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Original Article

Effects of Metformin on Prevention and Treatment of Biliary Tract Cancer: A Meta-analysis of Observational Studies

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Abstract

Background and objectives: Diabetes mellitus (DM) is an established risk for biliary tract cancer (BTC). Metformin, an anti-diabetes medication, has been reported for its association with a reduced risk of BTC. However, the controversy about metformin's benefit among epidemiological studies is unresolved. This study is to investigate metformin's effects on the development and progression of BTC.

Methods: Literature searches were performed, without language restriction, in Pubmed, Embase, and Web of Science, from their respective inceptions to February 28, 2023. All studies were screened by two researchers using Covidence. Quality assessment was performed using the Newcastle-Ottawa Scale. Meta-analyses were performed by a fixed and random-effect model using RevMan version 5.4.

Results: Nine observational studies met the inclusion criteria and were included in the meta-analysis. With pooled samples of 24,743,526 individuals from 4 case-control and 2 cohort studies, metformin was not associated with a decreased risk of BTC (pooled RR: 0.82, 95% CI: 0.42–1.59, p = 0.56). Sub-group analyses in each study design also revealed a null effect of metformin. Meta-analysis of 3 cohort studies reporting the association between metformin and survival of patients with BTC with a pooled sample of 1,163 patients showed a marginally significant effect of metformin on survival outcome improvement in both fixed effect models (pooled RR: 0.83, 95% CI: 0.68–1.00, p = 0.05), and random-effect model (pooled RR: 0.83, 95% CI: 0.68–1.01, p = 0.07).

Conclusions: Meta-analyses of available observational studies showed that metformin was neither significantly associated with decreased risk nor, survival improvement for BTC patients who had DM.

Keywords: Cholangiocarcinoma; Diabetes mellitus; Metformin; Risk factor; Survival.

Abbreviations: BTC, biliary tract cancer; CCA, cholangiocarcinoma; CI, confidence interval; DM, diabetes mellitus; eCCA, extrahepatic cholangiocarcinoma; GLP-1, glucagon-like peptide 1; HR, hazard ratio; iCCA, intrahepatic cholangiocarcinoma; IDH, isocitrate dehydrogenase; OR, odds ratio; RR, relative risk.

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Introduction

Diabetes mellitus (DM) is an established carcinogenesis risk for biliary tract cancers, i.e., cholangiocarcinoma (CCA) and carcinoma of the gallbladder.[1,2] The increased risk of CCA, both intrahepatic (iCCA) and extrahepatic (eCCA), and gallbladder malignancy are reported to be associated with DM in several observational studies, as well as in meta-analyses.[3,4] The association between DM and increased risk of CCA is speculated to result from the effects of hyperglycemia, insulin, insulin-like growth factor, and their receptors.[5–7] Recently, some anti-diabetic medications have been reported to potentially modify CCA risk in patients with DM.[8,9] Several observational studies have been carried out to determine

the effect of different anti-diabetic drugs on CCA risk modification. While exogenous insulin and sulfonylurea usage are not associated with an increased risk of CCA, incretin-based therapy was shown in one cohort study in the United Kingdom to have a modest association with the increased CCA risk in DM patients.[8] In contrast, the effects of incretin-based therapy are still controversial. Another case-control study conducted in Italy reported a null effect of incretin-based therapy on CCA development, neither in the patients using dipeptidyl peptidase-4 inhibitor nor in those using glucagon-like peptide 1 (GLP-1) receptor agonist.[10] In addition, preclinical studies of exendin-4, a GLP-1 receptor agonist, indeed inhibited CCA cell growth both *in vitro* and *in vivo*.[11,12] Contrariwise, DM patients using metformin were found likely to benefit from decreased CCA risk in both case-control and cohort studies.[9,13] These conflicting data have led to extensive epidemiological and molecular-level studies of metformin's effects on CCA development.

