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ORIGINAL RESEARCH

Effects of Pentoxifylline on Serum Markers of Diabetic Nephropathy in Type 2 Diabetes

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ABSTRACT

Objective: To investigate the effects of pentoxifylline (PTX) in combination with losartan compared to the high dose of losartan alone on serum markers of diabetic nephropathy such as HSP70, copeptin, CRP, and TNFα in patients with type 2 diabetes and nephropathy.

Methods: A single-center, randomized, double-blind, open-label clinical trial was conducted. Sixty-two patients were eligible and allocated to "PTX + losartan" and "high-dose losartan" arms of the trial using software for random number generation. The first arm received 400 mg PTX two times a day (BD) plus 50 mg losartan daily, while the second arm received 50 mg losartan two times a day (BD) for 12 weeks. Comparison of the biomarkers' levels before and after treatment was done using paired sample *t* test variance. ANCOVA was applied to evaluate the comparative efficacy of the two interventions. The effect size was calculated and reported for each biomarker.

Results: Urine albumin excretion (UAE), hs-CRP, and HbA1c significantly decreased in both trial arms compared to the baseline measures. Copeptin and TNFα showed significant differences (after vs before) only in the losartan group ($p = 0.017$ and $p = 0.043$, respectively). The losartan arm was more successful in reducing TNFα, copeptin, HSP70, systolic blood pressure (SBP), and diastolic blood pressure (DBP) values ($p = 0.045$, effect size = 7.3%; $p = 0.018$, effect size 10.1%; $p = 0.046$, effect size 4.7%, $p = 0.001$, effect size 23%; $p = 0.012$, effect size 10.2%, respectively) and the PTX arm was associated with a superior reduction of UAE and hs-CRP levels ($p = 0.018$, effect size 9.1%; $p = 0.028$, effect size 9.2%, respectively).

Conclusion: Add-on PTX to losartan may have more effective anti-inflammatory and anti-albuminuric roles and therefore may be more applicable in the management of diabetic nephropathy compared with high-dose losartan alone.

Trail Registration: Trial number IRCT 20121104011356N10.

Keywords: Pentoxifylline; Diabetic nephropathy; Diabetes type 2; Losartan

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Key Summary Points

Diabetic kidney disease (DKD), also known as diabetic nephropathy, is one of the severe causes of mortality and morbidity in patients with diabetes.

Plenty of serum markers are reported to be associated with renal lesions such as circulating TNF receptors, serum cystatin C, CRP, TNFα, kidney injury molecule-1 (KIM-1), *N*-acetyl-beta-D-glucosaminidase (NAG), liver-type fatty acid-binding protein (L-FABP), heat shock protein 70 (HSP70), and copeptin.

Pentoxifylline is an anti-inflammatory agent, which is a competitive nonselective phosphodiesterase inhibitor that raises intracellular cAMP, activates protein kinase A, inhibits TNF, and leukotriene, which may have effectiveness in chronic kidney disease.

Patients in the pentoxifylline arm experienced comparatively superior reductions in serum hs-CRP levels and UAE rates, and patients in the losartan arm recorded larger reductions in HSP70, TNFα, copeptin, SBP, and DBP.

Add-on pentoxifylline to losartan may be a more effective approach to reduce residual albuminuria and inflammation compared to high-dose losartan alone in the management of diabetic nephropathy.

INTRODUCTION

Diabetic kidney disease (DKD), also known as diabetic nephropathy, is one of the severe causes of mortality and morbidity in patients with diabetes [1]. It is characterized by elevated urine albumin excretion (UAE) or decreased glomerular filtration rate (GFR) or both of these conditions. DKD arises in 20–40% of patients with diabetes [2]. Early detection of symptoms and effective management may slow down or even arrest the progression of DKD. Several risk factors have been established for DKD, including hyperglycemia, hypertension, age, sex, and duration of diabetes. Plenty of serum markers have been reported to be associated with renal lesions such as circulating tumor necrosis factor (TNF) receptors, serum cystatin C, CRP, TNFα [3], kidney injury molecule-1 (KIM-1) [4], *N*-acetyl-beta-D-glucosaminidase (NAG) [5], liver-type fatty acid-binding protein (L-FABP) [6, 7], heat shock protein 70 (HSP70) [8], and copeptin [9]. In addition, osteopontin and N-terminal pro-brain natriuretic peptide (NT-proBNP) are potential risk biomarkers for diabetic disease aggravation [10]. Vascular complications in diabetes are highly associated with inflammation, which is triggered by several factors such as obesity. Adipose tissue inflammation may lead to local hypoxia due to rapid expansion of adipose tissue without sufficient vascular adaptation [11]. The renin–angiotensin system also has a significant role in inflammation, insulin resistance, and vascular damage [12–14]. Pentoxifylline (PTX) is an anti-inflammatory agent, which is a competitive nonselective phosphodiesterase inhibitor that raises intracellular cAMP, activates protein kinase A, and inhibits TNF, and leukotriene [15–17].

