Metformin reduces ovarian cancer risk in Taiwanese women with type 2 diabetes mellitus

Running title: Metformin and ovarian cancer

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Abstract

BACKGROUND: Whether metformin therapy affects ovarian cancer risk in Asian patients with type 2 diabetes mellitus (T2DM) has not been investigated.

METHODS: Data analysis was performed in 2014. The reimbursement databases of Taiwanese female patients with a new diagnosis of T2DM between 1998 and 2002 (*n*=479475) were retrieved from the National Health Insurance for follow-up of ovarian cancer until the end of 2009. Metformin was treated as a time-dependent variable; and of these patients, 286106 were never-users and 193369 were ever-users. A time-dependent approach was used to calculate ovarian cancer incidence and estimate hazard ratios by Cox regression for never-users (as referent group), ever-users, and subgroups of metformin exposure (tertiles of cumulative duration and cumulative dose).

RESULTS: During follow-up, 601 metformin ever-users and 2600 never-users developed ovarian cancer, representing an incidence of 49.4 and 146.4 per 100,000 person-years, respectively. The overall fully adjusted hazard ratio (95% confidence intervals) for ever- versus never-users was 0.658 (0.593-0.730). The fully adjusted hazard ratios for the first, second, and third tertiles of cumulative duration of metformin therapy were 1.169 (1.019-1.341), 0.761 (0.644-0.898) and 0.276 (0.225-0.340), respectively (*P*-trend <0.01); and 1.220 (1.067-1.395), 0.610

(0.513-0.725) and 0.305 (0.248-0.374), respectively (*P*-trend <0.01), for cumulative dose of metformin. In additional analyses, sulfonylureas but not the other antidiabetic drugs were associated with a reduced risk of ovarian cancer.

CONCLUSIONS: Metformin use is associated with a decreased risk of ovarian cancer.

Keywords: diabetes, epidemiology, metformin, ovarian cancer, Taiwan

Introduction

Ovarian cancer is more common in the white people than in Asian people [1,2]. It has a poor prognosis and high mortality, probably due to the late diagnosis with advanced disease [1,2]. Age and genetic factors are the two most well-established risk factors [2]. The mean age at diagnosis is approximately 60 years old, but patients with a hereditary tumor may be diagnosed 10 years earlier [2]. Although the average lifetime risk of ovarian cancer is low (<2%), women with a family history would have a lifetime risk of 4-5% if a single family member has the disease. The lifetime risk increases to 7% if two family members have the disease, and can be as high as 30% if a woman is a carrier of *BRCAI* or *BRCAII* mutation [2].

Increasing evidence suggests that metformin may exert anticancer effects in *in vitro* and animal studies via AMP-activated protein kinase (AMPK) dependent or independent pathways [3]. However, whether metformin may reduce the risk of ovarian cancer in humans has rarely been studied. There is only one case-control (1611 cases and 9170 controls) study aiming at specifically evaluating the risk of ovarian cancer associated with metformin by using the UK-based General Practice Research Database [4]. While comparing patients with metformin prescription of 1-9, 10-29 and ≥30 to patients with no prior use in the analysis including diabetes and non-diabetes patients, the adjusted odds ratios (95% confidence interval) were not significant: 0.97 (0.50-1.90),

0.61 (0.28-1.32) and 0.61 (0.30-1.25), respectively [4]. When analysis was restricted to patients with diabetes, there were only 85 cases and 480 controls, but the adjusted odds ratios (95% confidence interval) might be significant for the above comparisons: 0.59 (0.25-1.41), 0.38 (0.14-0.97) and 0.38 (0.15-0.94), respectively [4].

