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ORIGINAL RESEARCH ARTICLE

Dose-Dependent Relationship Between Long-Term Metformin Use and the Risk of Diabetic Retinopathy: A Population-Based Cohort Study

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Abstract

Background and Objective Recent research has raised concerns about the association between metformin treatment in patients with diabetes mellitus (DM) and an increased risk of diabetic retinopathy. We sought to investigate this relationship, specifically examining if metformin use affects diabetic retinopathy risk in a dose-dependent manner.

Methods This study was a secondary data analysis based on a nationwide population database in Taiwan. Patients with new-onset DM, an age of 20 years or older, and a diagnosis of type 2 DM received at any time during 2002–2013 were included in the study. Patients diagnosed with new-onset type 2 DM between 2002 and 2013 were enrolled as the study population. We divided them into two groups: those treated with metformin and those treated with sulfonylureas. A Cox proportional hazards model was employed to estimate the risk of diabetic retinopathy after 5 years of follow-up, including cumulative defined daily dose and intensity of metformin treatment.

Results A total of 241,231 patients received treatment with metformin, while 152,617 patients were treated with sulfonylureas. Compared with patients treated with sulfonylureas, patients who received metformin treatment, at a cumulative defined daily dose < 30, had a lower risk of diabetic retinopathy (adjusted hazard ratio = 0.77; 95% confidence interval 0.60–0.98). However, those with varying defined daily doses, especially at a higher metformin treatment level (> 25 defined daily dose), had a 2.43 times higher risk of diabetic retinopathy (95% confidence interval 1.37–4.30) compared with patients treated with sulfonylureas.

Conclusions Patients with DM treated with a lower cumulative dosage of metformin showed beneficial effects that were associated with a lower risk of diabetic retinopathy. In contrast, a higher intensity of metformin use had a greater risk of diabetic retinopathy.

Key Points

This study found that patients with type 2 diabetes mellitus who received a lower cumulative dosage of metformin were associated with a lower risk of diabetic retinopathy.

However, patients who received a higher intensity of metformin treatment had a greater risk of diabetic retinopathy.

Furthermore, age, sex, hyperlipidemia, and hypothyroidism were the risk factors of incident diabetic retinopathy in patients with type 2 diabetes.

Yu-Ching Li and Kuang-Hua Huang have contributed equally to this work and share first authorship.

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1 Introduction

Diabetic retinopathy (DR) is a common serious microvascular complication of diabetes mellitus (DM) and a major cause of vision loss in adults in developed countries [1]. Diabetic retinopathy is a chronic progressive microvascular complication of DM that typically occurs in two stages: an early stage, which involves non-proliferative DR, and an advanced stage, which involves proliferative DR, with clinically visible ophthalmic pathological changes and retinal neovascularization [2]. Diabetic retinopathy is associated with hyperglycemia, advanced glycation end products, hyperglycemia-induced oxidative stress, and low-grade inflammation [3], and its progression to the proliferative phase is a consequence of retinal inflammation, retinal neovascularization, and endothelial activation [4–6]. According to previous studies, in patients with DM, low vitamin B_{12} levels in serum are associated with an increased risk of DR [7].

Metformin is used in the treatment of patients with DM. According to a recent study [8], metformin exerts a positive effect against age-related diseases and DR through both 5′AMP-activated protein kinase (AMPK)-dependent and AMPK-independent pathways. In streptozotocin-induced diabetic rat models, metformin attenuates hyperglycemia-associated eye deterioration, partially by ameliorating inflammation, oxidative stress, vascular leakage, and retinal neovascularization [9]. Although many in vitro and animal studies have indicated the potential role of metformin in DR [10–12], whether these studies are applicable to patients with DM who undergo metformin treatment to slow down the progression of DR remains unclear. Clinically, although metformin considerably reduces the serum levels of vitamin B_{12} in patients with DM, these reduced levels substantially increase the risk of neuropathy [13]. This metformin-induced reduction in vitamin B_{12} levels may lead to an increase in the levels of homocysteine [14]. This increase in the serum levels of homocysteine may contribute to a reduction in retinal blood flow, thereby resulting in ischemic retinal vascular injury [15]. In regard to the association between metformin therapy and the risk of age-related macular degeneration (AMD), a Taiwanese study showed that metformin significantly reduces the risk of AMD in patients with type 2 DM, as evidenced by a lower incidence rate in ever users compared with never users [16].

In this study, we conducted a large-scale nationwide analysis to determine whether metformin use is associated with an increased risk of DR. We also used a nationwide database, namely the National Health Insurance Research Database (NHIRD) of Taiwan, to determine whether metformin use has a dose-dependent effect on the risk of DR in patients with DM.

