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Denosumab improves glycaemic parameters in postmenopausal osteoporosis patients with combined Type 2 diabetes mellitus

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ABSTRACT

This study aimed to determine whether RANKL inhibitors in postmenopausal osteoporosis patients with combined type 2 diabetes mellitus (T2DM) could improve their glucose metabolism index. First of all, 84 patients affected with postmenopausal osteoporosis with combined T2DM attending the Department of Endocrinology at the Third Hospital of Hebei Medical University were selected and randomized into two groups of 42 patients each. One group was given Denosumab 60 mg once every six months (denosumab group, D.G.), and the other group was given 2 mg ibandronate once every three months (ibandronate group, I.G.). Blood glucose parameters were compared before and after treatment in both groups and serum active GLP-1 levels and DPP-4 levels were also assessed. After treatment, there was no significant difference in fasting glucose between the two groups, but there was a significant decrease in fasting glucose in the Denosumab Group (D.G.) compared to before treatment. There was a significant difference in 2-hour postprandial glucose (2hPG) between the two groups after treatment, with the D.G. being lower than the ibandronate group (I.G.). Glycosylated haemoglobin (HbA1c) was lower in the D.G. than in the I.G. after treatment, but the difference between them was insignificant. In the D.G., serum active GLP-1 levels increased after treatment, and serum DPP-4 levels decreased. Serum GLP-1 and DPP-4 levels in the I.G. did not change compared with those before treatment. In conclusion, In the clinical management of postmenopausal osteoporosis patients with combined T2DM, the choice of RANKL inhibitors as anti-osteoporosis therapy may benefit their glycaemic parameters by elevating serum active GLP-1 levels and decreasing serum DPP-4 levels.

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Introduction

With the development of industrialization, people's living conditions have significantly improved, and chronic noncommunicable diseases have become an essential component of medical expenditures in many countries. Type 2 diabetes mellitus (T2DM) and osteoporosis, common chronic noncommunicable diseases, account for many patients in many hospital endocrinology departments. It is projected that by 2021, there will be approximately 536.6 million adults aged 20-79 years with diabetes worldwide, and global diabetes-related healthcare spending is estimated at $966 billion. The number of patients is expected to rise to 783.2 million by 2045 (1), in addition to the fact that approximately half of the people with diabetes are undiagnosed. The situation for osteoporosis is also not optimistic, and its prevalence is increasing yearly. In 2020, the worldwide prevalence of osteoporosis was 18.3%, among which the prevalence in women was 23.1% (2). It has been suggested that 1 in 2 postmenopausal women suffer from osteoporosis or experience at least one osteoporotic fracture in their lifetime (3).

The large population in China, coupled with the fact that T2DM patients account for the majority of all diabetic patients, has led to a very high absolute number of people with both T2DM and osteoporosis. Meanwhile, T2DM and osteoporosis have many connections in pathophysiological mechanisms and clinical practice. A member of the tumor necrosis factor superfamily, receptor activator of nuclear factor-κB ligand (RANKL), was found to bind to myeloid cells and act as a critical factor in activating and promoting osteoblast differentiation. At the same time, RANKL plays an essential role in glucose metabolism, and related studies have shown that RANKL can reduce muscle strength, decrease insulin sensitivity (4) and increase energy expenditure (5) by inducing the differentiation of preadipocytes to beige adipocytes in mice. In clinical practice, studies have shown that T2DM is an independent risk factor for an elevated risk of osteoporotic fractures (6), and this elevated fracture risk is associated with T2DM causing osteoporosis-related fractures through multiple factors (7). Studies have shown that in patients with T2DM, 41.3% have combined bone loss and 9.2% have combined osteoporosis, while the risk of hip fracture incidence in patients with T2DM is 1.7 times higher than that in nondiabetic patients (8). Therefore, for patients with T2DM combined with osteoporosis, clinical treatment may be potentially beneficial and have twice the effect with half the effort if the intrinsic connection between the two is fully considered and treatment is planned in an integrated manner.

RANKL inhibitors are fully humanized IG2 monoclonal antibodies to RANKL. In 2010, a RANKL inhibitor, Denosumab, was approved for marketing by the European Union and the U.S. Food and Drug Administration. After decades of research and clinical practice, the anti-osteoporotic effects of Denosumab are well established.

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In addition, bisphosphonates, such as zoledronic acid, alendronate, and ibandronate, are also commonly used in the treatment of osteoporosis. Therefore, for patients with T2DM combined with osteoporosis, choosing which anti-osteoporosis drug is more appropriate is a question that clinicians should consider.

