Metformin Attenuates Osteoporosis in Diabetic Patients with Carcinoma in Situ: A Nationwide, Retrospective, Matched-Cohort Study in Taiwan

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Abstract: Patients with diabetes are at increased risk of cancer development and osteoporosis. Metformin is an effective agent for diabetes management. Epidemiological studies have identified an association between metformin use and cancer prevention. This article outlines the potential for metformin to attenuate the rate of osteoporosis in diabetic patients with carcinoma in situ (CIS). From the National Health Insurance Research Database of Taiwan, 7827 patients with diabetes with CIS who were receiving metformin therapy were selected, along with 23,481 patients as 1:3 sex-, age- and index year-matched controls, who were not receiving metformin therapy. A Cox proportional hazard analysis was used to compare the rate of osteoporosis during an average of 15-year follow-up. Of the subjects who were enrolled, 801 (2.56%) had osteoporosis, including 168 from the metformin group (2.15%) and 633 from the without metformin group (2.70%). The metformin group presented a lower rate of osteoporosis at the end of follow-up ($p = 0.009$). The Cox proportional hazard regression analysis revealed a lower rate of osteoporosis for the metformin group (adjusted hazard ratio of 0.820; 95% confidence interval = 0.691–0.972, $p = 0.022$). Diabetic patients with CIS under metformin therapy presented lower osteoporosis rate than those who were not receiving metformin therapy.

Keywords: osteoporosis; diabetes; metformin; carcinoma in situ; National Health Insurance Research Database
1. Introduction

Patients with diabetes are at increased risk of cancer development [1] and associated with an increased risk of fragility fractures compared to the general population [2]. Type 2 diabetes mellitus (T2DM) and cancer share many risk factors, such as age, obesity, diet and physical inactivity [3]. The possible mechanisms for a direct link between T2DM and cancer include hyperinsulinemia [4], hyperglycemia [5] and inflammation [6,7].

Diabetes and cancer are clinical risk factors used for fragility fracture probability assessments across the general population [8,9]. Osteoporosis is a skeletal disorder that is characterized by low bone mass and compromised bone strength [10]. T2DM may affect bone metabolism and leads to osteoporosis [11] and cancer may affect both, through the direct effects of cancer cells on the skeleton and the deleterious effects of cancer-specific therapies on bone cells [9].

Metformin is an effective agent for T2DM management [12]. Furthermore, epidemiological studies have identified an association between metformin use and cancer prevention [13]. Fractures are a clinically important consequence of osteoporosis and result not only in disabilities, but also in excess mortality; however, the pathogenic mechanisms that underlie the relationship between osteoporosis, diabetes and cancer remain incompletely understood. The aim of this study was to determine the potential for metformin to attenuate the rate of osteoporosis in diabetic patients with carcinoma in situ using data from the Taiwan National Health Insurance Research Database (NHIRD), which is a nationwide health insurance database.

2. Materials and Methods

2.1. Data Sources

Our study used data from the NHIRD to investigate whether metformin therapy in diabetic patients with carcinoma in situ could lower osteoporosis rates, compared to a group of individuals who were not receiving metformin, over a 15-year period, from the outpatient Longitudinal Health Insurance Database (LHID) in Taiwan (2000–2015). The National Health Insurance (NHI) Program was launched in Taiwan in 1995, and as of June 2009, it included contracts with 97% of the medical providers in Taiwan, with approximately 23 million beneficiaries or more than 99% of the entire Taiwan population [14]. The NHIRD uses International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9-CM) codes to record diagnoses [15]. All diagnoses of T2DM, carcinoma in situ, and osteoporosis were made by a board-certified medical specialist. The Bureau of NHI randomly reviews the records of 1 in 100 ambulatory care visits and 1 in 20 in-patient claims, to verify the accuracy of the diagnoses [16]. Several studies have demonstrated the accuracy and validity of the diagnoses in the NHIRD [17,18].