2 Materials and Methods

2.1 Data Sources

In this study, we used a secondary database linked to the NHIRD, covering the period from 2001 to 2018. This database is maintained by the Health and Welfare Data Science Center of the Ministry of Health and Welfare of Taiwan. The NHIRD contains information on all beneficiaries enrolled in the National Health Insurance program of Taiwan, which is a government-run, single-payer national social insurance program that has been in place since 1995. The database also contains data on the health insurance claims of 99% of the entire Taiwanese population (approximately 23 million people). Disease diagnoses are established in accordance with the *International Classification of Diseases, Ninth Revision, Clinical Modification* (*ICD-9-CM*) and the *International Classification of Diseases, Tenth Revision, Clinical Modification* (*ICD-10-CM*). Typically, the NHIRD is used to obtain real-world data to support clinical decision making and healthcare policy making [17, 18]. In this study, we used data from the NHIRD to evaluate the risk of DR among patients with type 2 DM who underwent treatment with metformin.

2.2 Ethical Approval

This study was conducted in accordance with the Declaration of Helsinki. Data were obtained from the Longitudinal Health Insurance Database, which is maintained by the Health and Welfare Data Science Center and provides scrambled random identification numbers for insured patients to protect their privacy. The study protocol was approved by the Central Regional Research Ethics Committee of China Medical University, Taichung, Taiwan (approval no. CRREC-109-011). To protect the privacy of the participants, all data were anonymized. Because the database contains only deidentified data, the requirement for informed consent was waived.

2.3 Study Participants

Patients with new-onset DM, an age of 20 years or older, and a diagnosis of type 2 DM received at any time during 2002–13 were included in the study. The definition of patients with new-onset DM required meeting the following two criteria: (1) had no medical records of a DM diagnosis (ICD-9-CM code: 250) before and (2) had three above outpatient visits within 1 year with a primary diagnosis of DM. To reduce bias, the following patients were excluded: (1) patients with type 1 DM; (2) patients who received a diagnosis of DR before DM or a diagnosis of DR during

the first year after DM; (3) patients who were hospitalized within 1 year after receiving a diagnosis of DM; (4) patients who had not received any antihyperglycemic drugs; and (5) patients who had received a combination of metformin and sulfonylureas. After the exclusion, we divided patients into two groups: those treated with metformin and those treated with sulfonylureas. Metformin was assigned the Anatomical Therapeutic Chemical code A10BA02, while sulfonylureas were classified under the Anatomical Therapeutic Chemical code A10BB. A total of 393,848 patients with new-onset DM, who were treated with either metformin or sulfonylureas during the period from 2002 to 2013, were included in the study and divided into two groups according to the enrolled year to reduce the background bias: a case group (patients treated with metformin) and a comparison group (patients treated with sulfonylureas). The case group comprised 241,231 patients who were treated with metformin within the first year after receiving a diagnosis of DM, and the comparison group comprised 152,617 patients who were treated with sulfonylureas. Figure 1 depicts a flowchart of the patient selection.

2.4 Study Design

A cohort study with 5 years of follow-up was conducted to investigate the risk of DR in patients with DM who undergo treatment with metformin, compared with sulfonylurea use. To measure the intake of metformin, we used the defined daily dose (DDD), which is a standard measure of drug use and exposure. According to the World Health Organization, the DDD is the assumed average maintenance dose per day for adults. Notably, the DDD does not necessarily reflect the recommended or prescribed daily dose [19]. The DDD was created to address challenges related to dosage forms and provides a practical method for monitoring changes in medication usage, particularly in hospital settings where shifts in formulations or adjustments in pack sizes or fill volumes are common [20]. As with many average-based calculations, the DDD serves as a standardized unit of measurement reflecting a theoretical daily dose and is used to evaluate drug utilization. However, it is crucial to note that the DDD may not consistently correspond with the actual recommended or prescribed daily dose [20]. After an initial diagnosis of DM is established, the observation period before treatment with metformin is typically 1 year, with 2 g of metformin representing the baseline DDD [21]. In this study, we used two criteria for the dose-dependent analysis of metformin treatment and the risk of DR. We refer to related research about the division in cumulative DDDs (cDDDs) and the follow-up period of metformin treatment [22–24] and according to the actual usage of metformin users set the medication grade interval and follow-up duration. The first criterion was defined in accordance with the following four ranges of cDDDs for metformin treatment in the first year: < 30, 30–120, 120–240, and > 240. The second criterion was defined in accordance with the following four ranges of average monthly DDDs: < 5, 5–15, 15–25, and > 25. The date of the first prescription of metformin was the observation start date in patients who were treated with metformin. The date of the first prescription of sulfonylureas was the observation start date in patients who were treated with sulfonylureas. The included patients were regarded as having been continuously exposed to their cohort entry drug during the study period. All patients were followed up for 5 years and were followed from the observation start date until death, the use of another study drug (metformin or sulfonylureas), the incidence of DR, or the end of the observation, whichever occurred first.

