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Research Article

The Impact of Empagliflozin on Renal Function and Kidney Injury Markers in Patients with Diabetic Nephropathy

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Abstract

Background: Diabetic nephropathy affects approximately 50% of type 2 diabetes patients. Early detection of kidney disease is crucial to reducing deterioration of renal function. Reversing microalbuminuria towards normal showed beneficial effects in delaying the onset of renal impairment or even reversing the progression of the disease. Recently, empagliflozin, a sodium/glucose cotransporter-2 inhibitor, has received attention for its anti-inflammatory and reno-cardioprotective effects. ***Objective***: This interventional open-label randomized clinical trial aimed to evaluate the clinical outcome of empagliflozin as an add-on therapy for renal function parameters and other injury markers in type 2 diabetic nephropathy patients. ***Methods***: The study enrolled twenty-one type 2 diabetic patients with nephropathy and nineteen without nephropathy. Each group received empagliflozin 10 mg/day for 16 weeks as an add-on to the traditional treatment. Blood and urine samples were collected at baseline and at week 16 to evaluate the glycemic status, renal function, tubular injury markers, and inflammatory and oxidative stress markers. ***Results***: After 16 weeks, empagliflozin significantly reduced glycated hemoglobin A1c and urinary albumin/creatinine ratios in the nephropathy group. Compared with the non-nephropathy group, empagliflozin showed a significant increase in serum creatinine and a significant decrease in eGFRcr. Empagliflozin significantly reduced serum kidney injury molecule-1, cystatin C, interleukin-18, c-reactive protein, and malondialdehyde in both groups. ***Conclusions***: Adding empagliflozin to the traditional oral antidiabetic drugs in diabetic nephropathy improved albuminuria with a mild increment in serum creatinine. Empagliflozin also effectively reduced renal injury markers, as well as inflammatory and oxidative stress markers.

***Keywords*:** Albuminuria, Diabetic nephropathy, Empagliflozin, Type 2 DM.

تأثير إمباغليفلوزين على وظائف الكلى وعلامات إصابتها في المرضى الذين يعانون من اعتلال الكلية السكري

الخلاصة

الخلفية: يؤثر اعتلال الكلية السكري على حوالي 50٪ من مرضى السكري من النوع 2. الكشف المبكر عن أمراض الكلى أمر بالغ الأهمية للحد من تدهور وظائف الكلى. أظهر عكس بيلة الألبومين الدقيقة نحو وضعها الطبيعي آثارا مفيدة في تأخير ظهور القصور الكلوي أو حتى عكس تطور المرض. في الآونة الأخيرة، حظي إمباغليفلوزين، وهو مثبط للناقل المشترك للصوديوم/الجلوكوز-2 ، بالاهتمام لآثاره المضادة للالتهابات وحماية الكلى. **الهدف**: تهدف هذه التجربة السريرية العشوائية التدخلية المفتوحة التسمية إلى تقييم النتيجة السريرية لإمباغليفلوزين كعلاج إضافي لمعلمات وظائف الكلى وعلامات الإصابة الأخرى في مرضى اعتلال الكلية السكري من النوع 2. **الطريقة**: سجلت الدراسة واحدا وعشرين مريضا بالسكري من النوع 2 يعانون من اعتلال الكلية وتسعة عشر مريضا بدون اعتلال الكلية. تلقت كل مجموعة إمباغليفلوزين 10 ملغ / يوم لمدة 16 أسبوعا كإضافة للعلاج التقليدي. تم جمع عينات الدم والبول عند خط الأساس وفي الأسبوع 16 لتقييم حالة نسبة السكر في الدم، ووظائف الكلى، وعلامات الإصابة الأنبوبية، وعلامات الإجهاد الالتهابي والتأكسدي. **النتائج**: بعد 16 أسبوعا، قلل إمباغليفلوزين من الهيموغلوبين السكري A1c ونسب الألبومين/الكرياتينين البولية في مجموعة اعتلال الكلية. بالمقارنة مع المجموعة غير اعتلال الكلية، أظهر إمباغليفلوزين زيادة كبيرة في الكرياتينين في الدم وانخفاض كبير في ترشيح الكرياتين. إمباغليفلوزين قلل بشكل كبير من جزيء إصابة الكلى في الدم -1، والسيستاتين C، والإنترلوكين-18، والبروتين التفاعلي ج، ومالوندالديهيد في كلا المجموعتين. **الاستنتاجات**: إضافة إمباغليفلوزين إلى الأدوية المضادة لمرض السكر الفموية التقليدية في اعتلال الكلية السكري يحسن بيلة الألبومين مع زيادة خفيفة في الكرياتينين في الدم. كما قلل إمباغليفلوزين بشكل فعال من علامات الإصابة الكلوية، بالإضافة إلى علامات الإجهاد الالتهابية والتأكسدية.