Metformin (*N*, *N*-Dimethylbiguanide), classified as a biguanide anti-diabetic drug and previously recommended as a first-line drug in type 2 DM treatment by the American Association of Diabetes, is extensively used worldwide.[14] Metformin's primary effect is to activate the AMP-activated protein kinase thus in turn inhibiting the gluconeogenesis of hepatocytes. This effect prevents hyperglycemia in patients with DM and also helps sensitize insulin receptor signaling.[15]

The association between metformin use and decreased risk of hepatobiliary cancers has been studied in both hepatocellular carcinoma and BTC. One case-control study in the United States found that metformin was associated with a 60% reduced risk of iCCA in DM patients.[9] Molecular studies, both *in vitro* and *in vivo*, also support findings that metformin exerts a potent effect on the growth and aggressive phenotypes of CCA cells, and might be associated with prolonging survival in DM patients treated with metformin.[16,17] The anti-tumor effects of metformin appear broad and effective for both liver fluke- and non-liver fluke-associated CCA. Currently, metformin is registered for a clinical trial study on isocitrate dehydrogenase (IDH)-1 and IDH-2 mutation solid tumors, including iCCA.[18] However, over a wider range of BTCs, including eCCA and gallbladder carcinoma, metformin is not consistently effective in the prevention of BTC in DM patients.[19–24] In addition, BTC patients taking metformin for their DM treatment showed a discrepant effect among different populations.[25–27] Available systematic reviews and meta-analyses also show inconsistent results.[28,29] Thus, the benefit of using metformin for BTC prevention and as an add-on treatment, remains debatable and inconclusive.

This meta-analysis aimed to determine the effect of metformin on the prevention of BTC development among DM patients, as well as, the therapeutic benefit for patients who had BTC and were receiving metformin concurrently for DM. This clarification of metformin's effect will guide further translational and clinical studies as well as a prescribing regimen for anti-diabetic medication in patients with BTC.

Materials and methods

Data source and search strategies

All articles were searched for in PubMed, Web of Science, and Embase from their respective inceptions up to February 28, 2023, using the following search terms; [(metformin) or (antidiabetic)] and [(cholangiocarcinoma) or (bile duct cancer) or (biliary tract cancer) or (biliary carcinoma)] without language restriction. An additional manual search was also performed.

Inclusion and exclusion criteria

The epidemiological observational studies, both cohort and case-control, were included if they met the following criteria; 1) patients with diagnosed CCA or BTC, 2) metformin was prescribed for DM treatment in patients and, 3) relative risk; including Risk Ratio (RR), odds ratio (OR), hazard ratio (HR), were reported for BTC development, or overall survival of patients with BTC. The following studies were excluded; 1) non-clinical studies, *in vitro* and animal studies, conference abstracts, and reviews, 2) studies involving only gallbladder carcinoma which has a different etiology from other BTC and, 3) RR, OR, or HR and 95% confidence intervals (95% CI) were not reported for CCA, BTC risk development, or overall survival.

Data extraction and quality assessment

Screening and selecting of eligible studies were performed by two independent researchers (SS and CS) using Covidence software (Melbourne, Australia). First author, publication year, region, the subtype of CCA and other BTC, sample size, RR, OR and HR, were obtained. The discrepancies between the process of study selection were solved by consensus discussion with CCA research expert co-authors (SW and WS). The quality of included studies was evaluated using the Newcastle-Ottawa Scale.

Statistical analysis

The meta-analysis of all included studies was performed using RevMan 5.4 (Nordic Cochrane Centre, Copenhagen, Denmark) by using an inverse variance method, and the pooled RRs were then approximated. OR, RR, or HR, and 95% CI were used to compare outcomes between metformin and non-metformin groups. Statistical heterogeneity was assessed by I^2. The combined estimates were calculated and pooled under a random-effects model regardless of heterogeneity. Statistical significance was set at $p < 0.05$.

Results

Literature search and eligible studies

The literature search identified a total of 130 references from PubMed, 3,615 from Web of Science, and 2,097 from Embase. After removing duplications, 1,005 reports were included for screening. After in-depth evaluation 9 studies were eligible for meta-analysis. The PRISMA literature selection process is depicted in Figure 1. Table 1 shows the characteristics of studies included in the meta-analysis.[9,13,19,21,22,24–27]

Risk of bias for methodology assessment

All included studies were evaluated with Newcastle-Ottawa Scale tool and ranked with more than 6 stars, indicating the low bias risk in each study. The results for the risk of bias for the methodology are shown in Table 2.[9,13,19,21,22,24–27]

