

Effects of Empagliflozin/Linagliptin on Oxidative Stress Markers in Patients with Chronic Kidney Disease and Type 2 Diabetes: A Randomized, Open-Label, Controlled Trial

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Abstract

Background: Combined therapies using sodium-glucose cotransporter 2 inhibitors (SGLT2i) and dipeptidyl peptidase-4 inhibitors (DPP-4i) are expected to yield additive effects on glycemic control and reactive oxygen species (ROS) among patients with type 2 diabetes (T2DM). This study evaluated whether SGLT2i and DPP-4i could attenuate renal oxidative stress caused by chronic hyperglycemia.

Methods: A clinical trial was conducted among patients with T2DM and stage 3 chronic kidney disease (CKD). Patients were randomized 1:1 to receive either empagliflozin (10 mg/day)/linagliptin (5 mg/day) or standard treatment for 12 weeks. Renal oxidative stress and renal biomarkers were measured using urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) and the urine albumin-to-creatinine ratio.

Results: Forty-eight patients were analyzed. At week 12, the empagliflozin/linagliptin group showed a significant reduction in urinary 8-OHdG compared with the control group. Additionally, the empagliflozin/linagliptin group had a significant reduction in urinary albumin compared with the control group. The reduction in HbA1c with empagliflozin/linagliptin treatment was also superior to that observed with the control treatment. No between-group differences were observed regarding body weight, blood pressure, or estimated glomerular filtration rate at the end of follow-up. The proportion of subjects experiencing adverse events over 12 weeks was similar across treatment arms, with no hypoglycemic events requiring assistance.

Conclusions: Combined SGLT2i and DPP-4i treatment for 12 weeks improved renal oxidative stress and glycemic control in patients with T2DM and CKD. The treatment was well-tolerated.

Keywords: diabetic nephropathy; oxidative stress; sodium-glucose cotransporter 2 inhibitor; dipeptidyl peptidase-4 inhibitor; albuminuria

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การศึกษาแบบทดลองสุ่มถึงผลของยา Empagliflozin/Linagliptin ต่อภาวะ Oxidative Stress ในผู้ป่วยโรคไตเรื้อรังจากเบาหวาน

ศุภกรณ์ สืบศิริพงศ์, ศรัณยพงศ์ รุ่งประพันธ์ และ บัญชา สติระพจน์

แผนกโรคไต กองอายุรกรรม โรงพยาบาลพระมงกุฎเกล้าและวิทยาลัยแพทยศาสตร์พระมงกุฎเกล้า

บทคัดย่อ

บทนำ: การรักษาด้วยยา sodium-glucose cotransporter 2 inhibitors (SGLT2i) และ dipeptidyl peptidase-4 inhibitors (DPP-4i) คาดว่าช่วยเสริมฤทธิ์ในการควบคุมระดับน้ำตาลในเลือด และลดภาวะ reactive oxygen species (ROS) ในผู้ป่วยเบาหวานชนิดที่ 2 การศึกษานี้จึงประเมินผลของยา SGLT2i ร่วมกับ DPP-4i เพื่อลดภาวะ oxidative stress ภายในไตจากภาวะน้ำตาลในเลือดสูง

ระเบียบวิธีวิจัย: การศึกษาในผู้ป่วยเบาหวานชนิดที่ 2 ที่มีโรคไตเรื้อรังระยะที่ 3 โดยทดลองสุ่มให้ยา empagliflozin 10 มก./วัน ร่วมกับ linagliptin 5 มก./วัน เทียบกับการรักษามาตรฐานเป็นเวลา 12 สัปดาห์ แล้วประเมินภาวะ oxidative stress ภายในไตจากการตรวจวัด 8-hydroxy-2'-deoxyguanosine (8-OHdG) และแอลบูมินต่อครีเอตินินในปัสสาวะ