There have been previous clinical trials with PTX which, despite their small sample size, have shown statistically significant effects of this drug on stabilizing plaques, slowing the progression of atherosclerosis, and decreasing the risk and improving the outcome of vascular events. Studies suggest that PTX exerts these effects by reducing inflammatory markers and improving blood flow [18, 19]. However, further studies are required to assess the scope of benefits conferred by PTX on outcomes of end-organ damage in patients with diabetes. Some trials showed the benefits of PTX combined with angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin II receptor blockers (ARBs) in the treatment of DKD [19–27]. We designed this trial to investigate the effects of PTX in combination with losartan compared to the high dose of losartan alone on markers of diabetic nephropathy such as HSP70, copeptin,

hs-CRP, TNFα, and UAE in patients with type 2 diabetes and nephropathy.

METHODS

Design

A single-center, randomized, double-blinded clinical trial was conducted. Patients were recruited through the diabetes clinic of Vali-Asr hospital (Tehran, Iran) from August 2019 to February 2020 (IRCT 20121104011356N10). The study protocol is available in the Iranian Registry of Clinical Trials (https://en.irct.ir/trial/46758) and use of human data was in accordance with guidelines of the Helsinki Declaration of 1964 and its later amendments. Written informed consent was obtained from each subject regarding the privacy and anonymity of data collection. All individuals' experiments were approved by the National Institute for Medical Research Development (NIMAD) ethical committee (IR.NIMAD.REC.1398.193).

Subjects were found eligible if the following criteria were met: (1) diagnosis of type 2 diabetes mellitus based on American Diabetes Association (ADA 2019) criteria; (2) UAE rate at least 30 mg/24 h; (3) being on daily losartan 50 mg for at least 3 months. Exclusion criteria were (1) history of current infectious or malignant diseases, non-diabetic kidney disease including glomerulonephritis or tubulointerstitial diseases, retinal hemorrhage, acute myocardial infarction, or unstable angina; (2) history of cardiocerebrovascular or peripheral artery disease, and uncontrolled hypertension (i.e., systolic blood pressure [SBP] at least 140 mmHg and/or diastolic blood pressure [DBP] at least 90 mmHg); (3) history of anemia, hyperthyroidism, and hemodialysis; (4) baseline serum potassium concentration at least 5.5 meq/L; (5) estimated glomerular filtration rate (eGFR) less than 30 mL/min/1.73 m^2; (6) pregnancy; and (7) PTX intolerance. The sample size calculation and power calculation were done on the basis of the proper sample size formula of statistical superiority design, and α and β were 0.05 and 0.2, respectively [28].

Sixty-two patients were eligible and allocated to PTX and losartan arms of the trial using software for random number generation. The first arm received 400 mg PTX two times a day (BD) and 50 mg losartan daily, while the second arm received 50 mg losartan two times a day (BD). Subjects were instructed regarding the side effects of the medication. After 12 weeks, subjects returned for a follow-up visit and were interviewed and examined using the same protocol as the baseline. Written informed consent was obtained from each subject regarding confidentiality and anonymity of data collected, but the details and purpose of the study were not disclosed. Tehran University of Medical Sciences' board of ethics approved the study protocol.