Osteoporosis and T2DM interact, and controlling one is essential to controlling the other (9). RANKL signals the inflammatory NF-κB (nuclear factor κB) pathway, mainly from macrophages that infiltrate bone, pancreas, liver and muscle (10). RANKL also has an essential role in glucose metabolism. It can worsen muscle strength and insulin sensitivity by inducing beige adipocyte differentiation of preadipocytes in mouse models (11) and increase energy expenditure (12). A prospective population-based study in Italy confirmed that subjects diagnosed with T2DM showed higher serum concentrations of soluble RANKL at baseline than subjects who did not develop diabetes during a 15-year follow-up period (13). RANKL can induce insulin resistance in the liver by promoting inflammation (14). In the pancreas, RANKL elevates blood glucose by decreasing insulin production and increasing glucagon production (15), which may lead to pancreatic B-cell dysfunction (16). Kiechl et al. (Year). also confirmed the association of RANKL with impaired glucose tolerance (IGT) (17). In addition, research has shown that insulin resistance typical of T2DM also occurs in bone tissue (18). Megan M. Weivoda et al. (2023). identified DPP-4 (dipeptidyl peptidase-4) as an osteoclast-derived factor that may be associated with bone reconstruction and RANKL signalling energy metabolism (15). Therefore, we speculate that anti-osteoporosis drugs based on the RANKL pathway may have a role in glucose metabolism. For this reason, we designed this study to investigate whether Denosumab could improve glucose metabolism in osteoporosis patients with combined T2DM and investigate the possible mechanisms. Currently, in a retrospective cohort study in Denmark on the treatment of patients with diabetes combined with osteoporotic fractures, we found that denosumab has demonstrated superior therapeutic effects compared to alendronate (19). This initially establishes the value of denosumab for future clinical applications.

However, some researchers have disagreed that Denosumab can improve glycemia because some researchers believe that studies investigating RANKL inhibitors and diabetes cannot be considered qualified studies for various reasons (20). In addition, the concentration of RANKL was not consistent among individuals, and age and sex were both influential factors. For example, RANKL is positively correlated with age and OPG.RANKL is negatively correlated with age (21); healthy men have higher RANKL concentrations than healthy women, while the opposite is true for OPG (22). Meanwhile, none of the above studies included the Chinese population. Therefore, based on the above, we designed the present trial to investigate whether Denosumab could improve glycaemic-related indicators in postmenopausal osteoporosis patients with combined T2DM.

Materials and Methods

Research subjects

In this randomized controlled trial, we initially selected 90 subjects who attended the Third Hospital of Hebei Medical University from June 2021 to December 2021. They were all patients with T2DM combined with postmenopausal osteoporosis, and the subjects were randomized into the Denosumab and ibandronate groups (D.G. and I.G.) in a 1:1 ratio. Among them, two in the D.G. and one in the I.G. withdrew from the study on their own, and two in the I.G. and one in the D.G. were not reviewed in time, resulting in the inclusion of 84 subjects. Prior to the study, all patients were interviewed, clinically evaluated, and signed written informed consent after learning more about the trial process. Our study was conducted following the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee. Patients in the D.G. received Denosumab 60 mg/6 months, and the I.G. received ibandronate 2 mg/3 months. Anti-diabetic drugs were not adjusted, but patients using GLP-1 receptor agonists and DPP-4 inhibitor class drugs were omitted. Diabetes education (including diet and physical activity) was provided to patients for the six months of the trial. The flow and grouping of the study are detailed in Fig. 1.

Inclusion and exclusion criteria

The inclusion criteria were as follows: 1. postmenopausal women aged 45 to 90 years; 2. diagnoses of T2DM (1999 WHO diagnostic criteria) and osteoporosis (WHO diagnostic criteria); 3. glycosylated haemoglobin (HbA1c) between 6.0% and 8.0%; 4. blood glucose value not exceeding 13.9 mmol/L; and 5. body mass index (BMI) 18.00-34.00 kg/m^2. The exclusion criteria were as follows: 1. secondary osteoporosis; pituitary disease; post-hysterectomy and oophorectomy; organ failures such as heart, liver and kidney failure; tumours; uncontrolled thyroid and parathyroid disease; and any other diseases that may affect bone metabolism; 2. long-term use of glucocorticoids, birth control pills, adefovir or any medications that may affect bone metabolism; 3. women of childbearing age and men; 4. severe cognitive dysfunction, severe mental illness, etc.; 5. treatment with GLP-1 receptor agonists or DPP-4 inhibitor class drugs; and 6. inability or unwillingness to participate in this trial.