ผลการวิจัย: ผู้ป่วยจำนวน 48 รายเข้าร่วมการศึกษา หลังการรักษา 12 สัปดาห์ กลุ่มที่ได้รับ empagliflozin/linagliptin สามารถลดระดับ 8-OHdG ในปัสสาวะอย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับกลุ่มควบคุม นอกจากนี้กลุ่มที่ได้รับ empagliflozin/linagliptin สามารถลดระดับแอลบูมินในปัสสาวะอย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับกลุ่มควบคุม และสามารถลดระดับน้ำตาลสะสมในเลือดอย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับกลุ่มควบคุมเช่นกัน โดยไม่พบความแตกต่างกันของน้ำหนักตัว ความดันโลหิตและการทำงานของไตระหว่างผู้ป่วยทั้งสองกลุ่ม ผลข้างเคียงจากการรักษาในผู้ป่วยทั้งสองกลุ่มไม่มีความแตกต่างกัน รวมถึงการเกิดภาวะน้ำตาลในเลือดต่ำ

สรุป: การใช้ยา SGLT2i และ DPP-4i ร่วมกันเป็นระยะเวลา 12 สัปดาห์ สามารถเพื่อลดภาวะ oxidative stress ภายในไต และระดับน้ำตาลในเลือดในผู้ป่วยโรคไตเรื้อรังจากเบาหวาน ซึ่งอาจช่วยชะลอการไตเสื่อมจากโรคเบาหวาน โดยผู้ป่วยสามารถทนต่อการรักษาได้ดี

คำสำคัญ: โรคไตจากเบาหวาน; ภาวะ oxidative stress; ยา sodium-glucose cotransporter 2 inhibitor; ยา dipeptidyl peptidase-4 inhibitor; แอลบูมินต่อครีเอตินิน

Introduction

Approximately 40% of patients with type 2 diabetes mellitus (T2DM) develop chronic kidney disease (CKD), which presents a higher risk of mortality and often progresses to end-stage renal disease^{1,2}. Glycemic control is used to reduce diabetes-associated complications. However, evidence on the impact of intensive glycemic control in preventing macroalbuminuria and cardio-

vascular diseases, particularly myocardial infarction, remains limited³. Furthermore, the efficacy and safety of glucose-lowering agents for patients with both diabetes and CKD are still not well-established⁴. Among patients with T2DM receiving sulfonylureas or combined sulfonylureas and metformin, only about half achieve glycemic goals, with similar rates of treatment compliance⁵. Sodium-glucose cotransporter-2 (SGLT2)

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inhibitors and dipeptidyl peptidase-4 (DPP-4) inhibitors have shown promise in improving glycemic control with a low risk of hypoglycemia, along with additional benefits beyond glycemic control in patients with T2DM and CKD⁶.

Oxidative stress, chronic inflammation, and endothelial dysfunction form a vicious cycle between CKD and systemic complications. Elevated oxidative stress in T2DM and CKD is attributed to increased mitochondrial reactive oxygen species (ROS) driven by hyperglycemia⁷. Evidence suggests that SGLT2 and DPP-4 inhibitors may offer renoprotective effects beyond glycemic control^{8,9}. These renoprotective effects result from the antioxidant and anti-apoptotic properties of these agents, which benefit kidney and endothelial function^{10,11}. This has generated interest in inhibiting renal oxidative stress through combined therapy to prevent renal injury in T2DM with CKD, though this effect has not been extensively studied. Thus, our study aimed to assess the effect of combined SGLT2 inhibitor empagliflozin and DPP-4 inhibitor linagliptin on biomarkers of renal oxidative stress in patients with T2DM and CKD.

Methods

Study design

This study used a 12-week, single-center, randomized, open-label, controlled design among patients with T2DM and CKD stage 3 at Phramongkutklao Hospital in Bangkok, Thailand, between July 1, 2020, and December 31, 2020. Approval was obtained from the Institutional Review Board at the Royal Thai Army Medical Department (R063h/63), and the study was conducted following the Declaration of Helsinki. This study was registered in the Thai Clinical Trials Registry (TCTR2021316009: 22/03/2021). All patients provided written informed consent. Treatment protocols were randomly assigned using a computer-generated randomization procedure in blocks of four.