Assessment

During the initial visit, patients were interviewed according to a pre-designed questionnaire and underwent a thorough physical examination afterward. Subjects were asked about the drug history for diabetes and hyperlipidemia. A standard sphygmomanometer (Riester, Big Ben adults, Germany) was used to measured blood pressure. Subjects were asked to rest in a sitting position for at least 10 min; two readings with 5-min intervals were averaged. Height was measured by employing standard stadiometer, with subjects standing; the nearest 0.1 cm was recorded. Weight was assessed via a digital scale (Beurer, GS49, Germany); hence, only light-weight clothing was permitted. The Quetelet formula used to calculate body mass index (BMI), using the of weight in kilograms divided by height squared in meters (kg/m^2). The same examinations were performed at the 12-week follow-up visit.

Laboratory Evaluations

Subjects were instructed to fast overnight for at least 10 h at both initial and 12-week follow-up visits. The next morning, patients' venous blood samples were drawn and stored at − 70 °C in the hospital laboratory. Fasting plasma glucose (FPG) concentrations were

assessed by the enzymatic colorimetric method using the glucose oxidase (GOD) test. The percentage of glycated hemoglobin A1c (HbA1c) was determined using high-performance liquid chromatography (HPLC). Enzymatic methods (Pars Azmun commercial kits, Karaj, Iran) were employed to measure serum concentrations of total cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), and triglycerides (TG). Urine albumin concentrations were quantified by an immunoturbidimetric assay. Serum hs-CRP levels were quantitatively assessed by commercial kits (DRG kit, Germany) using the ELISA (enzyme-linked immunosorbent assay) method. Serum HSP70 levels were assessed using the ELISA method (ELISA kit, Bioassay Technology Laboratory China) with a sensitivity of 0.12 ng/mL (assay range 0.3–90 ng/mL) and interassay and intra-assay coefficient of variations of less than 8% and less than 10%, respectively. TNFα levels were measured using the ELISA method (ELISA kit, Bioassay Technology Laboratory China) with a sensitivity of 1.52 ng/mL (assay range 3–900 ng/mL) and interassay and intra-assay coefficient of variations of less than 8% and less than 10%, respectively. Copeptin levels were measured using the ELISA method (ELISA kit, Bioassay Technology Laboratory China) with a sensitivity of 0.024 ng/mL (standard range 0.05–20 ng/mL) and interassay and intra-assay coefficient of variations of less than 8% and less than 10%, respectively.

Statistical Analysis

To test the normality of our study population Kolmogorov–Smirnov and Shapiro–Wilk normality tests were performed and P–P plot and histogram were illustrated. The null hypothesis was rejected for all the variables; thus, they were normal. *T* test and chi-square analysis were performed to assess the demographic and laboratory data of the group of patients on PTX and losartan and the group of patients on high-dose losartan. Data were reported as mean ± standard deviation (SD) for continuous variables and as proportions for categorical variables. To assess the difference between levels of biomarkers (i.e., HSP70, copeptin, CRP, and TNFα) before and after the treatment in each group of patients, paired sample *t* test was used. Univariate analysis of covariance (ANCOVA) was applied to evaluate the comparative efficacy of the two interventions. The measured markers were entered as dependent variables, while the possible confounder categories and baseline measurements were treated as covariates. The independent variable was the intervention categories.

Model 1 was adjusted for baseline measurements. Model 2 controlled for baseline measurements, age, and gender. Model 3 controlled for baseline measurements, age, gender, BMI, and eGFR. Model 4 controlled for baseline measurements, age, gender, BMI, eGFR, SBP, and DBP.

The effect size was calculated from partial eta squared. The values of 1%, 6%, and 13.8% indicate small, medium, and large effect sizes, respectively. The statistically significant level was set at $p < 0.05$ for all tests. Interaction plots were illustrated. SPSS software version 25 for Windows was used for the statistical analysis.

RESULTS

The distribution of patients in the trial is shown in Fig. 1. A total 62 of the 71 initially screened patients met the inclusion criteria and were randomly assigned to PTX add-on losartan and high-dose losartan groups. One patient of the PTX group was missed during the follow-up. Five patients of the high-dose losartan were lost to follow-up; one of them discontinued the treatment and four of them were missed during the trial.

In total, 56 patients completed the 12 weeks of trial. Thirty and 26 patients remained in the trial in PTX add-on losartan and high-dose losartan groups, respectively. The baseline demographics, clinical, and laboratory characteristics of the two groups had no significant differences (Table 1).