In a review article evaluating the risk of various types of cancer associated with metformin use, the investigators secondarily collected information from published studies not primarily looking at the metformin-associated risk of ovarian cancer [3]. The odds ratio (95% confidence interval) for ovarian cancer associated with metformin use derived from one observational study [5] and two randomized controlled trials [6] was 0.69 (0.43-1.10) and 0.32 (0.06-1.66), respectively. In another recent meta-analysis including one observational study [4] and two clinical trials [6], the pooled odds ratio (95% confidence interval) was 0.67 (0.44-1.04) [7]. Therefore, although the risk of ovarian cancer associated with metformin use seems to be reduced, the estimated odds ratios are mostly not significant and findings are inconclusive.

Because no information is available for the Asian populations, the purpose of the present study was to evaluate whether metformin use in the Taiwanese women with type 2 diabetes mellitus (T2DM) would affect the risk of ovarian cancer. Specifically, the reimbursement databases of the National Health Insurance (NHI) were used and a new-user design and time-dependent approach for metformin use in data analyses were

applied in order to minimize the potential "prevalent user bias" [8,9] and "immortal time bias" [10,11]. Additionally, because a previous Hong Kong study showed that the use of sulfonylureas in the Chinese population with T2DM was associated with a lower risk of cancer and an interaction with metformin could be observed [12], the present study also evaluated the potential interaction between metformin and sulfonylureas on ovarian cancer risk. On the other hand, because a growing number of patients with T2DM are using insulin, the interaction between metformin and insulin was also evaluated.

Materials and Methods

The planned analysis of the reimbursement databases of all patients with a diagnosis of T2DM during the period from 1996 to 2009 was approved by the ethic review board of the National Health Research Institutes (registered approval number: 99274). Data analysis was performed in 2014.

Since March 1995 a compulsory and universal system of NHI was implemented in Taiwan. According to this system, all contracted medical institutes must submit computerized and standard claim documents for reimbursement. More than 99% of citizens are enrolled in the NHI, and over 98% of the hospitals nationwide are under contract with the NHI. The databases contain detailed records of every visit for each patient, including outpatient visits, emergency department visits, and hospital

admission. The identification information of the individuals was scrambled for the protection of privacy. Diabetes was coded as 250.XX and ovarian cancer as 183, based on the *International Classification of Diseases, Ninth Revision, Clinical Modification* (ICD-9-CM).

Figure 1 shows the flowchart for the procedures in selecting participants into the study, and these are further explained as follows. To create a cohort of female patients newly diagnosed with T2DM within the period of 1998-2002, male patients and patients with a diagnosis of diabetes at outpatient clinics during 1996 and 1997 in the available NHI databases were first excluded. The data of all female patients across the country who were newly diagnosed with diabetes and/or were under treatment with either oral antidiabetic drugs or insulin at outpatient clinics during the period of 1998-2002 were recruited (*n*=495033). After excluding patients with type 1 diabetes (*n*=1189), those with a diagnosis of ovarian cancer before the diagnosis of diabetes (*n*=1038), those with a duplicated identification number (*n*=35), unclear information on date of birth or sex (*n*=7366), or a follow-up duration of less than 6 months (*n*=11095), a total of 479475 patients with a diagnosis of newly-onset T2DM during 1998-2002 were identified.

Patients who had ever been prescribed metformin after entry were defined as ever-users (*n*=193369, 40.3%); never-users (*n*=286106, 59.7%) were defined as those

who had never been prescribed metformin. Cumulative duration (months) and cumulative dose (mg) of metformin use were calculated from the reimbursement databases. To evaluate a potential dose-response relationship between metformin and ovarian cancer, tertiles of cumulative metformin duration and dose were used for analyses. Exposure to other oral antidiabetic drugs (sulfonylurea, acarbose, pioglitazone, and rosiglitazone) and insulin was also similarly defined.

A number of comorbidities and covariates were determined as a status/diagnosis at the time of entry. These have been described in detail previously [13-17] and included nephropathy, hypertension, chronic obstructive pulmonary disease (a surrogate for smoking), stroke, ischemic heart disease, peripheral arterial disease, eye disease, obesity, dyslipidemia, urinary tract disease, and other cancers. Other medications included statins, fibrate, angiotensin-converting enzyme inhibitor and/or angiotensin receptor blocker, calcium channel blocker, aspirin, ticlopidine, clopidogrel, dipyridamole and non-steroidal anti-inflammatory drugs.