Study population

Eligible participants were aged 18 years or older, had an estimated GFR of 30 to 59 mL/min/1.73 m², were on stable glycemic treatment for T2DM, and had

glycosylated hemoglobin A1C between 7 and 10% for at least 12 weeks before the study. Exclusion criteria included recent changes in renin-angiotensin system inhibitors, acute kidney injury, malignancy, severe cardiovascular, pulmonary, or liver disease, chronic infection, pregnancy, limited life expectancy, or history of urinary or genital tract infection within 12 weeks. In the absence of preliminary data on the effects of empagliflozin/linagliptin on urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels, the sample size was estimated using anticipated oxidative stress improvement observed with dapagliflozin as an add-on therapy. A total of 22 participants per group was calculated to provide 80% power at a significance level of $\alpha = 0.05$ to detect a significant between-group difference in the change of urinary 8-OHdG levels¹².

Interventions

Eligible patients were randomly assigned to two groups: 24 patients received empagliflozin 10 mg/day and linagliptin 5 mg/day orally, while the other 24 received a placebo. Interventions lasted for 12 weeks, with data collected every 4 weeks.

Initial screening included medical history, physical examination, and routine blood tests. Every eight weeks, all patients were monitored regarding body weight, body mass index (BMI), blood pressure, symptoms, and signs of adverse effects and compliance with the medication. The subjects were provided with pills, and compliance was assessed by pill counts at follow-up visits. Adverse events, whether or not considered related to pioglitazone treatment, were monitored every four weeks.

Outcomes

The primary endpoint was the change from baseline in urinary 8-OHdG over 12 weeks. Other outcomes included changes in the urine albumin-to-creatinine ratio (UACR), estimated GFR, serum creatinine, high-sensitivity C-reactive protein (hs-CRP), fasting plasma glucose, and hemoglobin A1C. Urine 8-OHdG was determined using a two-site (NH₂-terminal/C-terminal) enzyme-linked immunosorbent assay (Novus Biologicals, LLC, Centennial, CO, USA).

Statistical Analysis

Data were expressed as percentages for categorical

variables and as mean $\pm$ standard deviation (SD) or 95% CI for continuous variables. The chi-square or Fisher's exact test and the Student's t-test or Mann-Whitney U test were used to compare differences between groups for categorical and continuous variables, respectively. Changes in continuous measures between baseline and study periods were tested using the paired t-test. Data were analyzed using SPSS Software, Version 15.0. All statistical tests were two-sided, with $P < 0.05$ considered statistically significant.

Results

A total of 64 patients in the outpatient clinic were

screened for eligibility, of whom 48 (75%) underwent randomization (Figure 1). Fourteen CKD patients were excluded for not meeting eligibility criteria, and two patients declined to participate. Patients with T2DM, mean age 69.0 ± 9.9 years, and mean GFR 43.4 ± 8.9 mL/min/ 1.73 m^2 met the entry criteria. Baseline characteristics were similar between groups, except for a significantly higher proportion using pioglitazone, hs-CRP, body weight, BMI, and triglycerides in the empagliflozin/linagliptin group (**Table 1**). Patients were adherent to study drugs, defined as $>80\%$ pill consumption throughout the study based on pill counts.