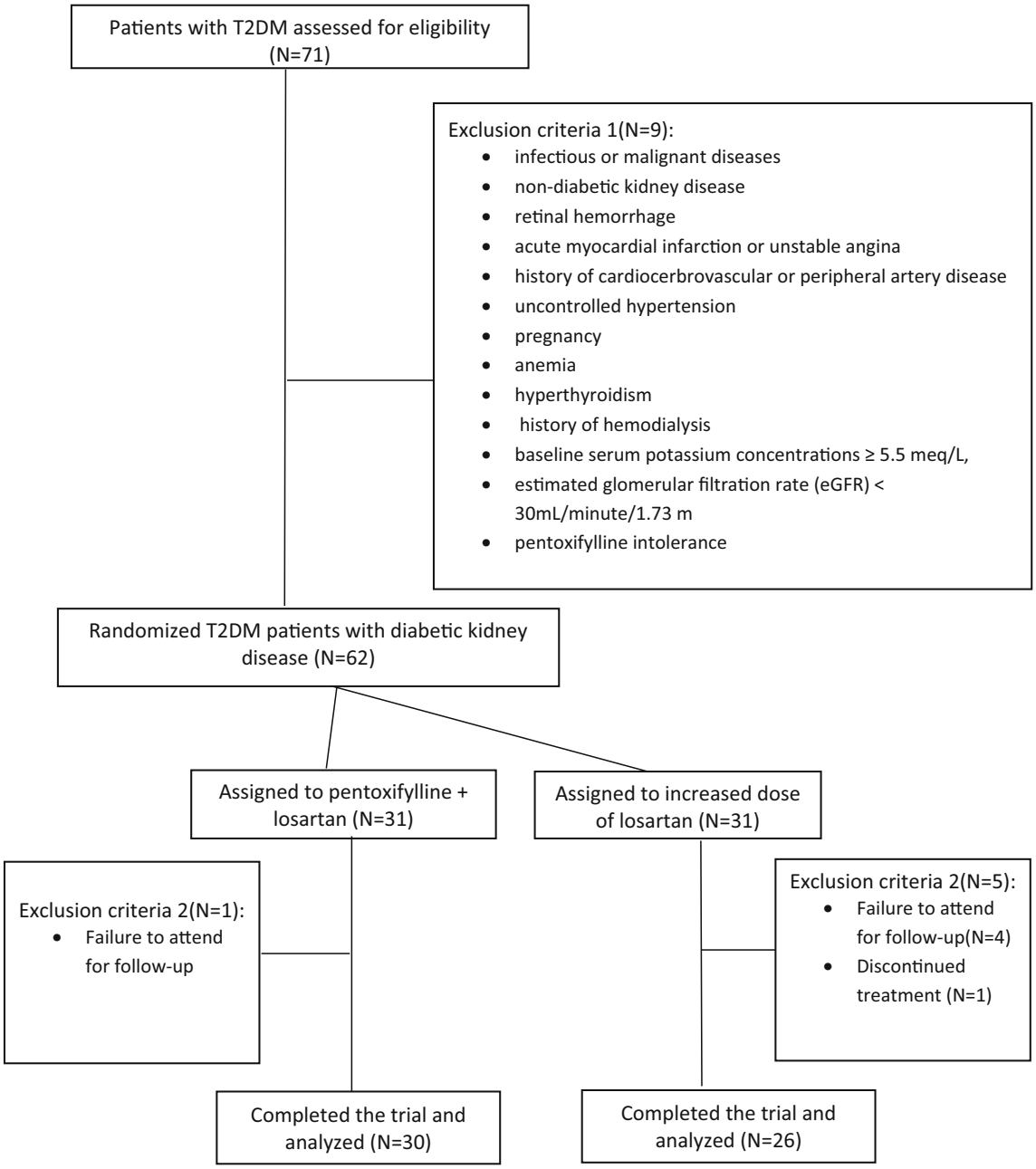

Fig. 1 Flowchart of study population selection

Changes in "Pentoxifylline Add-On Losartan" Arm

UAE declined compared with baseline during the trial (mean difference [95% CI] − 161.1 [− 216.91, − 105.3], $p < 0.001$). hs-CRP was another marker that reduced significantly (mean difference [95% CI] − 1.3 [− 1.87, 0.73], $p < 0.001$). There was also a significant reduction in HbA1c (mean difference [95% CI] − 0.28 [− 0.45, − 0.11], $p = 0.002$). There were no significant changes in HSP70, TNFα, copeptin, SBP,