Effects of metformin on the prevention of BTC

Among 9 included studies, 6 studies (2 case-control and 4 cohort studies) reported the effect of metformin on the development of BTC in DM patients. All case-control and cohort studies were included in a final meta-analysis with a pooled sample of 24,743,526 subjects. One study by de Jong *et al* included 2 different sub-cohorts according to designs for their analyses and thus both were included.[19] Significant heterogeneity among all studies was observed with an I^2 of 98%, $p < 0.001$, thus the random-effect model

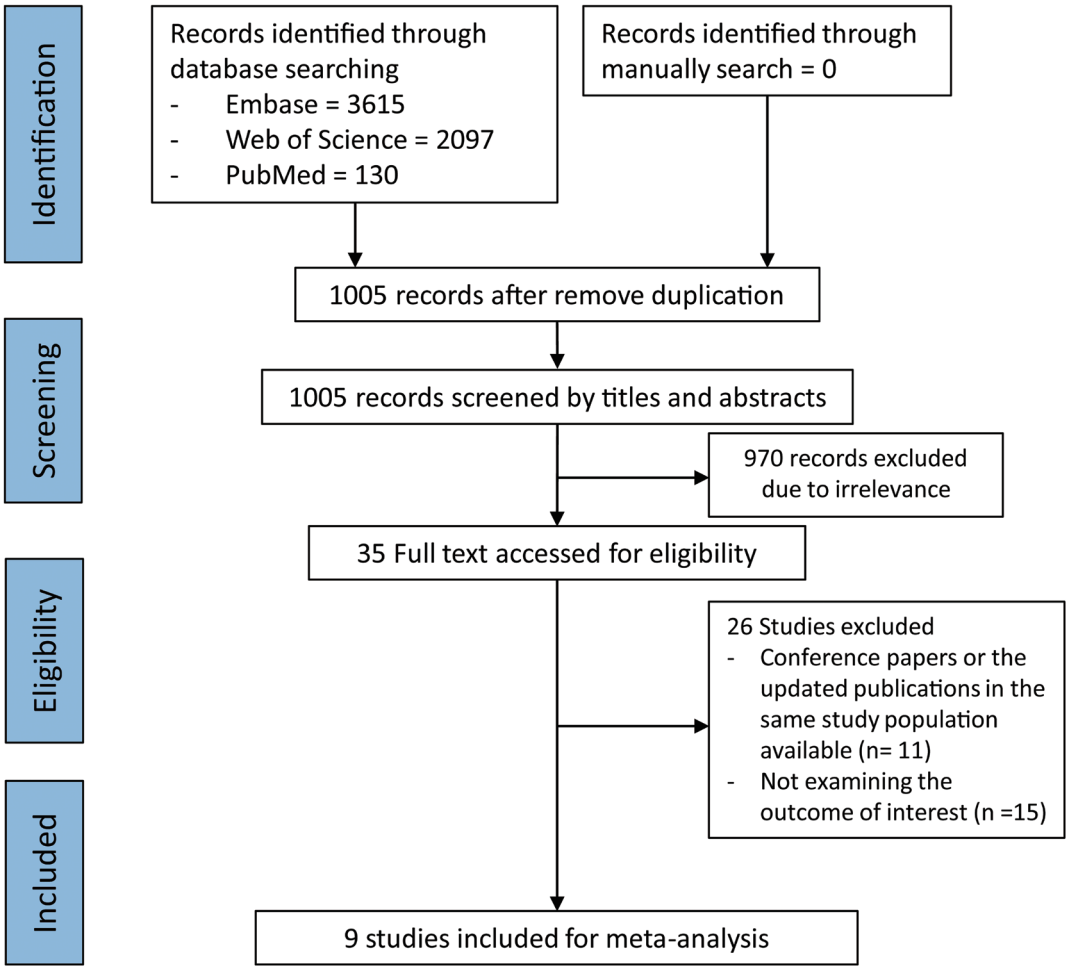

Fig. 1. Prisma flow of the study.

for meta-analysis was subsequently used. The results showed that metformin did not possess any preventive effect on BTC development, with pooled RR of 0.82 (95% CI: 0.42–1.59, $p = 0.56$). A forest plot of this meta-analysis is shown in Figure 2a.