2.2. Study Design and Sampled Participants

Our study was a retrospective matched-cohort design. Patients with diagnosed T2DM, carcinoma in situ and osteoporosis were selected from 1 January 2000 to 31 December 2015, according to ICD-9-CM 230.XX-234.XX (carcinoma in situ), ICD-9-CM 733.XX (osteoporosis) and ICD-9-CM 250.XX (T2DM). Furthermore, each enrolled patient was required to have made at least three outpatient visits within the study period, according to these ICD-9-CM codes whether or not they were receiving metformin therapy. Patients with osteoporosis diagnoses before 2000 and those less than 18 years of age were excluded. Furthermore, patients with diabetes who received thiazolidinedione or canagliflozin therapy were also excluded.

The covariates included Charlson comorbidity index (CCI), T2DM, sex, age, geographical area of residence (north, center, south and east of Taiwan) and urbanization level of residence (level 1 to 4). The urbanization level of residence was defined according to the population and various indicators of the level of development. Level 1 was defined as a population >1,250,000 and a specific economic, cultural, metropolitan and political development designation. Level 2 was defined as a population...
between 500,000 and 1,249,999 that played an important role in the political system, culture and economy. Urbanization levels 3 and 4 were defined as either populations between 149,999 and 499,999 or <149,999, respectively [19].

2.3. Outcome Measures

All of the study participants were followed from the index date until the onset of osteoporosis, from the NHI program, before the end of 2015.

2.4. Statistical Analysis

All statistical analyses were performed using SPSS software version 22 for Windows (SPSS, Inc., Chicago, IL, USA). chi-squared and t-tests were used to evaluate the distributions of categorical and continuous variables, respectively. A regression analysis of multivariate Cox proportional hazards was used to determine the risk of osteoporosis under metformin therapy in patients with diabetes and carcinoma in situ. The statistical analyses were presented as hazard ratio (HR) with ninety-five percent confidence interval (CI). The difference in the risk of osteoporosis for patients with diabetes and carcinoma in situ, with or without metformin therapy, was estimated using the log rank test—Kaplan-Meier method. A two-tailed test p-value less than 0.05 was considered to indicate statistical significance.

2.5. Ethics

Our study was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). The Institutional Review Board of Tri-Service General Hospital (TSGH) approved our study and waived the need for individual written informed consent (TSGH IRB No.2-105-05-082).

3. Results

Of the 61,307 enrolled patients, 11,151 were excluded and 50,156 patients with cancer and T2DM were included. Furthermore, 9030 of the included patients were receiving metformin therapy, of which 1203 patients had used metformin for less than 90 days and were excluded and 7827 were enrolled (case group). For the control group, we enrolled 41,126 individuals who were not receiving metformin therapy to represent the 1:3 sex-, age- and index year-matched control group and excluded 17,645 patients used metformin less than 90 days, for a final count of 23,481 subjects (control group) (Figure 1). Overall, the diabetic patients with carcinoma in situ under metformin therapy presented a lower rate of osteoporosis than those who were not receiving metformin (adjusted HR 0.820 (95% CI = 0.691–0.972, p = 0.022). Figure 2 shows the Kaplan-Meier analysis for the cumulative risk of osteoporosis in patient and control groups and the difference is statistically significant (log rank, p = 0.017).
Figure 1. Flowchart of study patient selection from the National Health Insurance Research Database in Taiwan. DM—Diabetes mellitus: ICD-9-CM 250; Carcinoma in situ: ICD-9-CM 230–234; Osteoporosis: ICD-9-CM 733.0; Metformin: ≥90 days.

Figure 2. Kaplan-Meier analysis of cumulative risk of osteoporosis among patients with diabetes mellitus with carcinoma in situ aged 18 and over, stratified by metformin use and analyzed using a log-rank test.
The baseline sex, age, comorbidities, location, urbanization, level of care and income of the study subjects and controls is presented in Table 1. Of the 31,308 adult diabetic patients with carcinoma in situ, 13,284 (42.43%) were male, 12,928 (41.29%) were ≥60 year of age and the mean age was 55.95 ± 14.28 years (Table 1). There were no significant differences between the metformin and control groups in age distribution, sex, comorbidities and covariates, after propensity-score matching.