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INTRODUCTION

Diabetic nephropathy (DN) is one of the frequent, burdensome, long-term complications of diabetes that leads to end-stage renal disease (ESRD) [1]. Diabetic nephropathy is characterized by elevated urinary albumin excretion, a reduced glomerular filtration rate (GFR) or both [2,3]. In type 1 diabetes mellitus (T1DM), chronic kidney disease (CKD) mostly progresses after ten years; however, in type 2 diabetes mellitus (T2DM), CKD may already be present at diagnosis. Up to three percent of people with T2DM have albuminuria at the time of diagnosis, since the early stages sometimes progress undetected or as prediabetes [4,5]. Approximately half of patients with T2DM and one-third of those with T1DM will develop DKD during their lifetimes [6]. Annually, one death will occur among every 20 people with DKD. It is well known that early detection of DN, along with aggressive management of its known risk factors, is crucial to reducing the deterioration, morbidity and mortality, as well as the social and economic burden [7]. The main goal of drug therapy is to keep renal function from getting worse by lowering microalbuminuria. This lower level of microalbuminuria can help delay the start of renal impairment or even stop the damage from getting worse [8]. Renin-angiotensin system (RAS) blockers, angiotensin-converting enzyme (ACE) inhibitors, and angiotensin II receptor blockers (ARB) are standard treatment options for DN, and many trials have demonstrated their safety and efficacy [9–11]. More recently, sodium/glucose cotransporter-2 inhibitors (SGLT2is) and non-steroidal mineral receptor antagonists (MRAs) have received attention for their anti-inflammatory and reno-cardioprotective effects [12]. For every patient with type 2 diabetes, the American Diabetes Association advises evaluating albuminuria and estimating GFR once a year [13]. Additionally, the European Society of Cardiology (ESC) and the European Association for the Study of Diabetes (EASD) also advise diabetic individuals to have their eGFR and albuminuria evaluated annually [14]. However, several biomarkers have been proven to be involved in renal repair after kidney injury, and they are supposed to be renal tubular injury markers even before clinical symptoms appear [15]. Several clinical trials have evaluated the safety and efficacy of empagliflozin, primarily focusing on cardio-renal outcomes. The 2008 Empagliflozin and Progression of Kidney Disease in Type 2 Diabetes Trial (EMPA-REG OUTCOME) found that when diabetic patients with high cardiovascular risk added empagliflozin to their standard treatment, the rate of clinically significant renal events decreased and kidney disease progression slowed compared to placebo [16]. Giving empagliflozin to people with chronic kidney disease in the 2020 EmPA-KIDNEY trial showed that compared to a placebo, empagliflozin made patients with chronic renal impairment less likely to have their kidney disease get worse and lowered the number of deaths from heart problems [17]. The significance of this study is that, to date, there has been no follow-up study regarding this SGLT2i recorded in Iraqi patients, with limited data available on the Middle Eastern population in general. The purpose of the present study is to evaluate the clinical efficacy of empagliflozin in type 2 diabetic nephropathy patients in terms of glycemic status, renal function, tubular injury, and inflammatory and oxidative stress markers.

METHODS

Study design

This is an interventional open-label randomized clinical trial that was conducted from May to December 2022 in Baghdad, Iraq. Ethical approvals were obtained from the institutions included in the work before the study commenced. All patients were fully informed about the study protocol and written consent was obtained from all participants before starting the study. All investigations and procedures carried out in this study involving human participants were in accordance with the 1975 Declaration of Helsinki and its later amendments.

Patients selection

Patients were enrolled in the study with the following criteria: T2DM, age between 18 and 70 years, on a combination of OADs (glimepiride, metformin and vildagliptin), and HbA1c (7%–10.5%) at the time of enrollment. Twenty-one patients were diagnosed with nephropathy (DN) and nineteen were without nephropathy (non-DN) and all the patients received empagliflozin 10 mg/day (Getz, Pakistan) for 16 weeks as an add-on to the traditional treatment. Glimepiride was down-titrated during the treatment period (if required) to mitigate the risk of recurrent hypoglycemic events. A specialist physician supervised all the mentioned steps.