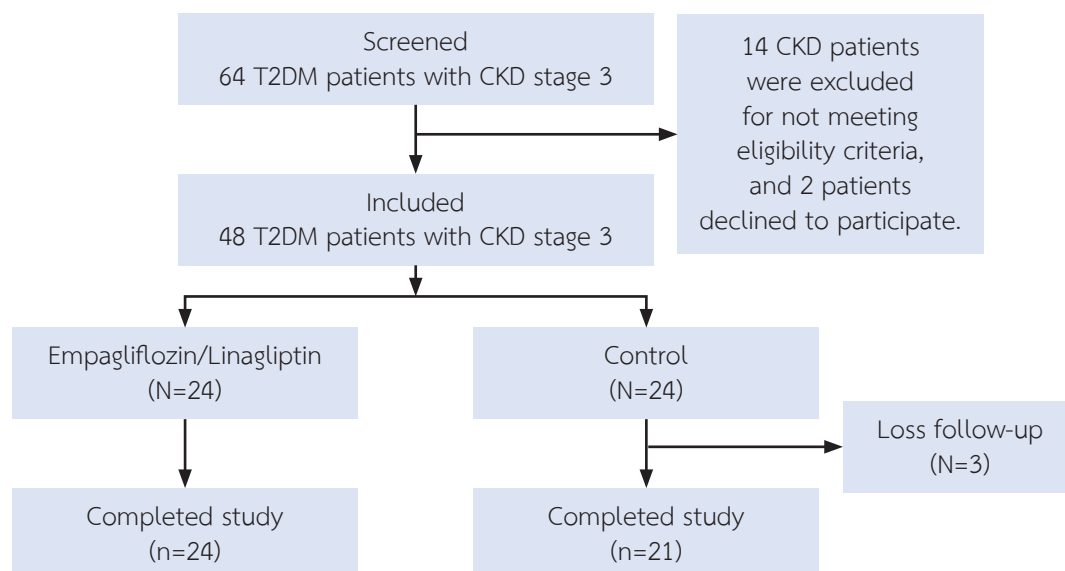


Figure 1 Flow chart of enrolled patients

T2DM, type 2 diabetes mellitus; CKD, chronic kidney disease

Table 1 Baseline characteristics between two groups

Parameters	Empagliflozin/linagliptin (N=24)	Placebo (N=21)	P value
Male (N, %)	16 (66.8%)	15 (71.4%)	0.731
Age (years)	67.4 $\pm$ 7.9	70.7 $\pm$ 11.7	0.27
Comorbidities (N, %)			
• Hypertension	21 (87.5)	21 (100)	0.236
• Dyslipidemia	22 (91.7%)	20 (95.2%)	1.000
• Coronary heart disease	4 (16.8%)	2 (9.5%)	0.670
• Congestive heart failure	1 (4.2)	-	1.000

Table 1 Baseline characteristics between two groups (continued)

Parameters	Empagliflozin/linagliptin (N=24)	Placebo (N=21)	P value
Current medications (N, %)			
• ACEI/ARB	15 (66.7)	14 (66.7)	0.965
• Calcium channel blockers	19 (79.2)	18 (85.7)	0.705
• Loop diuretics	1 (4.5)	1 (4.2)	0.950
• Metformin	15 (62.5)	9 (42.9)	0.188
• Sulfonylureas	15 (62.5)	14 (66.7)	0.771
• Thiazolidinediones	4 (16.7)	12 (57.1)	0.005
Body weight (kg)	75.7±11.8	67.0±10.5	0.006
Body mass index (kg/m ²)	28.4±4.7	25.5±4.2	0.015
Systolic blood pressure (mmHg)	139.6±19.6	141.7±19.2	0.721
Diastolic blood pressure (mmHg)	72.9±12.6	73.9±11.5	0.787
Glomerular filtration rate (mL/min/1.73 m ²)	45.3±8.8	41.1±8.7	0.116
Serum creatinine (mg/dL)	1.48±0.3	1.6±0.4	0.802
Fasting plasma glucose (mg/dL)	183.6±64.4	169.0±61.2	0.444
Hemoglobin A1C (%)	8.4±1.2	7.8±1.4	0.140
Urine albumin creatinine ratio (mg/gCr)	337.9±737.1	566.6±972.3	0.415
Urine 8-OHdG (ng/mg Cr)	49.7(30.2,121.19)	69.04(33.91,76.57)	0.699
LDL-cholesterol (mg/dL)	104.5±9.2	96.1±7.7	0.493
Triglycerides (mg/dL)	185.9±18.6	126.7±15.1	0.020

Comparison of Glycemic, Metabolic Profiles, and Blood Pressure Between Groups

Mean changes in hemoglobin A1C [-1.04%; 95%CI -1.48 to -0.61 vs. -0.43%; 95%CI -0.76 to -0.10, P=0.028] were significantly greater in the empagliflozin/linagliptin group compared to the placebo group after 12 weeks, despite no significant differences in blood pressure, body weight, fasting plasma glucose, uric acid, or LDL-cholesterol between groups (Table 2).

Comparison of Urine 8-OHdG Levels, Albumin, and Other Parameters Between Groups

After 12 weeks, the empagliflozin/linagliptin group showed significant reductions in mean urine albumin levels [-159.64 mg/gCr; 95% CI -318.23 to -1.06, P = 0.049] (Figure 2). Similarly, a significant difference in mean changes in urine 8-OHdG levels was observed between the groups [-53.90 ng/mg Cr; 95%CI -102.93 to -4.84, P = 0.034] (Figure 2). No significant differences in hs-CRP, serum creatinine, or estimated GFR were observed between groups (Table 3).