Table 1. Characteristics of study in the baseline.

| Variables                  | Total    | With     | Without  | p       |
|----------------------------|----------|----------|----------|---------|
| Gender                     |          |          |          | 0.999   |
| Male                       | 13,284   | 3321     | 9963     |         |
| Female                     | 18,024   | 3232     | 13,518   |         |
| Age (years)                | 55.95 ± 14.28 | 55.91 ± 14.36 | 55.96 ± 14.25 | 0.759   |
| Age groups (years)         |          |          |          | 0.999   |
| 18–49                      | 11,960   | 2990     | 8970     |         |
| 50–59                      | 6420     | 1605     | 4815     |         |
| ≥60                        | 12,928   | 3232     | 9696     |         |
| Low-income                 |          |          |          | 0.952   |
| Without                    | 30,998   | 7749     | 23,249   |         |
| With                       | 310      | 78       | 232      |         |
| Catastrophic Illness       |          |          |          | 0.206   |
| Without                    | 25,374   | 6382     | 18,992   |         |
| With                       | 5934     | 1445     | 4489     |         |
| Marital status             |          |          |          | 0.073   |
| Without                    | 12,841   | 3278     | 9563     |         |
| With                       | 18,467   | 4549     | 13,918   |         |
| Education (years)          |          |          |          | 0.683   |
| <12                        | 13,692   | 3407     | 10,285   |         |
| ≥12                        | 17,616   | 4420     | 13,196   |         |
| CCI_R                      | 0.61 ± 1.75 | 0.60 ± 1.70 | 0.61 ± 1.77 | 0.641   |
| Season                     |          |          |          | 0.866   |
| Spring (Mar–May)           | 8070     | 2037     | 6033     |         |
| Summer (Jun–Aug)           | 8425     | 2101     | 6324     |         |
| Autumn (Sep–Nov)           | 7896     | 1982     | 5914     |         |
| Winter (Dec–Feb)           | 6917     | 1707     | 5210     |         |
| Location                   |          |          |          | 0.659   |
| Northern Taiwan            | 13,289   | 3335     | 9954     |         |
| Middle Taiwan              | 8492     | 2150     | 6342     |         |
| Southern Taiwan            | 8227     | 2017     | 6210     |         |
| Eastern Taiwan             | 1253     | 315      | 938      |         |
| Outlets islands            | 47       | 10       | 37       |         |
| Urbanization level         |          |          |          | 0.877   |
| 1 (Highest)                | 12,423   | 3115     | 9308     |         |
| 2                          | 14,419   | 3603     | 10,816   |         |
| 3                          | 1346     | 324      | 1022     |         |
| 4 (Lowest)                 | 3120     | 785      | 2335     |         |
| Level of care              |          |          |          | 0.083   |
| Hospital center            | 16,949   | 4319     | 12,630   |         |
| Regional hospital          | 10,975   | 2668     | 8307     |         |
| Local hospital             | 3384     | 840      | 2544     |         |

P—chi-squared/Fisher’s exact test on category variables and t-test on continue variables.
At the end of the follow-up period, 801 enrolled subjects (2.56%) had osteoporosis, including 168 from the metformin group (2.15%) and 633 from the without metformin group (2.70%), as shown in Table 2. The metformin group was associated with a lower rate of osteoporosis at the end of the follow-up ($p = 0.009$). There were no significant differences between the metformin and control groups in age distribution, sex, comorbidities and covariates at the end of the follow-up period.

Table 2. Characteristics of study in the endpoint.