Follow-up started on the first day of diabetes diagnosis and ended on 31 December 2009, at the time of a new diagnosis of ovarian cancer, or at the date of the last reimbursement record. Exposure to metformin was treated as a time-dependent variable. Therefore, the metformin ever-users contributed person-years to the non-metformin group until they started using metformin, and after starting metformin,

to the metformin group.

The baseline characteristics of metformin never-users and ever-users were compared by Chi-square test and by Student's t test for age. The crude incidence density of ovarian cancer was calculated for metformin ever-users and never-users and for the different exposure subgroups. The numerator for the incidence was the number of patients with incident ovarian cancer during follow-up, and the denominator was the person-years of follow-up. Time-dependent Cox proportional hazards regression was performed to estimate the hazard ratios for ovarian cancer among metformin ever-users versus never-users, and for the various dose-response parameter subgroups. The following models were created: 1) adjusted for age; and 2) adjusted for all variables compared previously as baseline characteristics between ever-users and never-users (fully adjusted). Age was adjusted for as a continuous variable in the models.

For sensitivity analyses, traditional Cox regression models were created to evaluate 1) whether the association between metformin use and ovarian cancer risk could be consistently demonstrated; and whether the use of other antidiabetic drugs could also be associated with ovarian cancer (Model I); and 2) the potential interactions between metformin and sulfonylureas/insulin on ovarian cancer risk (Model II: metformin and sulfonylureas; Model III: metformin and insulin; and Model IV: metformin and sulfonylureas plus insulin). To create the above models, patients

with incident diabetes during 1998-2005 were recruited. An entry date was set on 1 January 2006 and patients with a prevalent diagnosis of ovarian cancer before this date were excluded. The remaining patients were then followed up for four years until 31 December 2009. All baseline characteristics plus diabetes duration at entry were included in the Cox model. For Model I, fully adjusted hazard ratios for each of the antidiabetic drugs were estimated. For Models II, III and IV, fully adjusted hazard ratios were estimated for the following four subgroups of patients, together with the interaction term between the use of metformin and another category of other antidiabetic drugs (i.e., sulfonylureas, insulin or sulfonylureas plus insulin, respectively): 1) patients not using metformin and not using sulfonylureas/insulin as the referent group, 2) patients using metformin but not using sulfonylureas/insulin, 3) patients not using metformin but using sulfonylureas/insulin, and 4) patients using metformin and the other category of antidiabetic drugs. Two-sided *P*-value for the interaction between metformin and sulfonylureas/insulin was assessed with the Wald test of the cross-product term in the model that included the two separate variables of metformin and sulfonylureas/insulin together with their cross-product term.

Analyses were conducted using SAS statistical software, version 9.3 (SAS Institute, Cary, NC). $P<0.05$ was considered statistically significant.

Results

Table 1 summarizes the baseline characteristics between metformin ever-users (*n*=193369) and never-users (*n*=286106). With the exception of rates of ticlopidine and clopidogrel use, all baseline characteristics differed significantly between the two groups.

Table 2 lists ovarian cancer incidence in metformin ever-users and never-users, and among the different tertiles of the dose-response parameters. The incidences of ovarian cancer in metformin ever-users and never-users were 49.4 and 146.4 per 100,000 person-years, respectively. With longer cumulative duration and higher cumulative dose, the incidence decreased correspondingly.

Table 3 shows the age-adjusted and fully adjusted hazard ratios for ovarian cancer with regards to metformin exposure by the time-dependent approach. For the overall hazard ratios comparing ever-users versus never-users, there was a significantly lower risk of ovarian cancer associated with metformin use in either the age-adjusted or the fully adjusted models. In the age-adjusted models of the dose-response analyses, metformin use was associated with a lower risk in all categories of exposure and with significant *P*-trends. In the fully adjusted models, although a significantly reducing risk was observed with increasing cumulative duration and cumulative dose, the first tertiles of the dose-response parameters showed a significantly higher risk associated

with metformin use.