Table 2 Comparison of the changes of glycemic, metabolic profiles and blood pressure before and after treatment between two groups

Mean changes	Empagliflozin/linagliptin (N=24)	Placebo (N=21)	P value
Mean fasting plasma glucose (mg/dL)			
Baseline	183.55±64.36	169.03±61.19	0.444
Week 12	147.46±45.27	162.9±55.92	0.312
Change from baseline (95% CI)	-36.1 (-65.53, -6.67)*	-6.12 (-32.65, 20.40)	0.128
Mean hemoglobinA1C (%)			
Baseline	8.45±1.2	7.85±1.44	0.140
Week 12	7.38±1.07	7.42±1.31	0.920
Change from baseline (95% CI)	-1.04 (-1.48, -0.61)*	-0.43 (-0.76, -0.10)*	0.028
Mean serum uric acid (mg/dL)			
Baseline	6.83±1.22	6.6±1.15	0.523
Week 12	7.11±1.62	6.63±1.22	0.270
Change from baseline (95% CI)	0.28 (-0.19, 0.75)	0.02 (-0.28, 0.33)	0.365
Median LDL-cholesterol (mg/gCr)			
Baseline	104.49±45.07	96.06±35.15	0.493
Week 12	107.01±41.67	102.25±23.65	0.635
Change from baseline (95% CI)	2.52 (-13.85, 18.88)	6.19 (-6.90, 19.28)	0.723
Mean systolic blood pressure (mmHg)			
Baseline	139.63±19.57	141.71±19.23	0.721
Week 12	134.57±20.92	142.48±16.28	0.172
Change from baseline (95% CI)	-5.39 (-15.57, 4.78)	0.76 (-8.95, 10.48)	0.370
Mean diastolic blood pressure (mmHg)			
Baseline	72.87±12.56	73.86±11.48	0.787
Week 12	70.17±12.12	73.29±11.37	0.386
Change from baseline (95% CI)	-2.44 (-9.23, 4.36)	-0.57 (-6.80, 5.65)	0.678
Mean body weight (kg)			
Baseline	75.66±11.75	67.02±10.52	0.013
Week 12	74.8±11.65	67.5±10.31	0.034
Change from baseline (95% CI)	-0.85 (-1.95, 0.24)	0.48 (-0.70, 1.65)	0.092

CI, confidence interval; *P <0.05

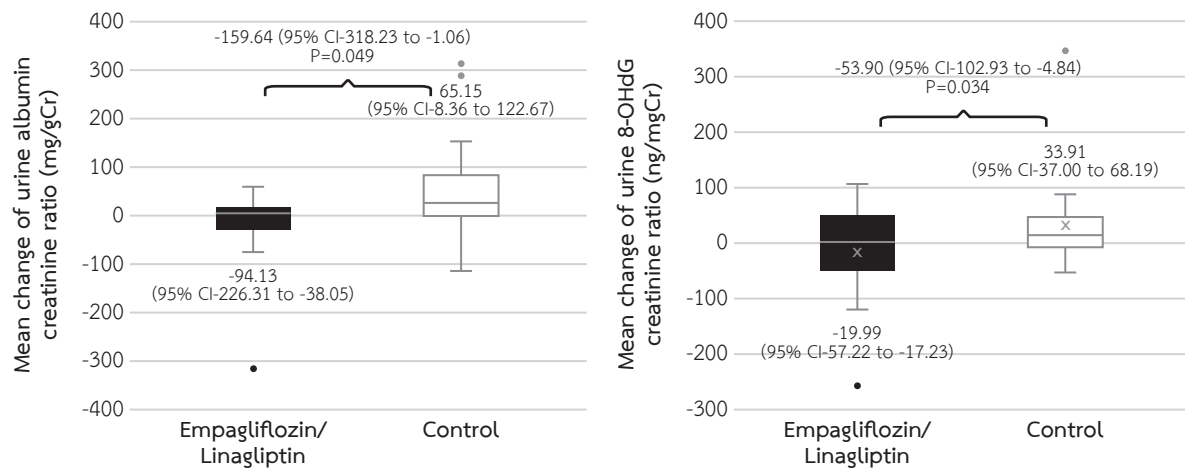


Figure 2 Baseline and final urine albumin and urine oxidative stress levels by treatment group