| Variables                     | Total        | Metformin With | Metformin Without | $p$   |
|-------------------------------|--------------|----------------|-------------------|-------|
|                               | $n$          | %              | $n$              | %    | $n$ | %    |
| Total                         | 31,308       | -              | 7827              | 25.00| 23,481 | 75.00| -    |
| Osteoporosis                  |              |                |                   |      |      |      | 0.009|
| Without                       | 30,507       | 97.44          | 7659              | 97.85| 22,848 | 97.30| -    |
| With                          | 801          | 2.56           | 168               | 2.15 | 633    | 2.70 | -    |
| Gender                        |              |                |                   |      |      |      | 0.999|
| Male                          | 13,284       | 42.43          | 3321              | 42.43| 9963   | 42.43| -    |
| Female                        | 18,024       | 57.57          | 4506              | 57.57| 13,518 | 57.57| -    |
| Age (yrs)                     | 61.37 ± 15.55| 61.32 ± 15.60 | 61.39 ± 15.54     | 0.707|
| Age groups (yrs)              |              |                |                   |      |      |      | 0.393|
| 18–49                         | 8106         | 25.89          | 2061              | 26.33| 6045   | 25.74| -    |
| 50–59                         | 6647         | 21.23          | 1625              | 20.76| 5022   | 21.39| -    |
| ≥60                           | 16,555       | 52.88          | 4141              | 52.91| 12,414 | 52.87| -    |
| Low-income                    |              |                |                   |      |      |      | 0.336|
| Without                       | 30,936       | 98.81          | 7726              | 98.71| 23,210 | 98.85| -    |
| With                          | 372          | 1.19           | 101               | 1.29 | 271    | 1.15 | -    |
| Catastrophic Illness          |              |                |                   |      |      |      | 0.652|
| Without                       | 22,517       | 71.92          | 5647              | 72.15| 16,870 | 71.85| -    |
| With                          | 8791         | 28.08          | 2180              | 27.85| 6611   | 28.15| -    |
| Marital status                |              |                |                   |      |      |      | 0.058|
| Without                       | 12,846       | 41.03          | 3283              | 41.94| 9563   | 40.73| -    |
| With                          | 18,462       | 58.97          | 4544              | 58.06| 13,918 | 59.27| -    |
| Education (years)             |              |                |                   |      |      |      | 0.659|
| <12                           | 13,687       | 43.72          | 3405              | 43.50| 10,282 | 43.79| -    |
| ≥12                           | 17,621       | 56.28          | 4422              | 56.50| 13,199 | 56.21| -    |
| CCI_R                         | 1.96 ± 3.70  | 1.99 ± 3.76    | 1.95 ± 3.68       | 0.320|
| Season                        |              |                |                   |      |      |      | 0.486|
| Spring                        | 7236         | 23.11          | 1782              | 22.77| 5454   | 23.23| -    |
| Summer                        | 7995         | 25.54          | 2017              | 25.77| 5978   | 25.46| -    |
| Autumn                        | 8780         | 28.04          | 2232              | 28.52| 6548   | 27.89| -    |
| Winter                        | 7297         | 23.31          | 1796              | 22.95| 5501   | 23.43| -    |
| Location                      |              |                |                   |      |      |      | 0.499|
| Northern Taiwan               | 12,321       | 39.35          | 3067              | 39.18| 9254   | 39.41| -    |
| Middle Taiwan                 | 9222         | 29.46          | 2340              | 29.90| 6882   | 29.31| -    |
| Southern Taiwan               | 8108         | 25.90          | 1993              | 25.46| 6115   | 26.04| -    |
| Eastern Taiwan                | 1548         | 4.94           | 399               | 5.10 | 1149   | 4.89 | -    |
| Outer islands                 | 109          | 0.35           | 28                | 0.36 | 81     | 0.34 | -    |
| Urbanization level            |              |                |                   |      |      |      | 0.727|
| 1 (Highest)                   | 10,135       | 32.37          | 2522              | 32.22| 7613   | 32.42| -    |
| 2                             | 14,118       | 45.09          | 3502              | 44.74| 10,616 | 45.21| -    |
| 3                             | 2131         | 6.81           | 537               | 6.86 | 1594   | 6.79 | -    |
| 4 (Lowest)                    | 4924         | 15.73          | 1266              | 16.17| 3658   | 15.58| -    |
| Level of care                 |              |                |                   |      |      |      | 0.590|
| Hospital center               | 12,771       | 40.79          | 3156              | 40.32| 9615   | 40.95| -    |
| Regional hospital             | 13,322       | 42.55          | 3340              | 42.67| 9982   | 42.51| -    |
| Local hospital                | 5215         | 16.66          | 1331              | 17.01| 3884   | 16.54| -    |

*$p$—chi-squared/Fisher’s exact test on category variables and $t$-test on continue variables.
The results of the Cox regression analysis of the factors associated with osteoporosis are shown in Table 3. The Cox proportional hazard regression analysis showed a lower rate of osteoporosis for patients receiving metformin therapy (adjusted hazard ratio of 0.820; 95% confidence interval = 0.691–0.972, \( p = 0.022 \)).