Methods

This randomized, open-label, parallel-design study included a Six-month treatment cycle. All participants were randomly assigned (by computer-generated random order) 1:1 to one of two treatment groups, I.G. (ibandronate was obtained from the Biomedical Engineering Centre of Hebei Medical University) or D.G. (Denosumab was obtained from Amgen, USA). All participants received the same diabetes education (including diet and exercise

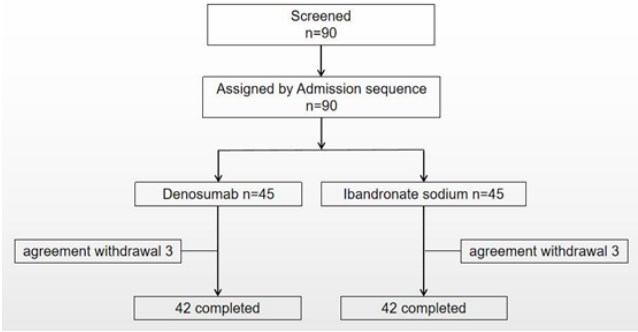

Figure 1. The grouping process of the study subjects.

instruction), and the diabetes medication regimen was not adjusted; patients using GLP-1 agonists or DPP-4 inhibitor class drugs were excluded. All participants were given anti-osteoporosis treatment according to the subgroup after assessing indications and excluding contraindications. Two participants in the I.G. developed fever, and one developed abdominal discomfort, which resolved independently without particular intervention. One participant in the D.G. developed muscle pain, which resolved without particular intervention. The remaining participants did not experience any adverse reactions, and no severe reactions occurred in either group.

Data collection

Sex, age, duration of diabetes, history of smoking and alcohol consumption, comorbidities, and medication history were assessed. Anthropometric measurements (height and weight) were performed. After 10 minutes of rest, the subject's blood pressure was measured twice in a seated position. BMI was calculated by dividing weight (kg) by the square of height (m^2). Serum and plasma were centrifuged (1000 g, 4 °C, 15 min) within 30 min after collection and stored at -80 °C. Fasting blood glucose (FBG), 2-hour postprandial blood glucose (2hBG), HbA1c, insulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood creatinine, blood urea nitrogen, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, 25-hydroxyvitamin D, parathyroid hormone, β-CTX, PINP, and thyroid function were measured by enzymatic assay in the laboratory of the Third Hospital of Hebei Medical University. Serum-active GLP-1 and DPP-4 levels were measured by enzyme-linked immunosorbent assay (ELISA) kits. BMD before and after treatment was measured with dual-energy X-ray absorptiometry (Horizon DXA system, manufacturer: Hological Inc., USA, Model: Discovery A) at the lumbar spine and both sides of the hip.

Outcome measures

The leading indicators in this study were changes in FBG, 2hBG, and HbA1c after six months of anti-osteoporosis drug treatment. Secondary indicators included serum GLP-1 and DPP-4 levels, measured at baseline and after three and six months of treatment, except for 2hBG, measured under fasting conditions.

Statistical analysis

Statistical analysis of the data counted or measured in the study was performed using SPSS 26.0 software (IBM USA). The distribution of the variables was analysed using the Kolmogorov–Smirnov normality test, with customarily distributed and continuous variables reported as the mean ± S.D. and nonnormally distributed variables reported as the median (interquartile range). Categorical variables are reported as proportions. Differences between the two groups were compared using independent t-tests for normally distributed variables and Mann–Whitney U tests for nonnormally distributed variables. Paired t-tests or Wilcoxon rank sum tests were performed for changes in the same group before and after the intervention. The chi-square or Fisher's exact test was used to analyse differences in proportions. P values less than 0.05 were considered statistically significant (two-tailed).

Results

Comparison of clinical baseline data

There was no statistically significant difference between the two groups in terms of clinical baseline data such as age, sex, and blood pressure (*P>0.05*), confirming comparability between both groups (Table 1).

Comparison of changes in BMD

Before treatment, there was no statistically significant difference in total BMD of the lumbar spine, femoral neck or hip joint between the two groups (*P>0.05*). After six months of treatment, the total BMD of the lumbar spine in the DG was 0.766 ± 0.109 g/cm^2, the total BMD of the femoral neck was 0.554 ± 0.086 g/cm^2, and the total BMD of the hip was 0.681 ± 0.115 g/cm^2. The total BMD of the lumbar spine in the I.G. was 0.795 ± 0.110 g/cm^2, the total

Table 1. Comparison of clinical baseline data.

