. Firstly, due to the availability of extensive and detailed records found in the national healthcare information system, information about comorbidities was recorded comprehensively. Within this database, detailed test results reflecting the severity and control of the diabetes were also easily accessible. This was important as the severity of the diabetes could well prove to be a potentially strong time-varying confounder. Moreover, the survival status of all patients was analyzed, and no single patient was lost to follow-up.

Several limitations should also be addressed. Because of the low sample size, it may not truly mirror the antineoplastic effects of metformin. One further weakness was the failure to obtain data on the duration of metformin exposure, including metformin dosing, to determine the dose-response relationship between the drug and the outcome. Moreover, the findings of this study may have been attributed to immortal time bias as the drug exposure was not treated as time-dependent. Lastly, this study only examines the impact of metformin on all-cause mortality and may not be reflective of cancer-related mortality as information about the specific cause of mortality was not readily available. However, the study was able to control for the potential confounding by adjusting for glycemic control (HBA1c), age, body mass index, and other comorbidities.

These limitations mean that there are areas in the research field that need to be improved especially with the advent of deep learning and new signaling pathways [44,45]. Both diabetes and CRC are heterogeneous diseases that require comprehensive and holistic approaches. An area that is worth exploring is the gut microbiome of diabetic or CRC patients (or dually affected) patients and healthy individuals [46,47]. The importance of the homeostasis of the gut microbiome was emphasized (reviewed in Lau et al. [48]). Diet impacts the gut microbiota, and gut microbiome modulation has been observed to ameliorate type 2 diabetes [49,50]. Evidence on how the ketogenic diet influences gut microbiome homeostasis and, therefore, cancer treatment and other diseases has been building [51–53]. The role of gut microbiome and utilization of the gut microbiome for the treatment of diseases have to be studied thoughtfully to enable effective treatments [54].

In line with the modulation of the gut microbiome for the prevention of these diseases, another area that has been commonly used by the general population is the consumption of supplements or traditional Chinese medicine or herbal medicine [55,56]. It is believed that there are anticancer potentials in certain supplements or beverages, and evidence on the effectiveness of these supplements has been collected. Nerolidol, a naturally occurring

sesquiterpene exuding floral odour, has been utilised in daily products and food. Interestingly, it is also a promising candidate for agricultural or medicinal use [57]. Similarly, Formononetin, derived from red clovers and a Chinese herb, is a 7-hydroisoflavones that exerts antitumorigenic effects via cell apoptosis and cell cycle arrest [58]. Citrus peel is a common Eastern snack containing Nobiletin, which is used to prevent CRC [59]. Resveratrol, found in red wine, possesses antioxidant, anti-inflammatory, and anticancer effects [60–62]. In addition, probiotics have been found to exert anticancer effects by modulating the gut microorganisms within the colon-associated biofilms [63]. Similarly, the usage of herbal medicine for diabetic management has been documented [64]. Therefore, the consumption of food and herbs is critical for cancer and diabetes treatment and prevention [65–72]. Another aspect that is frequently looked into is the knowledge and management of diabetes [73–76].

Gene and protein expression of both CRC cells and diabetic cells are crucial in basic research to determine the biology of cell states. The availability of genes and protein expression data led to genomics and proteomics data, which aggregate to multi-omics. An analysis of differential gene expression illustrated the critical genes that are responsible for lymph node metastasis in papillary thyroid carcinoma [77,78], while an example of how the sequencing results led to deeper insights via multi-omics is carried out by Azman et al. [79]. MicroRNA signatures in CRC have been analyzed based on functional and stage prediction [80–83]. Immunohistochemistry of tumour samples will aid in the diagnosis of cancer stages and statuses [84]. Another avenue to be explored is the therapy and treatment avenues, for example, potential cancer cure via targeting the membrane lipid and potential therapy by magnetic cellulose nanocrystal emulsions [85,86]. Thus, there are multifaceted areas of cancer research awaiting to be discovered.

5. Conclusions

Although many studies have tried to demonstrate the antitumor effects of metformin, the results have not been conclusive, raising unanswered questions about the antineoplastic effects of metformin. This study adds valuable information, as previous studies have noted an association between colorectal cancer and diabetes. Moreover, metformin is a relatively low-risk drug that is affordable. The evidence provided by this study does not support a significant association between metformin and colorectal cancer mortality. This study has several limitations, including small sample size, immortal time bias, failure to obtain specific information on metformin therapy, and all-cause mortality. Given the findings of this study, further studies are warranted to investigate the association between cancer progression and metformin usage. Larger powered trials are needed to further assess the impact of metformin on survival outcomes of colorectal cancer patients.

Author Contributions: YCL, SKL, and RP conceived and designed the study, and supervised the primary researcher. LLC aided in the analysis and interpretation of data. ABW collected the data. ABW and YCL wrote the final manuscript. LCM, YCL, and WHC edited the manuscript and all authors approved the final manuscript.

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