### Table 3. Factors of osteoporosis by using Cox regression.

| Variables                  | Crude HR | 95% CI       | 95% CI       | \( p \) | Adjusted HR | 95% CI       | 95% CI       | \( p \) |
|----------------------------|----------|--------------|--------------|--------|-------------|--------------|--------------|--------|
| Metformin                  |          |              |              |        |             |              |              |        |
| Without                    | 1        | 0.813        | 0.686        | 0.964  | 0.017       | 0.820        | 0.691        | 0.972  | 0.022 |
| With                       | 0.813    | 0.686        | 0.964        | 0.017  | 0.820       | 0.691        | 0.972        | 0.022  |       |
| Gender                     |          |              |              |        |             |              |              |        |
| Male                       | 0.662    | 0.497        | 0.883        | 0.005  | 0.576       | 0.431        | 0.770        | <0.001 |
| Female                     | 1        |              |              |        |             |              |              |        |
| Age groups (yrs)           |          |              |              |        |             |              |              |        |
| 18–49                      | 1        |              |              |        |             |              |              |        |
| 50–59                      | 2.827    | 1.648        | 4.851        | <0.001 | 3.113       | 1.813        | 5.345        | <0.001 |
| ≥60                        | 13.133   | 8.114        | 21.257       | <0.001 | 15.456      | 9.533        | 25.059       | <0.001 |
| Low-income                 |          |              |              |        |             |              |              |        |
| Without                    | 1        |              |              |        |             |              |              |        |
| With                       | 1.083    | 0.490        | 1.973        | 0.961  | 1.505       | 0.747        | 3.030        | 0.253  |
| Catastrophic Illness       |          |              |              |        |             |              |              |        |
| Without                    | 1        |              |              |        |             |              |              |        |
| With                       | 0.521    | 0.426        | 0.638        | <0.001 | 1.030       | 0.883        | 1.214        | 0.166  |
| Marital status             |          |              |              |        |             |              |              |        |
| Without                    | 1        |              |              |        |             |              |              |        |
| With                       | 1.234    | 0.724        | 2.013        | 0.306  | 1.305       | 0.896        | 2.284        | 0.299  |
| Education (years)          |          |              |              |        |             |              |              |        |
| <12                        | 1        |              |              |        |             |              |              |        |
| ≥12                        | 0.903    | 0.512        | 1.894        | 0.376  | 0.865       | 0.483        | 1.881        | 0.425  |
| CCI_R                      | 0.892    | 0.860        | 0.925        | <0.001 | 0.880       | 0.840        | 0.921        | <0.001 |
| Season                     |          |              |              |        |             |              |              |        |
| Spring                     | 1        |              |              |        |             |              |              |        |
| Summer                     | 0.946    | 0.778        | 1.150        | 0.576  | 0.993       | 0.817        | 1.208        | 0.946  |
| Autumn                     | 0.785    | 0.644        | 0.957        | 0.017  | 0.769       | 0.653        | 0.971        | 0.024  |
| Winter                     | 1.008    | 0.827        | 1.229        | 0.938  | 1.013       | 0.831        | 1.235        | 0.898  |
| Location                   |          |              |              |        |             |              |              |        |
| Northern Taiwan            | 1        |              |              |        |             |              |              |        |
| Middle Taiwan              | 1.318    | 1.111        | 1.563        | 0.002  |             |              |              |        |
| Southern Taiwan            | 1.151    | 0.958        | 1.383        | 0.113  | Multicollinearity with urbanization level |
| Eastern Taiwan             | 1.675    | 1.274        | 2.204        | <0.001 |             |              |              |        |
| Islands outer of Taiwan    | 1.584    | 1.290        | 1.993        | <0.001 |             |              |              |        |
| Urbanization level         |          |              |              |        |             |              |              |        |
| 1 (Highest)                | 0.525    | 0.374        | 0.736        | <0.001 | 0.560       | 0.420        | 0.827        | 0.002  |
| 2                          | 0.707    | 0.591        | 0.846        | <0.001 | 0.856       | 0.706        | 1.038        | 0.113  |
| 3                          | 0.725    | 0.598        | 0.878        | 0.001  | 0.912       | 0.733        | 1.135        | 0.410  |
| 4 (Lowest)                 | 1        |              |              |        |             |              |              |        |
| Level of care              |          |              |              |        |             |              |              |        |
| Hospital center            | 0.646    | 0.536        | 0.778        | <0.001 | 0.712       | 0.594        | 0.853        | <0.001 |
| Regional hospital          | 0.670    | 0.560        | 0.800        | <0.001 | 0.761       | 0.615        | 0.942        | 0.012  |
| Local hospital             | 1        |              |              |        |             |              |              |        |