Table 1 Baseline characteristics of the patients

	Patients on pentoxifylline + losartan ($N = 30$)	Patients on high dose of losartan ($N = 26$)	p value
Age (years)	58.47 ± 7.811	58.0 ± 9.867	0.844
Sex			
Female	8 (26.7%)	13 (50.0%)	0.099
Male	22 (73.3%)	13 (50.0%)	
Duration of diabetes (years)	14.90 ± 6.95	12.08 ± 6.019	0.113
BMI (kg/m^2)	31.425 ± 7.64	27.52 ± 3.53	0.207
SBP (mmHg)	136.83 ± 9.04	136.38 ± 12.55	0.87
DBP (mmHg)	80.54 ± 6.08	80.48 ± 6.3	0.97
FPG (mg/dL)	155.96 ± 47.2	158.5 ± 30.58	0.81
2hpp (mg/dL)	220.07 ± 68.8	202.41 ± 56.15	0.383
HbA1c (%)	8.07 ± 1.00	7.78 ± 1.12	0.31
Total cholesterol (mg/dL)	177.53 ± 38.51	172.50 ± 47.51	0.663
LDL (mg/dL)	98.20 ± 38.15	94.57 ± 39.24	0.728
HDL (mg/dL)	45.03 ± 10.81	39.50 ± 9.84	0.052
Triglycerides (mg/dL)	173.30 ± 76.35	188.53 ± 102.7	0.528
ALT (mg/dL)	22.0 ± 6.37	21.53 ± 4.15	0.754
AST (mg/dL)	19.73 ± 5.24	21.42 ± 7.33	0.321
UAE (mg/24 h)	350.3 ± 212.9	311.6 ± 269.8	0.68
eGFR (mL/min/1.73 m^2)	72.26 ± 23.4	76.55 ± 23.16	0.49
Serum creatinine (mg/dL)	1.22 ± 0.39	1.18 ± 0.32	0.247
Hyperlipidemia drug			
None	3 (10.0%)	4 (15.4%)	0.681
Atorvastatin	26 (86.7%)	21 (80.8%)	
Gemfibrozil	1 (3.3%)	0 (0.0%)	
Atorvastatin + gemfibrozil	0 (0.0%)	1 (3.8%)	
Antidiabetic drug			

and DBP compared to baseline (all p values were more than 0.05) (Table 2).

Changes in "High-Dose Losartan" Arm

Similar to the PTX group, UAE, hs-CRP, and HbA1c decreased significantly in this arm (mean difference [95% CI] − 79.36 [− 105.07, − 53.65] ($p < 0.001$), − 0.53 [− 0.80, − 0.26]

Table 1 continued

	Patients on pentoxifylline + losartan (*N* = 30)	Patients on high dose of losartan (*N* = 26)	*p* value
Metformin	2 (6.7%)	2 (7.7%)	0.481
Metformin + insulin	16 (53.3%)	8 (30.8%)	
Metformin + glibenclamide	4 (13.3%)	7 (26.9%)	
Insulin	7 (23.3%)	8 (30.8%)	
Glibenclamide	1 (3.3%)	1 (3.8%)	

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *BMI* body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *FPG* fasting plasma glucose, *HbA1c* hemoglobin A1c, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *2hpp* 2-h postprandial glucose, *UAE* urinary albumin excretion, *eGFR* estimated glomerular filtration rate

($p < 0.001$), and − 0.2 [− 0.34, − 0.05] ($p = 0.008$), respectively). In contrast to the PTX group, there were significant decreases in copeptin and TNFα serum levels (mean difference [95% CI] − 95 [− 171.7, − 18.3] ($p = 0.017$) and − 30 [− 59.2, − 0.98] ($p = 0.043$), respectively). Moreover, we observed a significant reduction in FPG, SBP, and DBP (mean difference [95%] CI − 12.9 [− 24.3, − 1.36] ($p = 0.03$), − 7.9 [− 10.98, − 4.82] ($p < 0.001$), and − 3.8 [− 6.1, − 1.57] ($p = 0.002$), respectively) (Table 2).