Outcome measurements

The study's outcomes measured the changes before and after treatment (weeks 0 and 16) in the following parameters: glycated hemoglobin A1c (HbA1c), urinary albumin to creatinine ratio (UACR), serum urea, creatinine, kidney injury molecule-1 (KIM-1), cystatin-c, malondialdehyde (MDA), high sensitive c-reactive protein (hs-CRP), interleukin-18 (IL-18), serum creatinine-based estimated glomerular filtration rate (eGFRcr) and serum cystatin-based-estimated glomerular filtration rate (eGFRcys). Serum urea and creatinine were measured using the enzymatic method with hexokinase on the Cobas C311 analyzer system (Roche Diagnostics, Germany) [18,19] while HbA1c was measured using the Tina-quant Hemoglobin A1c Dx Gen.3 assay on the Cobas C503 analyzer (Roche Diagnostics, Germany) [20]. Urinary ACR was measured using a DCA Vantage Analyzer (Siemens Diagnostics, Germany) [21]. Serum KIM-1, cystatin-c, MDA, hs-CRP, and IL-18 were measured using the ELISA technique (My BioSource, USA) [22–26]. The

estimated GFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation based on serum creatinine [27] and cystatin C [28].

Statistical analysis

Statistical analysis was performed using SPSS (version 29) and Microsoft Excel (2010). The chi-square test or Fisher's exact test was performed to test the significance of the difference between the non-continuous variables. A paired sample t-test was performed for comparison between before and after treatment values. An independent t-test was performed for comparison between the patients' groups. Bivariate correlation between the study variables was performed using Pearson's correlation coefficient. Predictions among the study variables were performed using multiple linear regression analysis. A *p*-value of <0.05 was considered statistically significant.

RESULTS

Table 1 displays the demographic and disease characteristics of the patients. The mean age of the study groups was 52.53±9.40 in non-DN and 54.24±7.75 in DN.

Table 1: Demographic and disease characteristics of the patients

Characters		Non-DN (no=19)	DN (no=21)	*p*-value
Age (year)	≤60	15(78.94)	17(80.95)	0.07
	>60	4(21.05)	4(19.05)	
Sex	Male	8(42.10)	11(52.38)	0.73
	Female	11(57.90)	10(47.62)	
Smoking	Yes	3(15.79)	2(9.52)	0.36
	No	16(84.21)	19(90.48)	
Educational level	Illiterate	4(21.05)	7(33.33)	0.06
	Primary	4(21.05)	0(0)	
	Secondary	9(47.37)	11(52.38)	
	College	2(10.53)	3(14.29)	
Residence	Urban	16(84.21)	13(61.90)	0.48
	Rural	3(15.79)	8(38.09)	
Duration of DM (year)	<5	6(31.57)	1(4.76)	0.02
	5-10	3(15.79)	4(19.04)	
	≥10	10(52.63)	16(76.19)	
Family History of DM	Yes	13(68.42)	17(80.95)	0.76
	No	6(31.58)	4(19.05)	
Comorbid disease history	Yes	11(57.89)	15(71.43)	0.11
	No	8(42.11)	6(28.57)	

Data are presented as number and percentage; Chi Square or Fisher's Exact test are used for data analysis; EMPA: Empagliflozin; DN: Diabetic nephropathy; non-DN: non-diabetic nephropathy; DM: Diabetes mellitus.

The mean BMI of the study groups was 34.64±5.28 in non-DN and 37.34±4.20 in DN. Table 2 displays the impact of empagliflozin on glycemic status, renal function parameters, tubular injury markers, and inflammatory and oxidative stress markers in both DN and non-DN groups. In the DN group, there is a significant reduction in UACR, an increase in serum creatinine, and a reduction in eGFRcr ($p<0.05$) compared to the non-DN group. The levels of cystatin-c, IL-18, hs-CRP, and MDA in the blood were significantly lower in both groups ($p<0.05$). Table 3 illustrates the bivariate correlations among renal function parameters and patients' demographics in DN. Estimated GFRcr negatively correlated with the patient's age ($p<0.01$) and duration of DM ($p<0.01$), while serum KIM-1 positively correlated with the duration of DM ($p<0.05$). Additionally, multiple linear regression analysis showed that the age and duration of DM are negative predictors for eGFRcr ($p<0.05$). While the duration of DM is a negative predictor for eGFRcys and a positive predictor for serum KIM-1 as well ($p<0.001$) (Table 4).