The hazard ratios derived from the traditional Cox regression models are shown in Table 4. In Model I, metformin and sulfonylureas were associated with a reduced risk of ovarian cancer with hazard ratio (95% confidence interval) of 0.546 (0.474-0.628) and 0.556 (0.484-0.639), respectively; but the other antidiabetic drugs were not significantly associated with ovarian cancer. In the analysis for the interaction between metformin and sulfonylureas/insulin in Models II, III and IV, all subgroups of users had a significantly lower risk of ovarian cancer while compared to the first subgroup (non-users of metformin and the other category of antidiabetic drugs). The *P*-values for the interaction terms were all significant ($P<0.01$).

Discussion

The present study is the first to show an inverse association between metformin use and ovarian cancer risk in female patients with T2DM in an Asian population. The dose-response relationship was well demonstrated using the parameters of cumulative duration and cumulative dose (Tables 2 and 3), and the reduced risk of ovarian cancer associated with metformin use was consistently shown in the sensitivity analyses (Table 4).

Age and family history are two well-established risk factors for ovarian cancer [2]. In the present study, age has been adjusted for in either the age-adjusted models or the

fully adjusted models (Tables 3 and 4). However, because of the lack of information, it was not possible to know whether the results could be confounded by family history or genetic parameters. It is deemed that such a confounding effect might be minimal because approximately 90% of the ovarian cancer cases are sporadic [18].

A recent study strongly indicated that obesity is an important risk factor for ovarian cancer in the Chinese women [19]. The adjusted hazard ratios (95% confidence interval) were 1.49 (1.05-2.13) and 2.42 (1.37-4.28) for a body mass index of 25.0-29.9 and ≥30 kg/m^2, respectively, while compared to a reference of 18.5-24.9 kg/m^2 [19]. A slightly but significantly higher risk in the first tertiles of the cumulative duration and cumulative dose of metformin in the fully adjusted models in the present study (Table 3) could be resulted from a residual confounding by obesity, because metformin is always prescribed to patients with obesity and users in the present study did show a higher prevalence of obesity diagnosis (Table 1). In clinical practice, we do not usually label a patient with the diagnosis of obesity unless he or she is rather obese (probably when the body mass index is >30 kg/m^2). Therefore, patients who used metformin with less obesity would not be labeled with a diagnosis of obesity, rendering a chance of residual confounding by obesity associated with metformin use. If such a residual confounding did exist, the estimated hazard ratios associated with metformin use (Table 3) would have been underestimated.

Metformin may show anticancer effects in various cancer cell types including the breast, glial cells, stomach, colon and pancreas [20]. With regards to ovarian cancer, metformin upegulates AMPK activity via liver kinase B1 (LKB1), resulting in an apoptotic effects on and reduced growth of ovarian cancer cells [21,22]. LKB1 activation may also cause cell cycle arrest of ovarian cancer cells independent of AMPK activity [21], probably through the inhibition of Bcl-2 expression [23]. Metformin may also exert an antiangiogenic effect on ovarian cancer in an *in vitro* and *in vivo* study [24].

The finding of a lower risk of ovarian cancer associated with the use of sulfonylureas (Model I, Table 4) might be contradictory to the general concept of a higher risk of cancer associated with sulfonylureas. While searching the literature, it was noted that some previous *in vitro* studies have demonstrated an anticancer effect of sulfonylureas on ovarian cancer. Yasukagawa et al. recently reported that glibenclamide may suppress cellular invasion in ovarian cancer cells by inhibiting the secretion of platelet-derived growth factor through its blockade of the ATP-sensitive K^+ channels [25]. El-Deeb et al. evaluated the antitumor effects of some novel cyclic arylsulfonylureas and has shown in a report in 2010 that the tested compounds may have a good inhibitory effect on the ovarian cancer cell line IGROV1 [26]. This finding was compatible with an earlier study by Taylor et al. published in 1992 [27].