HR—hazard ratio; CI—confidence interval; Adjusted HR—adjusted variables listed in table.

For the subgroups in which osteoporosis factors were stratified by the variables listed in Table 4, the Cox regression analysis showed that the male, age < 60 years, better income, without catastrophic illness, unmarried, longer education, live in the higher urbanization level and care in hospital center were associated with a much lower osteoporosis rate.
Table 4. Factors of osteoporosis stratified by variables listed in table by using Cox regression.

| Metformin With vs. Without (Reference) | Stratified Events | PYS | Rate (per 10^5 PYS) | Events | PYS | Rate (per 10^5 PYS) | Ratio | Adjusted HR | 95% CI | 95% CI | p |
|----------------------------------------|------------------|-----|---------------------|--------|-----|---------------------|-------|-------------|-------|-------|-----|
| Total                                  | 168              | 83,045.71 | 202.30            | 633    | 254,028.18 | 249.18            | 0.812 | 0.820       | 0.691 | 0.972 | 0.022 |
| Gender                                 | -                | -                | -                | -                | -                | -                | -    | -           | -     | -     | -    |
| Male                                   | 8                | 24,313.43      | 32.90             | 42     | 72,902.21  | 57.61             | 0.571 | 0.572       | 0.483 | 0.689 | 0.007 |
| Female                                 | 160              | 58,732.28      | 272.42            | 591    | 181,125.97 | 326.29            | 0.835 | 0.843       | 0.710 | 1.006 | 0.054 |
| Age groups (yrs)                       | -                | -                | -                | -                | -                | -                | -    | -           | -     | -     | -    |
| 18–49                                  | 1                | 16,823.43      | 5.94              | 16     | 48,608.18  | 32.92             | 0.181 | 0.180       | 0.150 | 0.218 | <0.001 |
| <0.001 50–59                           | 7                | 18,252.93      | 38.35             | 52     | 56,989.26  | 91.25             | 0.420 | 0.422       | 0.352 | 0.513 | <0.001 |
| ≥60                                     | 160              | 47,969.35      | 333.55            | 565    | 148,430.74 | 380.65            | 0.876 | 0.886       | 0.746 | 1.072 | 0.241 |
| Low-income                             | -                | -                | -                | -                | -                | -                | -    | -           | -     | -     | -    |
| Without                                | 166              | 82,125.32      | 202.13            | 626    | 251,334.43 | 249.07            | 0.812 | 0.818       | 0.682 | 0.954 | 0.019 |
| With                                   | 2                | 920.40         | 217.30            | 7      | 2693.75    | 259.86            | 0.836 | 0.845       | 0.703 | 0.998 | 0.047 |
| Catastrophic Illness                   | -                | -                | -                | -                | -                | -                | -    | -           | -     | -     | -    |
| Without                                | 148              | 63,571.83      | 232.81            | 562    | 192,669.45 | 291.69            | 0.798 | 0.806       | 0.672 | 0.958 | 0.020 |
| With                                   | 20               | 19,473.89      | 102.70            | 71     | 61,358.72  | 115.71            | 0.888 | 0.899       | 0.758 | 1.094 | 0.182 |
| Marital status                         | -                | -                | -                | -                | -                | -                | -    | -           | -     | -     | -    |
| Without                                | 70               | 38,033.11      | 184.05            | 295    | 120,005.45 | 245.82            | 0.749 | 0.754       | 0.632 | 0.891 | <0.001 |
| With                                   | 98               | 45,012.60      | 217.72            | 338    | 134,022.73 | 252.20            | 0.863 | 0.870       | 0.731 | 0.984 | 0.022 |
| Education (years)                      | -                | -                | -                | -                | -                | -                | -    | -           | -     | -     | -    |
| <12                                    | 88               | 38,948.45      | 225.94            | 320    | 122,940.53 | 260.29            | 0.868 | 0.867       | 0.721 | 0.979 | 0.023 |
| ≥12                                    | 80               | 44,097.26      | 181.42            | 313    | 131,087.65 | 238.77            | 0.760 | 0.765       | 0.634 | 0.910 | <0.001 |
| Season                                 | -                | -                | -                | -                | -                | -                | -    | -           | -     | -     | -    |
| Spring                                 | 38               | 18,725.29      | 202.93            | 155    | 57,375.11  | 270.15            | 0.751 | 0.759       | 0.632 | 0.902 | 0.001 |
| Summer                                 | 39               | 20,940.50      | 186.24            | 165    | 65,150.71  | 253.26            | 0.735 | 0.743       | 0.621 | 0.880 | <0.001 |
| Autumn                                 | 40               | 25,007.28      | 159.95            | 162    | 72,600.25  | 223.14            | 0.717 | 0.724       | 0.608 | 0.863 | <0.001 |
| Winter                                 | 51               | 18,372.65      | 277.59            | 151    | 58,902.11  | 256.36            | 1.083 | 1.094       | 0.924 | 1.286 | 0.388 |
Table 4. Cont.