In accordance with the *ICD-9-CM* code 362.0 and *ICD-10-CM* code E11.319, the criterion for DR was defined as three or more diagnoses within 1 year. The control variables used were sex, age, income, urbanization level, Diabetes Complications Severity Index (DCSI) score, and comorbidities. The comorbidities were hyperlipidemia (*ICD-9-CM* code: 272), chronic obstructive pulmonary disease (*ICD-9-CM* codes: 490-492 and 494-496), obesity (*ICD-9-CM* code: 278), chronic kidney disease (*ICD-9-CM* code: 585), anxiety (*ICD-9-CM* code: 300.0), depression (*ICD-9-CM* codes: 296.2 and 296.3), hypothyroidism (*ICD-9-CM* code: 244.9), hyperthyroidism (*ICD-9-CM* code: 242.9), and sleep disturbances (*ICD-9-CM* code: 780).

2.5 Statistical Analysis

All statistical analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC, USA). A Chi-square test was conducted to determine the differences in baseline characteristics between patients treated with and without metformin. After sex, age, income, urbanization level, DCSI score, comorbidities, and the patients enrolled year were adjusted for, the Cox proportional hazards model was used to estimate the difference in the risk of DR between patients treated with metformin. Hazard ratios (HRs) with 95% confidence intervals (CIs) were used. Two adjusted models were utilized to estimate the cDDD and intensity of metformin treatment (expressed as DDD/month). A *P*-value of <0.05 indicated statistical significance.

3 Results

Table 1 lists the baseline characteristics of all patients. The average age of all patients was 56.87 ± 12.43 years, and 27.59%, 15.93%, 15.89%, 12.94%, and 27.64% of the

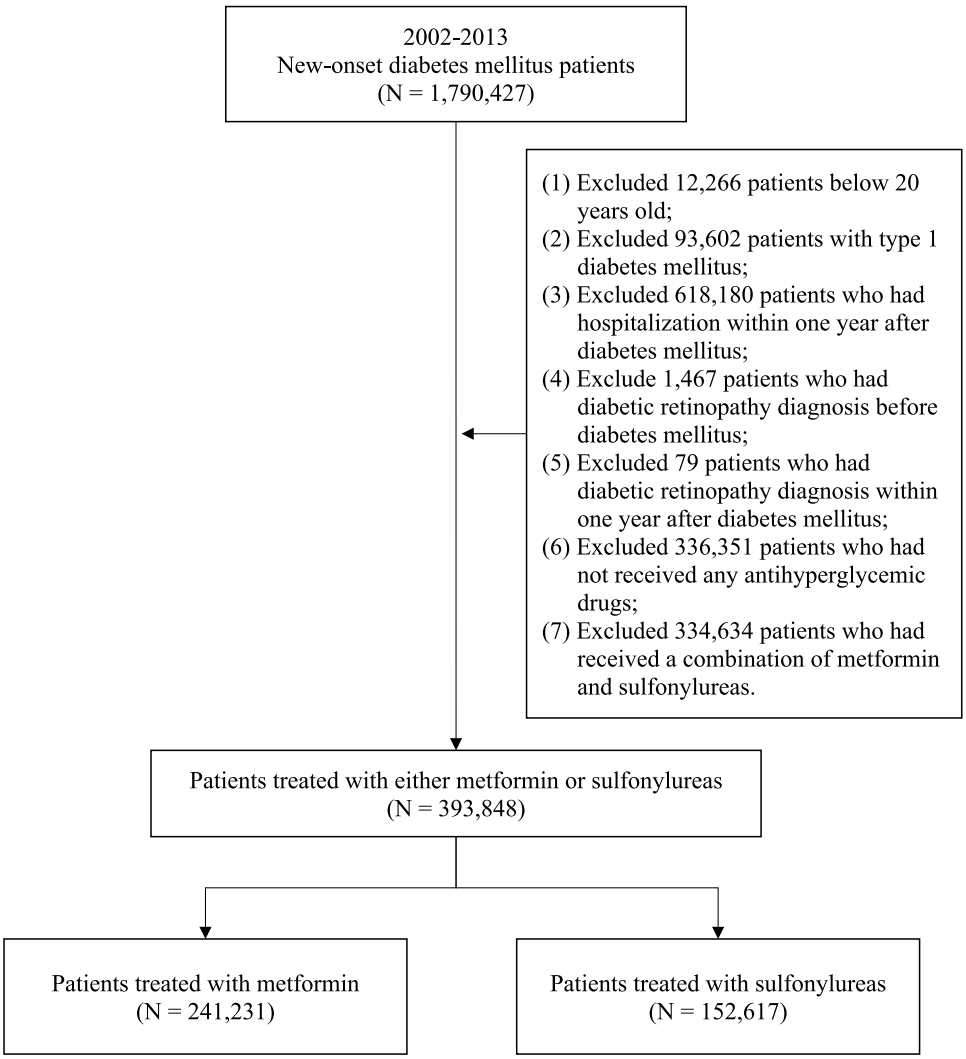

Fig. 1 Patient selection process

patients were 20–49, 50–54, 55–59, 60–64, and > 65 years of age, respectively. In addition, 52.07% of the patients were male.