	DG (n=45)	IG (n=45)	t/χ^2	P
Age	68.96±8.46	66.33±11.41	1.242	0.218
Diabetes duration (years)	9.77±7.81	9.42±7.53	0.216	0.829
Blood pressure (mmHg)				
SBP	139.74±12.13	140.38±9.64	0.277	0.782
DBP	86.64±10.18	85.43±7.58	0.640	0.524
Weight (kg)	65.25±8.48	67.62±13.60	0.992	0.324
BMI (kg/m2)	24.97±2.62	25.30±3.57	0.500	0.618
Smoking habit (yes/no)	4/38	2/40	0.718	0.397
menopause age (year)	50.57 (49.07,52.07)	49.90 (48.90,50.90)	1.542	0.086
ALT (U/L)	18.40(12.90,23.90)	20.62 (13.12,28.12)	1.062	0.345
AST (U/L)	19.43 (15.43,22.93)	21.29 (16.79,28.79)	0.694	0.451
TG (mmol/L)	1.29 (0.97,1.61)	1.32 (0.97,1.68)	0.225	0.677
TC (mmol/L)	4.84±1.06	4.48±0.83	1.794	0.076
HDL-c (mmol/L)	1.51±0.36	1.43±0.32	1.114	0.268
LDL-c (mmol/L)	2.76±0.73	2.52±0.61	1.692	0.094
PTH (pg/ml)	39.90±21.38	41.00±17.32	0.268	0.789
25-hydroxyvitamin D (ng/mL)	20.43±8.16	19.40±5.52	0.701	0.485

BMD of the femoral neck was 0.581 ± 0.077 g/cm^2, and the total BMD of the hip joint was 0.680 ± 0.091 g/cm^2. BMD increased in both groups compared to that before treatment (*P<0.05*), but there was still no significant difference between them (*P>0.05*) (Fig. 2).

Comparison of changes in bone metabolism

Before treatment, the differences in β-CTX and PINP between the two groups were not statistically significant (*P>0.05*). Six months after treatment, β-CTX and PINP were lower in both groups than before treatment (*P<0.05*). Both β-CTX and PINP at six months after treatment were lower in the D.G. than in the I.G. (*P<0.05*) (Fig. 3).

Comparison of changes in glycaemic parameters

Before treatment, FBG, 2hBG, and HbA1c were not different between the two groups (*P>0.05*). At three months and six months after treatment, FBG, 2hBG, and HbA1c were lower in the D.G. than before treatment (*P>0.05*) and lower than those in the I.G. (*P<0.05*). FBG, 2hBG, and HbA1c did not change dramatically at 3 and 6 months after treatment in the I.G. compared with the values before treatment (*P>0.05*) (Fig. 4).

Comparison of changes in GLP-1 and DPP-4 levels

Before treatment, no difference was seen in the GLP-1 and DPP-4 levels between the two groups (*P>0.05*), while GLP-1 was higher in the D.G. and DPP-4 was lower in the I.G. at three months and six months after treatment (*P<0.05*). After treatment, GLP-1 was consistently increased, and DPP-4 was decreased in the D.G. (*P<0.05*), whereas no changes in GLP-1 and DPP-4 levels, were observed in the I.G. (*P>0.05*) (Fig. 5).

Comparison of incidence of adverse reactions

No adverse effects, such as hypoglycaemia and hyperglycaemic crisis, were observed in either diabetes treatment group. On anti-osteoporosis treatment, two cases of fever and one case of abdominal discomfort were observed in the D.G., and one participant in the I.G. experienced muscle pain. No severe adverse reactions were observed in either group, with no significant differences in the incidence of adverse reactions between the two groups (*P>0.05*) (Table 2).

Discussion

This study reviews the application of Denosumab for six months in patients with T2DM combined with osteoporosis. This study has the advantage of being a randomized controlled trial in a completely realistic clinical setting. Additionally, we conducted this trial in a way that the various conditions were essentially the same in both groups. We also selected a previously uninvolved Chinese population to evaluate the effects of Denosumab on gly-

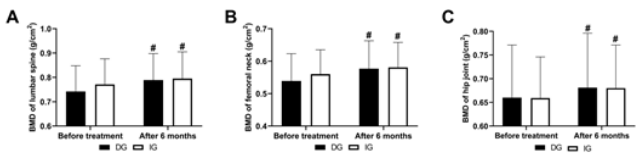

Figure 2. Comparison of BMD changes. (A) Comparison of total BMD of lumbar spine before and after treatment. (B) Comparison of total BMD of the femoral neck before and after treatment. (C) Comparison of total BMD of the hip joint before and after treatment. Note: # indicates a statistically significant difference compared to before treatment (*P<0.05*).