| Stratified        | Events | PYs       | Rate (per 10^5 PYs) | Events | PYs       | Rate (per 10^5 PYs) | Ratio | Adjusted HR | 95% CI  | 95% CI  | p     |
|-------------------|--------|-----------|---------------------|--------|-----------|---------------------|-------|--------------|---------|---------|-------|
| Urbanization      | -      | -         | -                   | -      | -         | -                   | -     | -            | -       | -       | -     |
| level             |        |           |                     |        |           |                     |       |              |         |         |       |
| 1 (Highest)       | 42     | 24,936.20 | 168.43              | 182    | 79,857.63 | 227.91              | 0.739 | 0.742        | 0.611   | 0.897   | <0.001|
| 2                 | 66     | 37,254.28 | 177.16              | 269    | 112,534.77| 239.04              | 0.741 | 0.749        | 0.620   | 0.903   | 0.002 |
| 3                 | 11     | 5966.48   | 184.36              | 36     | 18,479.15 | 194.81              | 0.946 | 0.953        | 0.798   | 1.138   | 0.265 |
| 4 (Lowest)        | 49     | 14,888.76 | 329.11              | 146    | 43,156.63 | 338.30              | 0.973 | 0.981        | 0.824   | 1.206   | 0.402 |
| Hospital center   | 54     | 31,037.93 | 173.98              | 214    | 97,437.34 | 219.63              | 0.792 | 0.799        | 0.635   | 0.950   | 0.009 |
| Regional hospital | 71     | 37,481.36 | 189.43              | 270    | 114,617.84| 235.57              | 0.804 | 0.812        | 0.682   | 0.964   | 0.018 |
| Local hospital    | 43     | 14,526.42 | 296.01              | 149    | 41,973.00 | 354.99              | 0.834 | 0.843        | 0.710   | 0.997   | 0.047 |

PYs—person-years; Adjusted HR—adjusted hazard ratio, adjusted for the variables listed in Table 3; CI—confidence interval.
4. Discussion

We found that diabetic patients with carcinoma in situ under metformin therapy presented lower osteoporosis rates than those who were not receiving metformin. The overall adjusted HR was 0.820 ($p = 0.022$), even after adjusting for comorbidities and other covariates. The Kaplan-Meier analysis revealed that the study subjects had a significantly lower 15-year risk of osteoporosis than the controls. This study is the first to indicate that diabetic patients with carcinoma in situ under metformin therapy have a lower osteoporosis risk in a nationwide, population-based study.