The average age of patients treated with metformin was 56.25 ± 12.59 years. With regard to comorbidities, 56,049 patients (23.23%) had hyperlipidemia, 12,071 patients (5.00%) had chronic obstructive pulmonary disease, 2530 patients (1.05%) were obese, 1126 patients (0.47%) had chronic kidney disease, 23,805 patients (9.87%) had anxiety, 1467 patients (0.61%) had depression, 1703 patients (0.71%) had hyperthyroidism, and 50,097 patients (20.77) had sleep disturbances. Significant differences were observed in the distribution of each comorbidity between patients treated with metformin and sulfonylureas ($P < 0.05$).

Table 2 presents the risk of DR at 5 years of follow-up. The average time to develop DR is 3.69 years in patients treated with metformin. After adjusting for relevant variables, compared with patients treated with sulfonylureas, we discovered that patients with DM who underwent metformin treatment at cDDDs of < 30, 30–120, 120–240, and > 240 for DR exhibited HRs of 0.77 (95% CI 0.60–0.98), 0.85 (95% CI 0.69–1.05), 0.90 (95% CI 0.69–1.17), and 1.41 (95% CI 0.91–2.19), respectively. Compared with patients treated with sulfonylureas, patients who underwent metformin treatment at DDDs of < 5, 5–15, 15–25, and > 25 per month for DR exhibited HRs of 0.82 (95% CI 0.66–1.01), 0.81 (95% CI 0.65–1.01), 1.06 (95% CI 0.79–1.44), and 2.43 (95% CI: 1.37–4.30), respectively. Compared with patients aged 20–49 years, those aged ≥ 65 years were at a higher risk of DR (HR: 1.55; 95% CI 1.23–1.94). In terms of comorbidities, patients with hypothyroidism had an increased risk of DR (HR: 2.61, 95% CI 1.34–5.06).

4 Discussion

Few large-scale, retrospective, cohort epidemiological studies have examined the relationship between metformin treatment and the risk of DR in patients with DM. In this study, we found that patients who received < 30 cDDD of metformin are associated with a lower risk of DR. However, those who received intensity of metformin treatment > 25 DDD/month experienced an increased risk of DR. In patients with DM, advanced age, a high DCSI score, and the presence of comorbidities such as hypothyroidism were associated with an increased risk of DR.

Generally, diabetes complications are either microvascular or macrovascular. In patients with DM, the most common microvascular complications include DR, diabetic nephropathy, and diabetic peripheral neuropathy, and the most common macrovascular complications include coronary artery disease, heart failure, stroke, and other peripheral vascular diseases [25, 26]. Diabetic retinopathy has a multi-factorial pathogenesis with a highly complex etiology. Both the duration of DM and the resulting hyperglycemia are regarded as the driving forces behind the progression of DR, particularly because the duration of DM is positively associated with the risk of DR [27]. Diabetic end-organ complications, such as DR, develop as a consequence of hyperglycemia, which progressively induces vascular disruption and initiates numerous pathological changes through multiple mechanisms. These mechanisms include the increased production of vascular endothelial growth factors, the formation of advanced glycation end products, the development of oxidative stress, the accumulation of intracellular sorbitol, and the activation of protein kinase C [28]. Although several pathways are involved in DR, the chronic exposure of retinal cells to hyperglycemia results in the accumulation of advanced glycation end products, which play a key role in retinopathy [29].

In our study, the comparison group includes patients receiving sulfonylureas. Findings from a network meta-analysis showed that sulfonylureas might be associated with a higher risk of DR compared with placebo, although the lower limit of the CI is nearly negligible [30]. Metformin is regarded as a first-line drug for the treatment of patients with DM. In addition to exerting a glucose-lowering effect, metformin has been demonstrated in in vivo and in vitro studies to exert a dose-dependent protective effect against oxidative stress and tight junction disorganization in retinal pigment epithelial cells and against angiogenesis and inflammation in retinal vascular endothelial cells [10–12, 31]. According to Nahar et al. [9], metformin attenuates histopathological ocular deterioration associated with hyperglycemia in streptozotocin-induced hyperglycemic rat models, partially by ameliorating vascular leakage, neovascularization, oxidative stress, and inflammation. An animal study found that DR was induced in mice treated with streptozotocin, regardless of metformin treatment. However, metformin appeared to alleviate the DR, possibly by reducing retinal neovascularization [12]. Moreover, according to a small prospective pilot clinical trial, metformin reduces the proliferation of human retinal pigment epithelial cells [32].