As a highly significant heterogeneity was observed among all studies, a sub-group analysis based on the research designs of the original studies was also conducted. Meta-analysis of 4 cohort studies was done with a pooled population of 6,188,954 subjects. A heterogeneity among the studies remained with an I^2 of 91%, $p < 0.001$, with the random effect model being used for meta-analysis. Metformin showed a null effect on risk modification of BTC with a pooled RR of 0.81 (95% CI: 0.48–1.37, $p = 0.44$) (Fig. 2b). Sub-group analysis of 2 case-control studies also showed a great heterogeneity with an I^2 of 98%, $p < 0.001$. Random effect model

Table 1. Characteristics of included studies

Study (Ref)	Region	Study design	Study period	No. of subjects
Chaiteerakij *et al*, 2013[9]	USA	Case-control	2000–2010	1206
de Jong *et al*, 2017[19]	Netherland	Cohort	1998–2011	57,621
Oh and Song, 2020[24]	South Korea	Cohort	2011–2015	66,627
Tseng, 2020[13]	Taiwan	Cohort	1999–2005	304,224
Sookaromdee and Wiwanitkit, 2020[21]	Thailand	Case-control	NR	18,547,869
Marcano-Bonilla *et al*, 2022[22]	Sweden	Cohort	NR	5,760,482
McNamara *et al*, 2015[26]	Canada	Cohort	1987–2013	913
Yang *et al*, 2016[25]	USA	Cohort	2001–2012	250
Casadei-Gardini *et al*, 2021[27]	Italy	Cohort	2005–2020	537

Table 2. New Castle-Ottawa Scale (NOS) for the assessment of the included studies

	Risk				
	Author (year)	**Selection**	**Comparability**	**Outcome**	**Total**
Case-control study					
1	Chaiteerakij (2013)[9]	***	**	***	8
2	Sookaromdee and Wiwanitkit (2020)[21]	***	**	**	7
Cohort study					
1	de Jong (2017)[19]	****	**	***	9
2	Oh and Song (2020)[24]	***	**	***	8
3	Tseng (2020)[13]	****	**	***	9
4	Marcano-Bonilla (2022)[22]	****	**	***	9

	Survival				
	Author (year)	**Selection**	**Comparability**	**Outcome**	**Total**
Cohort study					
1	McNamara (2015)[26]	***	*	***	7
2	Yang (2016)[25]	***	**	***	8
3	Casadei-Gardini (2021)[27]	***	**	***	8

meta-analysis was used to analyze the effects of metformin on the risk of BTC in a pooled population of 18,554,572 subjects. No effect of metformin on the risk of BTC was observed (RR: 0.69, 95% CI: 0.06–7.47, $p = 0.76$) (Fig. 2c).

Effects of metformin on survival outcome of patients with DM

The effect of metformin on the survival of patients with BTC was further analyzed in another 3 studies with a pooled population of 1,700 individuals. A minimal and non-significant heterogeneity among the studies was observed with an I^2 of 7%, $p = 0.34$. By the random effect meta-analysis, metformin showed a marginal benefit for patients with BTC, who were prescribed this medication for their concurrent DM, with a pooled RR of 0.83 (95% CI: 0.68–1.01, $p = 0.07$) (Fig. 3a). The marginal beneficial effect of metformin on BTC patients' survival was also consistent in the fixed effect model meta-analysis with a pooled RR of 0.83 (95% CI: 0.68–1.00, $p = 0.05$) (Fig. 3b).

Discussion

The association of DM and increased BTC risk, especially CCA, has been consistently reported in several studies over the past decade.[3,4,9] However, the effect of anti-diabetic medication on the modification of CCA risk is still controversial among different DM drug treatment groups.[2] The authors of the current study have reviewed research that showed metformin has a promising role in CCA prevention in DM patients as well as a potential role for add-on treatment in patients with CCA.[2] A breakthrough case-control study in the United States by Chaiteerakij *et al* reported an association between taking metformin and a 60% reduced CCA risk in patients with DM.[9] In addition, the results of many preclinical studies on the molecular mechanisms underlying the inhibitory effects of metformin on CCA cells also support the epidemiological observations.[16,17,30–32] However, observational studies in other regions are inconsistent with Chaiteerakij *et al*'s findings.[19,20] Since metformin seemed highly promising for repurposing as a CCA chemoprevention agent, as well as, an add-on medication for CCA treatment, this systematic review and meta-analysis also investigated metformin's effectiveness globally.