Table 3 Comparison of the changes of renal function, renal oxidative stress and inflammation before and after treatment between two groups

Mean changes	Empagliflozin/linagliptin (N=24)	Placebo (N=21)	P value
Mean serum creatinine (mg/dL)			
Baseline	1.48±0.28	1.59±0.44	0.331
Week 12	1.51±0.29	1.55±0.49	0.721
Change from baseline (95% CI)	0.03 (-0.06, 0.13)	-0.03 (-0.11, 0.04)	0.263
Mean estimated GFR (mL/min/1.73 m²)			
Baseline	45.34±8.82	41.11±8.66	0.116
Week 12	44.05±9.4	43.62±10.89	0.887
Change from baseline (95% CI)	-1.29 (-4.83, 2.25)	1.11 (-0.97, 3.19)	0.257
Median high sensitivity C-reactive protein (pg/mL)			
Baseline	3.59±3.76	1.67±1.78	0.033
Week 12	7.63±15.55	1.72±2.09	0.078
Change from baseline (95% CI)	4.04 (-2.71, 10.79)	0.05 (-0.84, 0.94)	0.274
Mean urine albumin creatinine ratio (mg/gCr)			
Baseline	337.93±737.13	566.59±972.34	0.415
Week 12	243.8±583.06	632.1±1019.51	0.185
Change from baseline (95% CI)	-94.13 (-226.31, -38.05)*	65.51 (8.36, 122.67)*	0.049
Mean urine 8-OHdG/creatinine ratio (ng/mg Cr)			
Baseline	89.98±87.98	63.1±39.31	0.186
Week 12	77.91±39.36	97.01±101.85	0.428
Change from baseline (95% CI)	-19.99 (-57.22, 17.23)	33.91 (-37.00, 68.19)	0.034

CI, confidence interval; *P <0.05

Throughout the 12-week study, no serious adverse events, related or unrelated to empagliflozin/linagliptin, were reported, including drug-induced urinary tract infection, pancreatitis, or severe hypoglycemia. One patient in the empagliflozin/linagliptin group and two in the placebo group experienced nausea and vomiting. Two patients in each group reported fatigue. All patients continued treatment through the end of the study.

Discussion

This is the first randomized, controlled trial to evaluate the effects of empagliflozin/linagliptin on renal oxidative stress in patients with T2DM and CKD. After 12 weeks of treatment, patients showed reductions in hemoglobin A1C, urine 8-OHdG, and albumin levels, without significant differences in renal function or inflammation between groups. These results provide proof of concept that empagliflozin/linagliptin improves glycemic control related to renal oxidative stress in patients with T2DM and CKD.

Oxidative stress plays a significant role in developing diabetic complications, particularly in settings of uncontrolled hyperglycemia¹³, and renal oxidative stress is directly linked to podocyte damage, tubulointerstitial injury, and diabetic kidney progression¹⁴. Advanced diabetic microvascular complications have shown significantly higher serum 8-OHG levels, contributing to microvascular complications in diabetes¹⁵. Urinary 8-OHG levels have been reported as a predictive marker for incident albuminuria during long-term follow-up¹⁶. Combined SGLT2 and DPP-4 inhibitors target several pathophysiological pathways and are associated with improved glycemic control and reduced renal risks in T2DM¹⁷. This study demonstrated that urinary 8-OHG excretion decreased following empagliflozin/linagliptin treatment compared to the control group in patients with CKD and T2DM. To our knowledge, this is the first randomized clinical trial demonstrating the preventive effect of empagliflozin/linagliptin treatment on renal oxidative injury in T2DM. The experimental model also supports that empagliflozin or linagliptin attenuates diabetic kidney disease by inhibiting renal oxidative

stress and enhancing renal autophagy¹⁸⁻²⁰. Empagliflozin reduced high glucose-induced oxidative stress, inhibited human renal proximal tubular epithelial cell migration, and epithelial-to-mesenchymal transition²¹. Furthermore, combined empagliflozin and linagliptin enhanced glomerular autophagy and decreased renal apoptosis in an animal model of T2DM²², and linagliptin partially attenuated oxidative stress markers in pilot clinical studies²³.