Multiple studies have indicated that metformin is associated with reduced plasma vitamin B_{12} levels [33–35]. This metformin-induced reduction in vitamin B_{12} levels may lead to an increase in the levels of homocysteine [14]. This increase in the serum levels of homocysteine may contribute to a reduction in retinal blood flow, thereby resulting in ischemic retinal vascular injury [7, 15]. Hyperhomocystenemia has been proposed as a potential risk factor for the progression and development of DR [36].

A study indicated patients with DM who undergo metformin treatment, particularly high doses and long durations above 4 years, are associated with vitamin B_{12} deficiency [37]. Another study also implied that a higher cumulative exposure to metformin and longer metformin use were strongly linked with a greater risk of vitamin B_{12} deficiencies [38]. Long-term and a high dose or higher cumulative use of metformin has been found to be closely associated with biochemical B_{12} deficiency as well as hyperhomocysteinemia [39]. Thus, the cDDDs of metformin were divided into < 30, 30–120, 120–240, and > 240, and the treatment intensities of metformin were divided into < 5, 5–15, 15–25, and > 25 DDDs/month, and follow-up periods of 5 years were used after the initiation of metformin treatment. In this large-scale retrospective cohort study, we discovered that patients with DM who received < 30 cDDD of metformin are associated with a lower risk of DR, while patients who received metformin > 25 DDD/month experienced an increased risk of DR. Diabetic retinopathy is one of the common microvascular complications in patients with DM [25, 26], a long-term and higher cumulative dosage of metformin among patients with DM may result in an increased risk of developing DR. Several clinical studies also suggest metformin use plays a protective role in DR that is correlated with a decreased risk of DR development. A study showed that in patients with DM, treatment with metformin was associated with a lower risk of DR compared with treatment using other oral anti-hyperglycemic agents [40]. A population-based cohort study found that metformin treatment in patients with DM was associated with a reduced risk of non-proliferative DR, and a potential role was found for a decreased sight-threatening DR risk in patients who had previously been diagnosed with non-proliferative DR [41]. Another population-based study indicated that long-term metformin use was associated with a significant reduction in the severity of DR in patients with DM [3]. Patients with DM who received lower doses

Table 1 Baseline characteristics of study subjects

Variables	Total		Sulfonylurea		Metformin		*P* value
	N	%	*N*	%	*N*	%	
Total	393,848	100.00	152,617	100.00	241,231	100.00	
Sex							< 0.001
Female	188,755	47.93	69,350	45.44	119,405	49.50	
Male	205,093	52.07	83,267	54.56	121,826	50.50	
Age (years)							< 0.001
20–49	108,649	27.59	39,109	25.63	69,540	28.83	
50–54	62,758	15.93	24,537	16.08	38,221	15.84	
55–59	62,598	15.89	23,182	15.19	39,416	16.34	
66–64	50,980	12.94	19,496	12.77	31,484	13.05	
65+	108,863	27.64	46,293	30.33	62,570	25.94	
Mean ± SD	56.87 ± 12.43		57.84 ± 12.12		56.25 ± 12.59		
Income level (NTD)							< 0.001
≤ 21,000	200,080	50.80	91,219	59.77	108,861	45.13	
21,001–33,000	94,115	23.90	29,014	19.01	65,101	26.99	
≥ 33,001	99,653	25.30	32,384	21.22	67,269	27.89	
Urbanization							< 0.001
Level 1	103,290	26.23	36,777	24.10	66,513	27.57	
Level 2	128,190	32.55	47,651	31.22	80,539	33.39	
Level 3	65,329	16.59	25,375	16.63	39,954	16.56	
Level 4	56,035	14.23	24,056	15.76	31,979	13.26	
Level 5	8771	2.23	4227	2.77	4544	1.88	
Level 6	16,895	4.29	7717	5.06	9178	3.80	
Level 7	15,338	3.89	6814	4.46	8524	3.53	
DCSI score							< 0.001
0	259,051	65.77	103,403	67.75	155,648	64.52	
1	75,727	19.23	27,357	17.93	48,370	20.05	
≥ 2	59,070	15.00	21,857	14.32	37,213	15.43	
Enrolled year							< 0.001
2002	29,924	7.60	22,078	14.47	7846	3.25	
2003	28,337	7.19	19,624	12.86	8713	3.61	
2004	29,232	7.42	18,360	12.03	10,872	4.51	
2005	27,363	6.95	15,830	10.37	11,533	4.78	
2006	28,468	7.23	14,522	9.52	13,946	5.78	
2007	30,367	7.71	13,268	8.69	17,099	7.09	
2008	31,152	7.91	10,807	7.08	20,345	8.43	
2009	34,409	8.74	10,187	6.67	24,222	10.04	
2010	35,097	8.91	8350	5.47	26,747	11.09	
2011	36,681	9.31	7294	4.78	29,387	12.18	
2012	39,983	10.15	6417	4.20	33,566	13.91	
2013	42,835	10.88	5880	3.85	36,955	15.32	
Hyperlipidemia							< 0.001
No	317,021	80.49	131,839	86.39	185,182	76.77	
Yes	76,827	19.51	20,778	13.61	56,049	23.23	
COPD							< 0.001
No	374,653	95.13	145,493	95.33	229,160	95.00	
Yes	19,195	4.87	7124	4.67	12,071	5.00	
Obesity							< 0.001
No	390,900	99.25	152,199	99.73	238,701	98.95	