Comparative Efficiency of Interventions

The comparative effects of the two treatment arms in reducing target outcome measures were assessed with ANCOVA modelling and the results are presented in Table 3. The PTX intervention was associated with a superior reduction of UAE and hs-CRP levels compared with the losartan intervention ($p = 0.018$, effect size 9.1%; $p = 0.028$, effect size 9.2%, respectively) and the effect size increased in multivariable adjusted model 4 (model 4: $p = 0.012$, effect size 11.3%; $p = 0.02$, effect size 11.4%, respectively). The losartan arm was more successful in reducing TNFα, copeptin, SBP, and DBP values compared with the PTX arm in the baseline ANCOVA model and after controlling for possible confounders in multivariable adjusted models (baseline model: $p = 0.045$, effect size 7.3%; $p = 0.018$, effect size 10.1%; $p = 0.001$, effect size 23%; $p = 0.012$, effect size 10.2%, respectively) and the effect size increased in multivariable adjusted model 4 (model 4: $p = 0.022$, effect size 10.7%; $p = 0.021$, effect size 10.8%; $p = 0.001$, effect size 25%; $p = 0.007$, effect size 12.6%, respectively). The losartan arm was more efficient in reducing HSP70 compared with the PTX arm after controlling for possible confounders in multivariable adjusted models (model 2: $p = 0.046$, effect size 4.7%, model 3: $p = 0.041$, effect size 4.7%; and model 4: $p = 0.039$, effect size 8.6%) (Table 3). There was no significant difference in HbA1c reduction between the two groups ($p = 0.96$).

DISCUSSION

This study explored the cooperative effects of pentoxifylline add-on losartan and high dose of losartan on serum markers of diabetic nephropathy after 3 months of intervention. Patients in the pentoxifylline experienced comparatively superior reductions in serum hs-CRP levels and UAE rates, and patients in the losartan arm recorded larger reductions in HSP70, TNFα, copeptin, SBP, and DBP measures.

Heat shock proteins (HSP) have protective effects against stressful conditions [29], and there are abnormalities of HSPs in patients with type 2 diabetes [30]. Nakhjavani et al. showed that HSP70 is increased in patients with type 2

Table 2 Mean changes of measured markers in pentoxifylline group and losartan group

Variable	Pentoxifylline group				Losartan group			
	Baseline	After 3 months	Mean difference [95% CI]	*p* value	Baseline	After 3 months	Mean difference [95% CI]	*p* value
HSP70 (ng/mL)	34 ± 16.1	36.9 ± 17.8	2.99 [− 4.9, 10.9]	0.22	31.7 ± 16.7	29.9 ± 11.9	− 1.7 [− 9.1,5.7]	0.63
TNFα (ng/L)	198.5 ± 61.4	217.5 ± 84.5	18.9 [− 8.9, 46.7]	0.17	232.6 ± 100.2	202.5 ± 98.9	− 30 [− 59.2, − 0.98]	0.043
Copeptin (ng/mL)	400.8 ± 101.3	404.7 ± 162.9	3.88 [− 68, 75.8]	0.91	417.4 ± 184.3	322.3 ± 54.1	− 95 [− 171.7, − 18.3]	0.017
hs-CRP (mg/L)	2.35 ± 1.89	1.05 ± 1.04	− 1.3 [− 1.87, − 0.73]	0.001	1.62 ± 0.92	1.08 ± 0.86	− 0.53 [− 0.80, − 0.26]	0.001
UAE (mg/24 h)	350.3 ± 212.9	189.23 ± 110.18	− 161.1 [− 216.91, − 105.3]	0.001	311.6 ± 269.8	232.23 ± 220.3	− 79.36 [− 105.07, − 53.65]	0.001
HbA1c %	8.07 ± 1.00	7.78 ± 0.81	− 0.28 [− 0.45, − 0.11]	0.002	7.78 ± 1.12	7.5 ± 1.06	− 0.2 [− 0.34, − 0.05]	0.008
FBS (mg/dL)	155.96 ± 47.2	152.3 ± 42.2	− 3.6 [− 14.3, 7.1]	0.49	158.5 ± 30.58	145 ± 29.14	− 12.9 [− 24.3, − 1.36]	0.03
SBP (mmHg)	136.83 ± 9.04	135.16 ± 7.75	− 1.67 [− 3.46, 0.11]	0.065	136.38 ± 12.55	128.48 ± 10.96	− 7.9 [− 10.98, − 4.82]	0.001
DBP (mmHg)	80.54 ± 6.08	80 ± 5.1	− 0.54 [− 2.09, 1.00]	0.47	80.48 ± 6.3	76.64 ± 8.01	− 3.8 [− 6.1, − 1.57]	0.002
GFR	72.26 ± 23.4	79.07 ± 28.16	6.81 [− 7.05, 20.68]	0.32	76.55 ± 23.16	74.86 ± 21.59	− 1.68 [− 14.3, 10.93]	0.78