DISCUSSION

In the current study, empagliflozin effectively reduced the UACR, which could be a consequence of changes in charge and/or size selectivity of the glomerular filtration barrier [29,30], resulting in a reduction in intraglomerular pressure and an improvement in tubular reabsorptive capacity [31]. As expected, the EMPA-REG OUTCOME trial showed that different doses of EMPA were helpful for people with normal, micro, and macro albuminuria in the short and long term [32]. A recent systematic review clarified that SGLT2is were consistently better at improving UACR compared with GLP-1 and DPP4i, and confirmed that SGLT2i decreased the risk for albuminuria onset by 16–20% and for albuminuria progression by 27–48% [33]. This study found an inverse correlation between UACR and eGFRcr, linking albuminuria to advanced age, longer duration of diabetes, and poor glycemic control, all of which predict renal function decline through correlation with eGFRcys [34–36]. Researchers have linked an elevated urine albumin excretion rate, even in the early microalbuminuric range, to higher cardiovascular morbidity and mortality as well as progressive renal failure in both diabetic and nondiabetic patients [37]. Current findings reported an increase in serum creatinine and a decrease in eGFRcr during the study period, consistent with the study of Mozawa *et al*., which found EMPA induced a decline in eGFRcr within the first 4 weeks and recovered again after 24 weeks [38]. The action of SGLT2i, which induced a modest decline in the filtration rate (approximately 3-5 mL/min), is commonly attributed to the activation of TGF by high sodium levels in the proximal tubule. This activation leads to reversible intrarenal hemodynamic effects, such as afferent vasoconstriction, which decreases intraglomerular pressure and provides the proposed renoprotective effect of SGLT2i [39,40]. However, due to the influence of other parameters on serum creatinine, the assessment of renal function solely based on creatinine may lead to misdiagnosis and underestimation, potentially rendering the patient ineligible for certain anti-diabetic medications. Current findings regarding CysC were consistent with the results of a meta-analysis and post-hoc analysis; eGFR values based on serum CysC were higher than those based on serum creatinine [41,42]. These studies, as also confirmed previously by the KDIGO [43], recommended the use of CysC in

combination with creatinine for accurate estimation of renal function, especially for those with eGFRcr ranging from 45 to <60 mL/min/1.73 m2. EMPA lowered serum CysC levels when eGFRcys levels went up. In the study by Mozawa *et al*., EMPA raised serum CysC levels within 12 weeks and then started to drop them after that, which showed that eGFRcys levels went up during the same time period as this study [38].

Table 2: Effect of Empagliflozin on glycemic, renal function, inflammatory and oxidative stress markers in diabetic nephropathy and non-diabetic nephropathy

Variables		non-DN (n=19)	DN (n=21)	*p*-value[a]
HbA1c (%)	Pre-	9.03±1.29	10.58±1.28	0.00
	Post-	7.85±0.74	8.33±1.00	0.03
p-value[b]		0.001	0.001	
UACR	Pre-	11.65±6.19	135.71±74.63	<0.001
	Post-	13.95±11.5	78.86±74.11	<0.001
p-value[b]		0.22	0.003	
Creatinine (mg/dl)	Pre-	0.77±0.29	0.93±0.39	0.17
	Post-	0.74±0.15	0.98 ± 0.43	0.07
p-value[b]		0.48	0.001	
eGFRcr (ml/min)	Pre-	93.98±24.73	92.91±26.55	0.68
	Post-	93.83±23.86	82.65±29.16	0.19
p-value[b]		0.45	0.040	
Urea (mg/dl)	Pre-	24.25±7.45	25.26±2.52	0.27
	Post-	25.95±6.79	25.81±4.59	0.12
p-value[b]		0.85	0.25	
KIM-1 (pg/ml)	Pre-	763.11±199.98	1057.64±542.80	0.024
	Post-	384.79±51.22	385.48 ± 39.15	0.96
p-value[b]		<0.001	<0.001	
Cystatin C (ng/ml)	Pre-	511.46±139.59	517.87±115.43	0.70
	Post-	322.15±37.98	333.28±30.32	0.14
p-value[b]		0.003	<0.001	
eGFRcys (ml/min)	Pre-	131.5±21.53	132.82±19.55	0.89
	Post-	163.17±12.49	156.18±10.42	0.046
p-value[b]		<0.001	<0.001	
IL-18 (pg/mL)	Pre-	239.12±71.74	242.20±65.95	0.87
	Post-	92.89±11.71	93.68±10.29	0.85
p-value[b]		0.001	<0.001	
hs-CRP (µg/ml)	Pre-	3.15±5.60	3.20±3.71	0.76
	Post-	0.84±4.35	1.08±2.59	0.14
p-Value[b]		0.001	<0.001	
MDA (nmol/ml)	Pre-	27.81±7.54	29.95±5.71	0.75
	Post-	17.91±3.82	19.29±3.31	0.19
p-value[b]		0.001	<0.001	

Data presented as mean ± SD; [a] Independent *t*-test; [b] Paired Samples *t*-test; DN: Diabetic nephropathy; non-DN: non-diabetic nephropathy; EMPA: Empagliflozin; UACR: Urinary albumin to creatinine ratio; eGFRcr: serum creatinine-based estimated glomerular filtration rate; KIM-1: Kidney injury molecule-1; eGFRcys: serum cystatin c-based estimated glomerular filtration rate; IL-18: Interleukin-18; hs-CRP: Highly sensitive c-reactive protein; MDA: Malondialdehyde.