Table 1 (continued)

Variables	Total		Sulfonylurea		Metformin		*P* value
	N	%	*N*	%	*N*	%	
Yes	2948	0.75	418	0.27	2530	1.05	
CKD							< 0.001
No	391,722	99.46	151,617	99.34	240,105	99.53	
Yes	2126	0.54	1000	0.66	1126	0.47	
Anxiety							< 0.001
No	359,222	91.21	141,796	92.91	217,426	90.13	
Yes	34,626	8.79	10,821	7.09	23,805	9.87	
Depression							< 0.001
No	391,867	99.50	152,103	99.66	239,764	99.39	
Yes	1981	0.50	514	0.34	1467	0.61	
Hypothyroidism							< 0.001
No	392,371	99.62	152,395	99.85	239,976	99.48	
Yes	1477	0.38	222	0.15	1255	0.52	
Hyperthyroidism							< 0.001
No	391,722	99.46	152,194	99.72	239,528	99.29	
Yes	2126	0.54	423	0.28	1703	0.71	
Sleep disturbance							< 0.001
No	316,530	80.37	125,396	82.16	191,134	79.23	
Yes	77,318	19.63	27,221	17.84	50,097	20.77	

CKD chronic kidney disease, *COPD* chronic obstructive pulmonary disease, *DCSI* Diabetes Complications Severity Index, *SD* standard deviation, *NTD* New Taiwan Dollar

of metformin may have had milder disease severity, potentially influencing their risk of DR. Conversely, those taking higher or longer cumulative doses of metformin might have experienced more severe DM, which could counteract the protective effects of metformin therapy on DR. However, as our data are sourced from Taiwan's NHIRD, representing a relatively homogeneous population, the findings may vary across different racial and ethnic groups. The mechanisms connecting a cumulative metformin dosage to the risk of DR remain unclear, necessitating well-designed large-scale studies to evaluate the potential benefits and risks of metformin therapy for DR in patients with DM.

Metformin was recently shown to protect against AMD. Metformin inhibited oxidative stress on human retinal pigment epithelium cells through stimulating the AMPK signaling pathway in an AMD mouse model [42]. The proposed geroprotective effects of metformin have been attributed to the antioxidant and anti-inflammatory effects that can protect the retinal pigment epithelium cells against the lesions of early AMD [43]. A large-scale study found that metformin use was associated with a decreased odds of AMD development, with a 42% reduction, but not with other medications [44]. A systematic review and meta-analysis found that treatment with metformin is not associated with a significant lower risk of AMD development [45]. These studies suggest that metformin may have a protective role in AMD development or progression in those who are at risk that is correlated with a decreased risk of AMD development.

In this study, we discovered that patients with DM and hypothyroidism are at an increased risk of DR. This finding is compatible with earlier studies that have shown an increased risk of sight-threatening DR in patients with DM and subclinical hypothyroidism [46] and a 2.13-fold higher risk of DR in patients with DM and subclinical hypothyroidism according to a meta-analysis [47]. Several studies also supported that thyroid function abnormalities are associated with diabetic microvascular complications [48, 49] and an animal study suggested that metformin treatment may suppress thyroid-stimulating hormone and increase the secretion of free triiodothyronine and free thyroxine [50]. The reduction in thyroid-stimulating hormone may explain the lower risk of nodular goiter [51] and thyroid cancer [52] associated with metformin use observed in earlier human studies conducted in Taiwan. Because low levels of free thyroid hormone may be an independent risk factor for microvascular complications in patients with euthyroidism and DM [53], the increased levels of thyroid hormones associated with metformin use might have attenuated the development of DR.