Our literature search across 3 databases, identified 6 observational studies (2 case-controls and 4 cohorts) that reported the relative risk of BTC and/or CCA development. Two case-control studies reported the ORs and showed the opposite outcomes of metformin on CCA risk to each other.[9,21] Notably, these 2 studies were conducted in different regions where the known risk factors are different.[33,34] The other studies were cohort studies that reported the HR for the development of a group of BTC, including all subtypes of CCA (iCCA and eCCA) as well as gallbladder carcinoma, based on International Classification of Disease (ICD) coding systems used in the primary databases.[13,19,20] All 6 reports (case-control and cohort studies), with one study possessing 2 sub-cohorts, were included in the final meta-analysis.[19] This meta-analysis showed that metformin was not associated with a modified risk for BTC development. Sub-group analyses classified by the research designs of the original studies (case-control *vs*. cohort studies) also consistently showed that metformin was not associated with a modification of BTC risk among the included population. Significant heterogeneity was observed among all studies. This could be due to the biological heterogeneity of cancer subtypes all grouped as biliary tract cancers in the original studies, *e.g.*, gallbladder carcinoma has a different etiology to CCA and is more aggressive.[35,36] Even in the CCA group, the iCCA and eCCA could also originate from different cell types and be associated with different risk factors.[37] These factors are potential confounders in our analysis and need to be considered in interpreting the results. As the original studies did not report relative risk for each BTC subtype, sensitivity analysis, could not be carried out in the present meta-analysis.