Therefore, the findings of a lower risk of ovarian cancer associated with metformin and sulfonylureas in the present study (Tables 3 and 4) and the inhibitory effects of sulfonylureas on ovarian cancer cell growth in previous *in vitro* studies [26,27] provide a good rationale for future exploration on the combined use of metformin and sulfonylureas for the prevention and treatment of ovarian cancer.

Studies on the effect of insulin use and ovarian cancer risk are still rare. Theoretically, insulin has the potential to cause ovarian cancer via the stimulation of insulin-like growth factor 1 (IGF-1) receptor [28] or through its effects on the regulation of other hormones [29]. However, earlier studies did not confirm that the use of insulin for diabetes treatment [29] or serum C-peptide level [30] was associated with an increased risk of ovarian cancer. Recent studies also did not find that the level of IGF-1 was predictive for ovarian cancer [31]. On the contrary, a higher level of IGF-1 in pregnancy was associated with a lower risk of ovarian cancer [32] and patients with ovarian cancer and higher level of IGF-1 might have better prognosis [33]. The findings of the present study suggested that neither insulin use (Model I, Table 4) nor its combination with metformin and sulfonylureas (Model IV, Table 4) increases the risk of ovarian cancer. Taken together, the findings on ovarian cancer risk related to the use of sulfonylureas and insulin in the present study (Table 4) were consistent with what had been primarily reported in studies by other investigators.

These somewhat relieved the concern of a potential risk of ovarian cancer related to sulfonylureas and/or insulin therapy in patients with T2DM.

This study has several strengths. The databases included all claim records on outpatient visits, emergency department visits, and hospital admission. We derived the diagnoses of ovarian cancer from all these sources. Cancer is considered a severe morbidity by the NHI and most medical co-payments can be waived. Furthermore, there is a low drug cost-sharing required by the NHI and patients from a low-income household, veterans or patients with prescription refills for chronic disease are exempted from the drug cost-sharing. Therefore, the detection rate of ovarian cancer would tend not to differ among different social classes. The use of medical records also reduced the potential bias related to self-reporting.

The study limitations included a lack of actual measurement data for confounders such as family history, lifestyle, diet, and genetic parameters. In addition, we could not consider the potential impact of biochemical data such as levels of glucose, insulin, C-peptide and IGFs. Another limitation is the lack of information on the pathology, grading, and staging of ovarian cancer. Finally it is worth to stress that the interpretations of the findings of the present study should be cautious because it is an observational study and bias or residual confounding may not be completely excluded.

In summary, the present study suggests that metformin use is associated with a

decreased risk of ovarian cancer in female patients with T2DM.

Author Contributions

C.H. researched data and wrote manuscript.

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Conflict of interest: None

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Table 1. Baseline characteristics between never-users and ever-users of metformin

Variables	Metformin				*P*
	Never-users		Ever-users		
	n	%	*n*	%	
n=479475	286106		193369		
Age (years)*	54.95	16.67	56.55	12.28	<0.0001
Hypertension	34707	12.13	34656	17.92	<0.0001
Chronic obstructive pulmonary disease	7666	2.68	3799	1.96	<0.0001
Stroke	8093	2.83	4524	2.34	<0.0001
Nephropathy	12912	4.51	3385	1.75	<0.0001
Ischemic heart disease	8614	3.01	6123	3.17	0.0022
Peripheral arterial disease	3434	1.20	2166	1.12	0.0113
Eye disease	750	0.26	696	0.36	<0.0001
Obesity	1066	0.37	1096	0.57	<0.0001
Dyslipidemia	42157	14.73	23894	12.36	<0.0001
Urinary tract disease	14456	5.05	11090	5.74	<0.0001
Other cancers	18439	6.44	7935	4.10	<0.0001
Statin	2695	0.94	3832	1.98	<0.0001
Fibrate	2393	0.84	5522	2.86	<0.0001
Angiotensin converting enzyme inhibitor/angiotensin receptor blocker	10577	3.70	15054	7.79	<0.0001
Calcium channel blocker	11884	4.15	12520	6.47	<0.0001
Sulfonylurea	32137	11.23	174344	90.16	<0.0001
Insulin	5386	1.88	44525	23.03	<0.0001
Acarbose	3381	1.18	57219	29.59	<0.0001
Pioglitazone	969	0.34	36003	18.62	<0.0001
Rosiglitazone	1377	0.48	38777	20.05	<0.0001
Aspirin	6166	2.16	6529	3.38	<0.0001
Ticlopidine	319	0.11	197	0.10	0.3190
Clopidogrel	25	0.01	19	0.01	0.6997
Dipyridamole	6023	2.11	5184	2.68	<0.0001
Non-steroidal anti-inflammatory drugs (excluding aspirin)	50833	17.77	33102	17.12	<0.0001