The major strength of the present study was the use of a population-based design. Study subjects were selected from the entire population of Taiwan and our sample was highly

Table 2 Five-year follow-up of incident diabetic retinopathy in patients with new-onset diabetes mellitus with metformin

Variables	Five-year follow-up of incident diabetic retinopathy						
	Events				Adjusted model		
	N	%	IR	*P*-value	HR	95% CI	*P*-value
Total	668	0.17	0.34				
cDDD of metformin use				< 0.011			
Sulfonylurea (ref.)	136	0.09	0.18		1		
DDD < 30	138	0.20	0.39		0.77	0.60–0.98	0.032
DDD 30–120	269	0.23	0.46		0.85	0.69–1.05	0.140
DDD 120–-240	101	0.22	0.43		0.90	0.69–1.17	0.424
DDD > 240	24	0.31	0.62		1.41	0.91–2.19	0.122
Intensity of metformin use				< 0.001			
Sulfonylurea (ref.)	136	0.09	0.18		1		
< 5	260	0.22	0.43		0.82	0.66–1.01	0.067
5–15	197	0.21	0.42		0.81	0.65–1.01	0.060
15–25	62	0.25	0.51		1.06	0.79–1.44	0.687
> 25	13	0.54	1.07		2.43	1.37–4.30	0.002
Sex				0.021			
Female (ref.)	350	0.19	0.37		1		
Male	318	0.16	0.31		0.84	0.72–0.98	0.025
Age (years)				< 0.001			
20–49 (ref.)	142	0.13	0.26		1		
50–54	92	0.15	0.29		1.10	0.84–1.43	0.498
55–59	113	0.18	0.36		1.25	0.97–1.60	0.082
66–64	124	0.24	0.49		1.64	1.28–2.09	< 0.001
≥ 65	197	0.18	0.36		1.55	1.23–1.94	< 0.001
Income level (NTD)				< 0.001			
≤ 21,000 (ref.)	204	0.10	0.20		1		
21,001–33,000	260	0.28	0.55		1.17	0.97–1.42	0.102
≥ 33,001	204	0.20	0.41		1.15	0.94–1.40	0.179
Urbanization				< 0.001			
Level 1 (ref.)	246	0.24	0.48		1		
Level 2	191	0.15	0.30		0.63	0.52–0.76	< 0.001
Level 3	99	0.15	0.30		0.61	0.49–0.77	< 0.001
Level 4	79	0.14	0.28		0.59	0.46–0.77	< 0.001
Level 5	16	0.18	0.37		0.79	0.48–1.32	0.375
Level 6	23	0.14	0.27		0.60	0.39–0.92	0.019
Level 7	14	0.09	0.18		0.38	0.22–0.66	< 0.001
DCSI score				0.069			
0 (ref.)	420	0.16	0.32		1		
1	152	0.20	0.40		1.20	0.99–1.45	0.055
≤ 2	96	0.16	0.33		0.93	0.74–1.17	0.523
Enrolled year[a]	–	–	–	–	1.70	1.62–1.78	< 0.001
Hyperlipidemia				0.181			
No (ref.)	524	0.17	0.33		1		
Yes	144	0.19	0.38		0.80	0.67–0.97	0.022
COPD				0.318			
No (ref.)	641	0.17	0.34		1		
Yes	27	0.14	0.28		0.92	0.62–1.35	0.661
Obesity				0.369			
No (ref.)	661	0.17	0.34		1		

Table 2 (continued)

Variables	Five-year follow-up of incident diabetic retinopathy						
	Events				Adjusted model		
	N	%	IR	*P*-value	HR	95% CI	*P*-value
Yes	7	0.24	0.48		1.31	0.62–2.77	0.477
CKD				0.749			
No (ref.)	665	0.17	0.34		1		
Yes	3	0.14	0.28		0.66	0.21–2.09	0.481
Anxiety				0.655			
No (ref.)	606	0.17	0.34		1		
Yes	62	0.18	0.36		1.04	0.79–1.36	0.793
Depression				0.046			
No (ref.)	661	0.17	0.34		1		
Yes	7	0.35	0.71		1.80	0.85–3.84	0.127
Hypothyroidism				< 0.001			
No (ref.)	659	0.17	0.34		1		
Yes	9	0.61	1.22		2.61	1.34–5.06	0.005
Hyperthyroidism				0.835			
No (ref.)	664	0.17	0.34		1		
Yes	4	0.19	0.38		0.97	0.36–2.60	0.955
Sleep disturbance				0.031			
No (ref.)	559	0.18	0.35		1		
Yes	109	0.14	0.28		0.70	0.57–0.87	< 0.001

cDDD cumulative defined daily dose, *CI* confidence interval, *CKD* chronic kidney disease, *COPD* chronic obstructive pulmonary disease, *DCSI* Diabetes Complications Severity Index, *DDD* defined daily dose, *HR* hazard ratio, *IR* incidence rate per thousand person-years, *NTD* New Taiwan Dollar, *ref.* reference

[a]As a continuous variable

representative and sufficient to mitigate selection bias. Additionally, long follow-up periods of 5 years were used after the initiation of metformin treatment, and our data yielded findings of sufficient statistical power regarding the relationship between metformin use and the risk of DR.