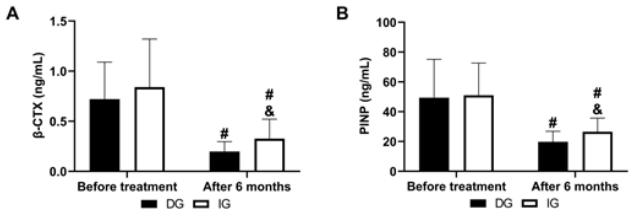

Figure 3. Comparison of changes in bone metabolism. (A) Comparison of β-CTX before and after treatment. (B) Comparison of PINP before and after treatment. Note: # indicates a statistically significant difference compared to before treatment (*P<0.05*), & indicates a statistically significant difference compared to D.G. (*P<0.05*).

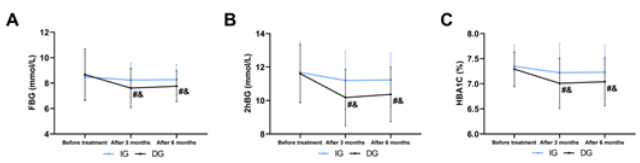

Figure 4. Comparison of changes in glycaemic parameters. (A) Comparison of FBG changes during treatment. (B) Comparison of changes in 2hBG during treatment. (C) Comparison of Hba1c changes during treatment. # indicates a statistically significant difference compared to before treatment (*P<0.05*), & indicates a statistically significant difference compared to I.G. (*P<0.05*).

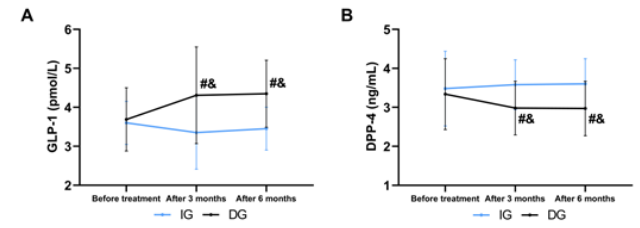

Figure 4. Comparison of changes in GLP-1 and DPP-4 levels. (A) Comparison of GLP-1 changes during treatment. (B) Comparison of DPP-4 changes during treatment. # indicates a statistically significant difference compared to before treatment (*P<0.05*), & indicates a statistically significant difference compared to I.G. (*P<0.05*).

Table 2. Adverse effects during treatment.

	Fever	Abdominal discomfort	Muscle pain	Total incidence rate (%)
D.G. (n=45)	1 (2.22)	1 (2.22)	0 (0.0)	4.44%
I.G. (n=45)	0 (0.0)	0 (0.0)	1 (2.22)	2.22%
χ^2				0.345
P				0.557

caemic parameters in osteoporosis patients with combined T2DM and to explore the possible mechanisms of action. Because China has a large population base and is in a period of rapid economic development, this study has more obvious significance for the Chinese population.

Compared to bisphosphonates, Denosumab is a relatively new anti-osteoporosis drug, and its anti-osteoporosis effects are well-established. There are similar but imperfect or controversial previous studies on whether Denosumab affects improving blood glucose. In this study, we selected postmenopausal patients with osteoporosis as subjects, which removed the interference of sex. Nikooyeh B et al. (2013) suggested that the improvement of glucose tolerance in patients with osteoporosis may be related to their administration of vitamin D (23), which may improve fatty liver and hepatic insulin resistance (24). Therefore, we achieved a balance of serum 25-hydroxyvitamin D levels between both groups in our trial design. In addition, we excluded patients with high blood glucose and glycosylated haemoglobin. To balance hypoglycaemic drugs, we did not adjust the regimen to avoid hyperglycaemic crises as much as possible.