*Age is expressed as mean under the column of "*n*" and standard deviation under the column of "%".

Table 2. Incidence of ovarian cancer by metformin exposure

Metformin use	Case number observed	Incident cases of ovarian cancer	Person-years	Incidence rate (per 100,000 person-years)
Never-users	446824	2600	1775476.4	146.4
Ever-users	193369	601	1216103.5	49.4
Cumulative duration (months)				
Never-users	446824	2600	1775476.4	146.4
<14.7	63707	326	278005.8	117.4
14.7-46.4	63900	176	384548.3	45.8
>46.4	65762	99	553549.5	17.9
Cumulative dose (mg)				
Never-users	446824	2600	1775476.4	146.4
<407,000	63789	338	277637.6	121.7
407,000-1,532,000	63825	160	389208.5	41.1
>1,532,000	65755	103	549257.4	18.8

Table 3. Metformin exposure and hazard ratios for ovarian cancer using time-dependent approach

Metformin use	Age-adjusted			Fully adjusted*		
	HR	95% CI	*P*	HR	95% CI	*P*
Ever-users	0.341	(0.310-0.377)	<0.01	0.658	(0.593-0.730)	<0.01
Cumulative duration (months)						
<14.7	0.872	(0.762-0.998)	<0.05	1.169	(1.019-1.341)	<0.01
14.7-46.4	0.397	(0.338-0.466)	<0.01	0.761	(0.644-0.898)	<0.01
>46.4	0.128	(0.104-0.156)	<0.01	0.276	(0.225-0.340)	<0.01
P-trend			<0.01			<0.01
Cumulative dose (mg)						
<407,000	0.942	(0.825-1.076)	0.38	1.220	(1.067-1.395)	<0.01
407,000-1,532,000	0.354	(0.299-0.420)	<0.01	0.610	(0.513-0.725)	<0.01
>1,532,000	0.131	(0.108-0.160)	<0.01	0.305	(0.248-0.374)	<0.01
P-trend			<0.01			<0.01

Referent group: never-users of metformin; HR: hazard ratio, CI: confidence intervals

*Adjusted for all variables in Table 1

Table 4. Traditional Cox regression models evaluating the effects of various antidiabetic drugs and the interaction between metformin and sulfonylureas/insulin on ovarian cancer risk