The add-on therapeutic effects of metformin in BTC patients

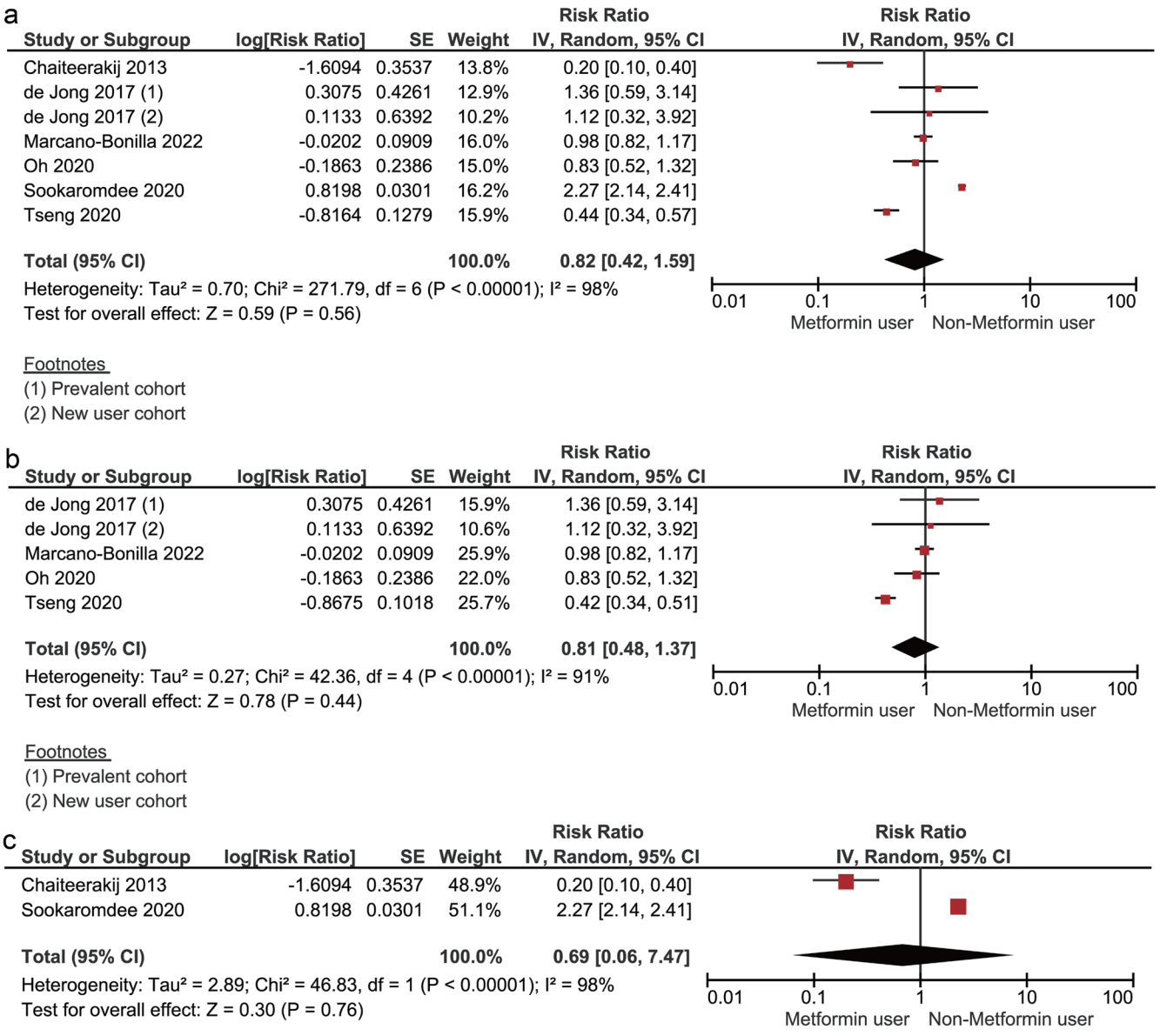

Fig. 2. Effects of metformin on the risk of BTC. (a) Metformin showed a null effect on risk modification of BTC in a pooled meta-analysis of all studies. Subgroup analyses of cohort studies (b) and case-control studies (c) show consistent results that metformin is not associated with reduced BTC development. BTC, biliary tract cancer.

receiving standard treatment were also meta-analyzed. From 3 included studies with minimal and non-statistically significant heterogeneity, metformin showed null effects on overall survival in 2 cohorts, and another cohort showed a benefit on overall survival in BTC patients who were prescribed metformin.[25–27] The meta-analysis thus showed a marginal benefit of metformin on the overall survival of BTC patients. Although metformin is very promising for BTC treatment in preclinical studies, the lack of efficacy and discrepancies in metformin's effects on BTC between preclinical and human studies may result from several factors as discussed in a recent review by the current authors.[38] First, the *in vitro* studies used a relatively high dose of metformin at a millimolar scale which could increase the risk of adverse effects in humans at the same concentration. Thus *in vitro* dosage may not be directly translatable. Second, metformin seems beneficial for cancers that originated from the tissues with high expression of its transporters and in tissues with high accumulation capacity, e.g., the liver and small intestine. Conversely, BTC and CCA are desmoplastic by nature, thus this factor could be a barrier to metformin's activity. Third, all patients in the included studies were at the late stage of BTC and metformin dosages in clinical practice also vary across the patients depending on their glycemic status, renal function, and other indications or contraindications. These could be confounding factors that make metformin less beneficial in a setting of observational clinical studies. Last, observational studies are limited by the nature of the study designs. To affirm whether metformin is beneficial for BTC treatment, randomized controlled trials need to be carried out. At the time of our search, one phase Ib clinical trial of metformin