Considering FBG, our results revealed that the mean decrease in FBG in the DG was 1.06 mmol/L after three months of treatment, and this change was roughly maintained at six months. While previous studies showed no significant difference in FBG after denosumab treatment compared to that before treatment, our findings differed slightly, suggesting a significant decrease in FBG within the denosumab group compared to that before treatment. Interestingly, there was no observed difference between the FBG of the two groups after three months and six months of treatment. Our analysis suggests that this may be related to the fact that there was a slight decrease in FBG in the I.G., although none of the patients in either group changed their glucose-lowering medications but was educated about diabetes. Thus, they may have had a lifestyle change, which also resulted in an average decrease in FBG of approximately 0.25 mmol/L in the I.G. regarding the change in 2hBG, our study showed that after treatment, 2hBG in the D.G. changed significantly compared with the I.G., and the difference was statistically significant. The results of our study also showed that after three months of treatment, the mean HbA1c decreased by 0.28% in the D.G. This change was largely maintained after six months; although our study showed no significant difference in HbA1c after treatment between the two groups, this is still consistent with previous findings that subjects treated with RANKL inhibitors had a more significant decrease in HbA1c levels (25). We speculate that this may be related to our choice of the subject population, as we excluded patients with very high blood glucose, which led to a predominance of FBG in terms of contribution to HbA1c. There was no noticeable difference in FBG between both groups after treatment; although 2hBG in the D.G. was lower than that in the I.G., there was no significant difference in HbA1c between the two groups. In addition, we measured the circulating levels of active GLP-1 and DPP-4 in both groups, and the changes in the D.G. were also significant. , Therefore, we hypothesize that the mechanism of blood glucose improvement by Denosumab could be achieved by decreasing the circulating levels of DPP-4 and increasing the levels of GLP-1 in patients. It is well known that both GLP-1 and DPP-4 are targets for treating diabetes. Inhibition of DPP-4 prevents the hydrolytic inactivation of GLP-1, which increases insulin synthesis and secretion, reduces glucagon release and lowers blood glucose.

Moreover, we also analysed the osteoprotective effects of both drugs in this population, although this was not the focus. As we expected, no difference was found in the osteoprotective effect of these two regimens, and the increase in BMD was essentially the same. Our study demonstrated further support for the safety of both regimens, as there was no significant difference between both groups in the incidence of adverse reactions, consistent with the results of previous studies, which showed no significant differences between RANKL inhibitors and ibandronate in terms of the incidence of fractures, allergies, eczema, hypocalcaemia or serious infections, malignancies or cardiac disease (26). This denotes that it is not necessary to give preference to bisphosphonates, at least when considering anti-osteoporosis effects; in addition, subcutaneous administration of Denosumab is easy to use.

RANKL is associated with macroangiopathy in diabetic patients, and patients with diabetic macroangiopathy have higher concentrations of RANKL in their bodies (27). Trials have also shown that RANKL is involved in macroangiopathy in diabetic patients. Due to the short duration of the trial, we expect more extended studies to assess whether Denosumab can benefit patients with osteoporosis in combination with T2DM when treating their macrovascular lesions (28,29).

There are still some limitations to our study. First, this study is single-centre, and the sample size was not large enough, which may have limited the statistical power in analysing certain parameters. Second, although all patients were educated about diabetes, there was self-control of diet and physical activity. Third, some of the patients' circumstances, such as occupation, education, income, and place of residence, were not balanced. Fourth, for practical reasons, GLP-1 and DPP-4 were not measured at 2 hours after a meal. Fifth, patients with abnormal glucose tolerance were not studied. A study by Blanca T (30) et al. revealed an association between RANKL inhibitors and FBG, HbA1c and HOMA1-IR (insulin resistance index), and this association was particularly pronounced in the impaired glucose tolerance group. Because of the number of cases, we did not recruit a certain number of patients with abnormal glucose tolerance.

For patients with osteoporosis combined with T2DM, the choice of RANKL inhibitors seems more appropriate for their medical condition. RANKL inhibitors helped to enhance their BMD and, more importantly, significantly improved their glycaemic parameters by a mechanism that may be related to elevating their serum GLP-1 levels and decreasing their DPP-4 levels. In contrast, denosumab is expected to be a new treatment option for T2DM combined with osteoporosis in the future, providing patients with more effective treatment effects and treatment safety, which is of great significance for improving the quality of clinical care.

Declarations

Ethics approval and consent to participate

The present study was indicated by the Ethics Committee of The Third Hospital of Hebei Medical University (Approval No:2020-003-1).

Conflict of interest

The authors declare to have no conflict of interest.

Authors' contribution statement

YK.L designed the study; K.W. wrote and revised the manuscript, L.G, C.L, and XX.B., collected, analyzed and interpreted data; YW.T supervised the research. All authors agreed to be accountable for all aspects of the work and approved the final submitted manuscript.

Finding

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Data availability statement

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

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