Model/antidiabetic drug		Case number		Hazard ratio (95% confidence interval)	*P*
Model I					
Variable	Interpretation	*n/N* for users	*n/N* for non-users		
Metformin	Yes vs. no	747 / 397512	1512 / 328856	0.546 (0.474-0.628)	<0.01
Sulfonylureas	Yes vs. no	767 / 429517	1492 / 296851	0.556 (0.484-0.639)	<0.01
Insulin	Yes vs. no	179 / 99865	2080 / 626503	0.950 (0.796-1.134)	0.57
Acarbose	Yes vs. no	176 / 97259	2083 / 629109	1.003 (0.843-1.194)	0.97
Pioglitazone	Yes vs. no	46 / 30783	2213 / 695585	0.814 (0.600-1.104)	0.19
Rosiglitazone	Yes vs. no	148 / 92614	2111 / 633754	0.848 (0.702-1.026)	0.09
		n	*N*		
Model II					
Metformin	Sulfonylureas				
No	No	1399	259290	1.000	
Yes	No	93	37561	0.408 (0.329-0.507)	<0.01
No	Yes	113	69566	0.435 (0.356-0.530)	<0.01
Yes	Yes	654	359951	0.313 (0.274-0.358)	<0.01
				P-interaction <0.01	
Model III					
Metformin	Insulin				
No	No	1497	321031	1.000	
Yes	No	583	305472	0.527 (0.457-0.608)	<0.01
No	Yes	15	7825	0.504 (0.301-0.843)	<0.01
Yes	Yes	164	92040	0.557 (0.445-0.696)	<0.01
				P-interaction <0.01	
Model IV					
Metformin	Sulfonylureas plus insulin				
No	No	1507	324798	1.000	
Yes	No	588	307698	0.387 (0.344-0.436)	<0.01
No	Yes	5	4058	0.372 (0.154-0.900)	<0.05
Yes	Yes	159	89814	0.410 (0.333-0.505)	<0.01
				P-interaction <0.01	

n = case number of ovarian cancer

N = case number followed

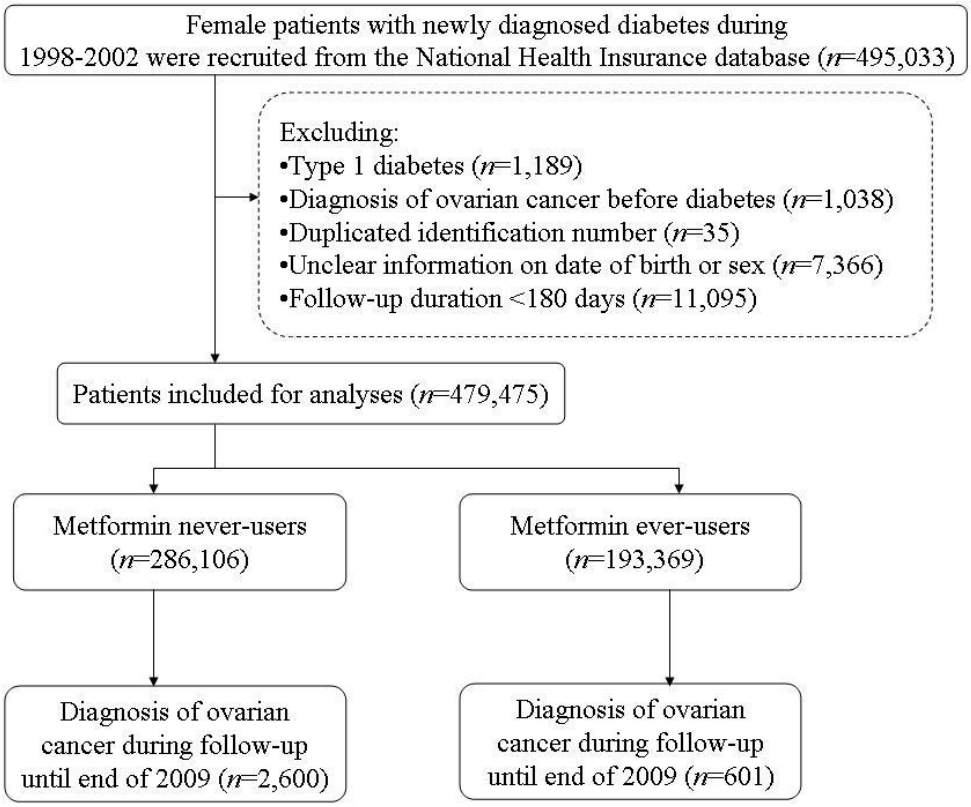

Figure 1. Flowchart for selection of participants into the study