This study has several limitations. First, we were unable to collect data on the family history of DR among our patients. We were also unable to collect data on lifestyle-related characteristics that are pertinent to the risk of DR, such as smoking, alcohol consumption, glycated hemoglobin levels, body mass index, physical activity, personal history, and dietary habits, which are potential confounding factors. Poorly controlled blood sugar levels are strongly associated with the occurrence of DR and are also reflected in other complications. Because of the absence of data on patients' blood sugar control in the NHIRD, we used the DCSI to adjust for diabetes severity among patients. Higher DCSI scores indicate poorer blood sugar control, thereby minimizing potential bias in the study's findings. Second, the NHIRD did not have laboratory records including serum levels of B_{12} and homocysteine, which limits the current study. Third, we were uncertain of whether all patients had taken their prescribed antidiabetic medications. Because of the Taiwan National Health Insurance, physicians may prescribe antidiabetic medications to patients even if their symptoms are mild and do not yet require pharmacological intervention to lower blood sugar levels. While some patients may indeed have mild symptoms that can be managed through diet or exercise alone, we are unable to identify such patients, which is a study limitation. Fourth, our diagnoses of DR and other comorbidities were wholly dependent on *ICD-9-CM* and *ICD-10-CM* codes. Nonetheless, to verify the accuracy of diagnoses, the National Health Insurance Bureau of Taiwan randomly reviews patient charts and interviews patients. Fifth, other anti-diabetic medications (such as insulin, dipeptidyl peptidase 4 inhibitors, and sodium-glucose cotransporter-2 inhibitors) and other drugs (such as aspirin and fibrate) that may be associated with the risk of DR development, which were not included in the present study, may influence the outcomes of the research. Finally, this study is an epidemiological study. Although many risk factors of DR have been controlled to confirm the correlation between metformin use and the risk of developing DR, there are still many potential risk factors that cannot be included, hence this study's results cannot explain the causal relationship. Large randomized controlled prospective studies are needed for a complete assessment of the association between metformin treatment and the risk of DR.

5 Conclusions

Patients with DM who received a lower cumulative dosage of metformin showed beneficial effects that were associated with a lower risk of DR, while higher metformin use had a greater risk of DR. In these patients, advanced age, a high DCSI score, and the presence of hypothyroidism are comorbidities associated with an increased risk of DR. Furthermore, the severity degree of DR is also an important issue. It is necessary to conduct further research to investigate the long-term and higher cumulative dosage of metformin and DR severity.

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Declarations

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Conflicts of interest/competing interests Yu-Ching Li, Kuang-Hua Huang Yih Yang, Shuo-Yan Gau, Tung-Han Tsai, and Chien-Ying Lee have no conflicts of interest that are directly relevant to the content of this article.

Ethics approval This study was conducted in accordance with the Declaration of Helsinki. Data were obtained from the Longitudinal Health Insurance Database, which is maintained by the Health and Welfare Data Science Center and provides scrambled random identification numbers for insured patients to protect their privacy. The study protocol was approved by the Central Regional Research Ethics Committee of China Medical University, Taichung, Taiwan (approval no. CRREC-109-011).

Consent to participate To protect the privacy of the participants, all data were anonymized. Because the database contains only deidentified data, the requirement for informed consent was waived.

Consent for publication Not applicable.

Availability of data and material The database used to support the findings of this study was provided by the Health and Welfare Data Science Center, Ministry of Health and Welfare under license and so cannot be made freely available. Requests for access to these data should be made to the Health and Welfare Data Science Center (https://dep.mohw.gov.tw/dos/cp-5119-59201-113.html).

Code availability All statistical analyses in the present study were conducted using SAS version 9.4 (SAS Institute, Cary, NC, USA).

Authors' contributions All authors have participated in this study and have reviewed and agreed with the final manuscript. Y-CL: conceptualization, methodology, data curation, writing (original draft), writing (review and editing). K-HH: conceptualization, methodology, funding acquisition, writing (original draft), writing (review and editing). YY: methodology, data curation, formal analysis. S-YG: formal analysis. T-HT: formal analysis. C-YL: conceptualization, methodology, validation, funding acquisition, writing (original draft), writing (review and editing). All authors read and approved the final version.

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