Prior research has found that patients with diabetes have a relatively high risk of fracture [20], and one systematic review that showed that patients with diabetes have up to a three-fold greater fracture risk than the average person, with hip fracture being the largest [21]. Cancer is a major risk factor for osteoporosis, which is a common bone disease characterized by reduced bone mass and increased risk of fracture. Furthermore, these factors are associated both with the direct effects of cancer cells on the skeleton and the deleterious effects of cancer-specific therapies on bone cells [9]. One review article showed that certain key factors, osteoprotegerin (OPG)/ receptor activator of NF-κB ligand (RANKL)/receptor activator of NF-κB (RANK), underlie the molecular mechanism of osteoclastogenesis [22], and the anti-human RANKL monoclonal antibody has been successfully applied to the treatment of osteoporosis and cancer-related bone disorders [23,24].

Metformin is the preferred initial pharmacologic medicine for T2DM treatment, unless there are contraindications [25]. First-line metformin therapy has beneficial effects on HbA1C and weight [26] and may reduce the risk of cardiovascular events and death [27]. Patients with diabetes who received thiazolidinedione or canagliflozin therapy were excluded, due to the potential risk of osteoporosis [28]. Some studies have reported that older age, diabetes, and cancer under chemotherapy were associated with increased fracture risk, which was associated with elevated bone resorption [29,30].

Will metformin treatment reduce the incidence of osteoporosis in diabetic patients with carcinoma in situ? No study has investigated this in the past, and therefore, we used a cohort investigation to evaluate this issue. We found that the diabetic patients with carcinoma in situ under metformin therapy presented lower osteoporosis rates than those who were not receiving metformin. Evans and other scholars first proposed that metformin could reduce the cancer risk of patients with diabetes through epidemiological studies [31], in accordance with our results.

The impact of metformin on osteoporosis has been studied in the past. In vitro data on metformin suggest a protective effect on bones [32] and that metformin improves bone quality and decreases the risk of fractures in patients with diabetes, in addition to improving glycemic control and insulin sensitivity [33]. Recent studies have shown that metformin can be osteogenic in vitro through the activation of AMP-activated protein kinase (AMPK), which results in osteoblastic cells differentiation, bone matrix synthesis and osteoblasts proliferation [34,35]. An in vivo study in mice showed that metformin enhances osteoblast proliferation and inhibits osteoclast differentiation to attenuate cancellous bone loss [36]. Furthermore, molecular research has found that metformin reduces RANKL and stimulates OPG expression in osteoblasts, which further inhibits osteoclast differentiation and prevents bone loss in ovariectomized rats [37]. Furthermore, metformin has been found to promote the proliferation of murine preosteoblasts by regulating the AMPK-mechanistic target of rapamycin 2 (mTOR2) and the AKT-mTORC1 signaling axis [38]. Additionally, metformin promotes the proliferation and differentiation of murine preosteoblasts by regulating the expression of sirtuin 6 (sirt6) and oct4 [39].

We therefore hypothesized that diabetic patients with carcinoma in situ who are on metformin therapy may present attenuated cancellous bone loss through metformin regulation of AMPK signaling in preosteoblasts, which reduces RANKL and stimulates OPG expression. Hence, metformin could be associated with reduced osteoporosis. Our findings are similar to many studies that have shown that females and older patients have a greater risk of osteoporosis. Clinical factors could be associated with those projected by demographic changes, with regards to age- and sex-specific risks [40]. Otherwise, the reasons why the subgroups that lived in higher urbanized areas and received therapy in hospital centers showed lower rates of osteoporosis are unknown and warrant further studies.
The present study has a few limitations. First, our study lacks analyses of disease duration, disease severity and patient parameters, such as body weight, BMI and waistline. Hip fracture risk is increased in patients with diabetes, whereas BMD is increased in patients with T2DM [41]. While the pathophysiological mechanism is only partially understood, a common complication may explain the increased fracture risk, whereas BMI may ameliorate the increased fracture risk in patients with T2DM [42]. Finally, a longer follow-up period may be necessary to clarify the osteoporosis risk for diabetic patients with carcinoma in situ.

In conclusion, diabetic patients with carcinoma in situ under metformin therapy presented a lower osteoporosis rate than those who were not receiving metformin therapy, and this effect may be attributed to the decreased levels of proinflammatory factors and the potential for metformin to modulate molecular pathways involved in cancer cell signaling and metabolism.

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