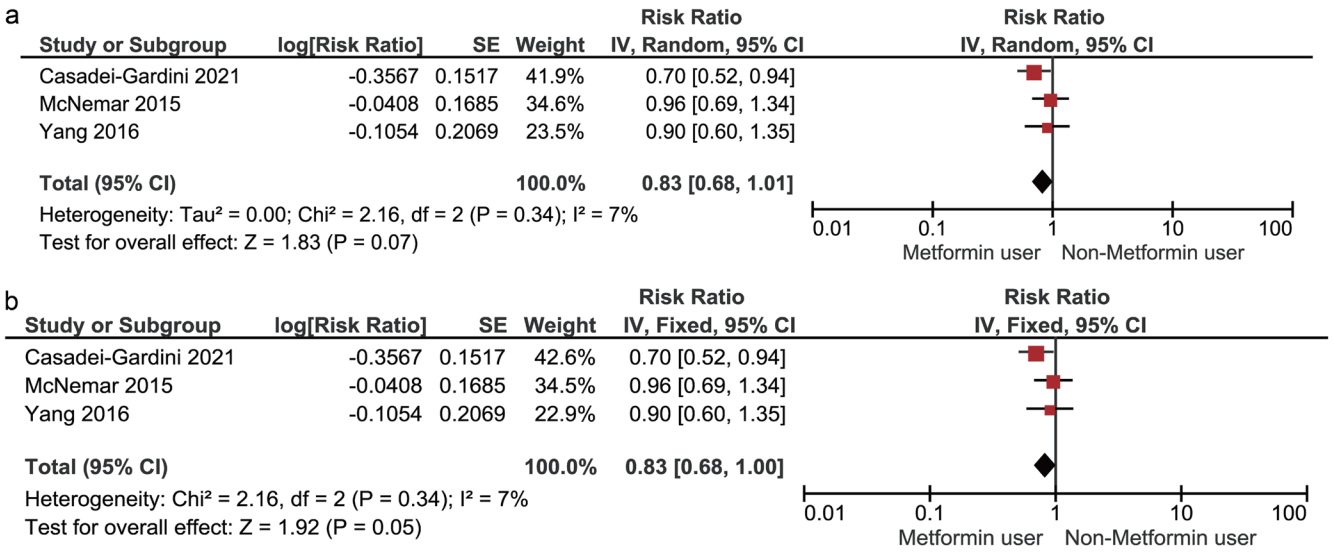

Fig. 3. Effects of metformin on overall survival of BTC. Random-effect (a) and fixed-effect (b) meta-analyses of the effects of metformin on the improvement of overall survival in BTC patients show a trend of favoring metformin treatment with a marginal statistical significance.

and chloroquine in 12 patients with isocitrate dehydrogenase-1 mutated iCCA was located.[39] It reported a poor clinical response of the tumor to both drugs, though they were well tolerated by the patients.[39] Due to great heterogeneity among BTC subtypes, more experimental research is needed for more definitive conclusions.

Even though this meta-analysis did not support a beneficial effect of metformin on CCA prevention, the estimated RR of 0.82 favored an 18% lower risk of patients with DM who used this drug over the other medications. Moreover, metformin also shows a high potential for improving survival outcomes in BTC patients by favoring a reduced risk of mortality with an RR of 0.83 and with a marginal statistical significance ($p = 0.05$) in a fixed model meta-analysis. Metformin has been consistently reported in a series of population-based cohort studies to be associated with a significantly lower risk of CCA and cancers that are highly malignant and located in organs associated with the biliary tract system, including hepatocellular carcinoma, pancreatic cancer, and gastric cancer.[13,40–42] Due to lowering the risk for such cancers, unless contraindicated metformin should remain in a major position in the treatment of type 2 DM in clinical practice both in the general population and patients who have BTC.

This systematic review and meta-analysis are the updated report on the effects of metformin on BTC, both on a carcinogenesis risk and a benefit on survival outcome. However, several limitations need consideration. First, the number of studies was limited at the time this meta-analysis was done. Second, sensitivity analysis for subtypes of BTC with heterogenous biological backgrounds could not be done due to the limitation in the data. Finally, CCA and BTC are associated with different risk factors in different regions. Therefore, when the numbers of primary studies are sufficient, further meta-analyses with sensitivity analysis of BTC subgroups classified by various factors are needed.

Conclusion

This meta-analysis of observational studies suggests that metformin does not provide any chemopreventive effects against BTC development in patients who have DM as an underlying disease. Neither does metformin for DM treatment appear to confer any benefit for the survival of BTC patients with DM. To affirm the results of the present study, a meta-analysis of a greater number of studies as well as randomized control trials are needed.

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Conflict of interest

The authors have no conflicts of interest related to this publication.

Author contributions

Conceptualization (SS, WS, SW, CS), methodology (SS, LW, SN, CS), formal analysis (LW, SN, CS), supervision (WS, SW), writing first draft (SS, CS), All authors have made a significant contribution to this study and have approved the final manuscript.

Data sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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