Large randomized controlled trials have demonstrated that canagliflozin, dapagliflozin, and empagliflozin lower the risk of kidney outcomes and albuminuria in patients with established cardiovascular disease or multiple cardiovascular risk factors and CKD²⁴⁻²⁶. Conversely, DPP-4 inhibitors have shown potentially renoprotective effects, though not consistently. Saxagliptin and linagliptin decreased the risk of albuminuria progression among patients with cardiovascular disease and CKD^{27, 28}. Both classes have been reported to reduce urinary albumin excretion, with additional benefits expected from combining the agents beyond the advantages of each compound alone. Experimental studies indicate that DPP-4 inhibitor treatment prevents tubulointerstitial injury by inhibiting kidney fibrosis through the endothelial-mesenchymal transition, and adding DPP-4 inhibitors to SGLT2 inhibitors significantly enhanced attenuation of the inflammasome, kidney dysfunction, and renal pathological changes in diabetic mice^{29, 30}. This suggests that combined treatment might be more effective in protecting against tubulointerstitial injury in T2DM with CKD. Consistent with these findings, empagliflozin/linagliptin reduced urine albumin levels in patients with T2DM and CKD.

Combined SGLT2 and DPP-4 inhibitors enhanced glycemic control and were proven safe in managing T2DM³¹. In line with reports on hemoglobin A1C reduction and low risk of hypoglycemic events with SGLT2/DPP-4 inhibitors^{32, 33}, our study showed that this combination resulted in a greater mean hemoglobin A1C reduction of -1.04%, with baseline hemoglobin A1C at 8.4%, and no severe hypoglycemia was reported. The complementary mechanisms of SGLT2 and DPP-4 inhibitors address

multiple pathophysiological aspects of T2DM. Combined therapy showed potential benefits for weight loss and blood pressure reduction, as SGLT2 inhibitors promote weight and blood pressure reduction while DPP-4 inhibitors are weight-neutral³². Our results indicated that combined SGLT2 and DPP-4 inhibitors tended to reduce body weight (-0.85 kg; 95% CI -1.95 to 0.24) and systolic blood pressure (-5.39 mmHg; 95% CI -15.57 to 4.78). Based on current evidence, SGLT2/DPP-4 inhibitors as combination therapy may offer benefits beyond glycemic control and good tolerability profiles, including a low risk of hypoglycemia without significant changes in laboratory parameters³⁴.

This study has several limitations. First, it was a single-center study, and the control group did not include DPP-4 inhibitors for comparison of urine 8-OHG and albumin levels. Second, the small sample size and lack of blinding could introduce detection and performance bias. Third, while the effects of empagliflozin/linagliptin on urine 8-OHG were observed, we could not determine the impact on clinical endpoints related to renal oxidative pathology in patients with CKD. Further research is needed to confirm these results and evaluate long-term clinical outcomes.

Conclusions

Our study demonstrated that empagliflozin/linagliptin, as an add-on to standard therapy, significantly improved glycemic control and reduced renal oxidative stress in patients with T2DM and CKD. This combination therapy may have a therapeutic role in managing renal oxidative stress in this population.

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Statement of Ethics

The study was approved by the Ethics Committee of the Institutional Review Board at the Royal Thai Army

Medical Department (R063h/63) and was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants.

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Author Contributions

S.S. and S.R. conceived the study idea. S.S. and B.S. completed the analysis and drafted the manuscript with contributions from all authors. N.N. and B.S. reviewed and supervised the work. All authors contributed to the article, and the final version of the manuscript was reviewed and approved by all authors.

Data Availability Statement

The data that support the findings of this study are not publicly available due to the risk of participant identification and the need to protect the privacy of research participants. Further inquiries can be directed to the corresponding author and will be made available upon reasonable request.

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