In clinical practice, eGFR and proteinuria are the most common ways to diagnose DN and track its progression. However, it is thought that estimates of GFR may only show late functional changes in the kidney and not early structural changes [44]. Furthermore, a significant proportion of renal impairment occurs in a non-albuminuric state or before the onset of microalbuminuria [45,46]. The present study found a positive correlation between serum KIM-1 and the duration of diabetes, and a negative correlation with eGFRcr in DN patients. In addition, poor glycemic control predicts elevated KIM-1 levels. A previous study, which found elevated urinary KIM-1 levels in DN subgroups (normo-, micro-, and macroalbuminuria) and chronic kidney disease (stages 2-4), reported high serum levels of KIM-1 in both DN and non-DN groups, suggesting that KIM-1 may predict renal injury secondary to DM in the early period, independent of albuminuria [47]. As with other studies, empagliflozin lowered serum KIM-1 levels [31,48], which suggests that this drug might be able to protect the kidneys from early signs of DN [49], heal tubular cells, and reverse histopathological changes in the kidneys.

Table 3: Correlations among renal function parameters and patient's demographics in diabetic nephropathy

Demographics		Renal Function Parameters			
		UACR	eGFRcr	eGFRcys	KIM-1
Age	*r*	0.06	-0.43	0.10	0.12
	p	0.70	0.00	0.54	0.44
Duration of DM	*r*	0.18	-0.84	0.26	0.39
	p	0.27	0.00	0.10	0.01
BMI	*r*	-0.19	0.15	0.03	-0.04
	p	0.23	0.34	0.87	0.82
UACR	*r*	1	-0.44	0.12	0.01
	p		0.00	0.27	0.95
eGFRcr	*r*	-0.44	1	-0.123	-0.28
	p	0.00		0.649	0.01
eGFRcys	*r*	0.12	-0.123	1	0.09
	p	0.27	0.649		0.42
KIM-1	*r*	0.01	-0.28*	0.09	1
	p	0.95	0.01	0.42	

Pearson's correlation used to find Correlation coefficient (*r*); DM: Diabetes mellitus; BMI: Body mass index; UACR: Urinary albumin to creatinine ratio; eGFRcr: serum creatinine-based estimated glomerular filtration rate; eGFRcys: serum cystatin c-based estimated glomerular filtration rate; KIM-1: Kidney injury molecule-1.

Study limitations

This study has some limitations since it is a single-center study, which could potentially limit the generalizability of the findings. Because this is the first study on empagliflozin as an add-on to three OADs in Iraqi patients, the small sample size means that the current study could be considered a pilot study, and thus it is highly recommended to conduct another study on a larger sample with a multi-center.

Table 4: Predictors for renal and glycemic parameters in diabetic nephropathy

Variables		UACR	eGFRcr	eGFRcys	KIM-1	HbA1c
UACR	β	1	-0.23	-0.02	0.18	-0.22
	p		0.34	0.93	0.45	0.42
eGFRcr	β	-0.23	1	-0.06	0.28	0.39
	p	0.34		0.78	0.20	0.12
eGFRcys	β	-0.02	-0.06	1	0.17	0.34
	p	0.93	0.78		0.47	0.07
KIM-1	β	0.18	0.28	0.17	1	-0.24
	p	0.45	0.20	0.47		0.21
PPG	β	-0.04	-0.06	-0.38	0.43	0.47
	p	0.88	0.79	0.01	0.01	0.04

Multiple Linear regression analysis (beta coefficient) used to find regression coefficient (β); UACR: Urinary albumin to creatinine ratio; eGFRcr:, serum creatinine-based estimated glomerular filtration rate; eGFRcys: serum cystatin c-based estimated glomerular filtration rate; KIM-1: Kidney injury molecule-1; PPG: Postprandial glucose.

Conclusion

In diabetic nephropathy, adding empagliflozin to traditional oral antidiabetic drugs improved albuminuria with a slight increase in serum creatinine. Empagliflozin also effectively reduced renal injury markers, as well as inflammatory and oxidative stress markers.

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Conflict of interests

No conflict of interests was declared by the authors.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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