Table 3 Results of ANCOVA for between-group comparisons

Variable	Model 1				Model 2	Model 3	Model 4			
	Adjusted 3-month mean (95% CI)		Effect size %	*p* value	*p* value	*p* value	Adjusted 3-month mean (95% CI)		Effect size %	*p* value
	Pentoxifylline	Losartan					Pentoxifylline	Losartan		
HSP70 (ng/mL)	36.78 (31.22, 42.34)	30.22 (24.36, 36.08)	4.7	0.11	0.046	0.041	37.91 (32.27, 43.55)	28.96 (22.99, 34.92)	8.6	0.039
TNFα (ng/L)	228.93 (202.84, 255.02)	189.36 (161.300, 217.43)	7.3	0.045	0.021	0.019	233.8 (206.19, 261.58	183.65 (153.70, 213.60)	10.7	0.022
Copeptin (ng/mL)	404.77 (358.55, 450.98)	322.30 (272.65, 371.95)	10.1	0.018	0.025	0.025	408.26 (358.61, 457.90)	318.27 (264.64, 371.90)	10.8	0.021
log hs-CRP (mg/L)	− 0.22 (− 0.33, − 0.11)	− 0.04 (− 0.154, 0.074)	9.2	0.028	0.027	0.023	− 0.219 (− 0.32, − 0.11)	− 0.042 (− 0.14, 0.06)	11.4	0.02
log UAE (mg/24 h)	2.19 (2.13, 2.259)	2.30 (2.24, 2.37)	9.1	0.018	0.013	0.021	2.19 (2.12, 2.25)	2.31 (2.24, 2.37)	11.3	0.012
HbA1c%	7.67 (7.53, 7.81)	7.70 (7.56, 7.84)	0.2	0.73	0.98	0.97	7.68 (7.54, 7.83)	7.68 (7.54, 7.82)	0.1	0.96
SBP mmHg	135 (132.86, 137.14	128.63 (126.49, 130.77	23	0.001	0.001	0.001	135.23 (133.03, 137.42)	128.41 (126.21, 130.6	25	0.001
DBP mmHg	79.97 (78.16, 81.78)	76.66 (74.85, 78.48)	10.2	0.012	0.008	0.007	80.225a (78.32, 82.12)	76.420a (74.52, 78.31)	12.6	0.007

Model 1: baseline; Model 2: controlled for baseline, age, and gender; Model 3: controlled for baseline, age, gender, BMI, and eGFR; Model 4: controlled for baseline, age, gender, BMI, eGFR, systolic blood pressure, diastolic blood pressure but variable SBP was adjusted with diastolic blood pressure and DBP was adjusted with systolic blood pressure in model 4

diabetes and could be a potential diagnostic factor in such patients [30]. Recent studies showed that HSP molecules play a role in the pathogenesis of diabetic nephropathy [31, 32], and there is an increase in serum HSP70 levels in patients with albuminuria [8]. Plasma copeptin is a surrogate marker of vasopressin associated with a decline in kidney function and progression of diabetic nephropathy in patients with type 1 and type 2 diabetes [33, 34]. Inflammatory parameters are increased in patients with diabetes, and both serum and urinary inflammatory parameters are independently associated with proteinuria in diabetic nephropathy [35]. Indeed, diabetes is now considered as an inflammatory disease. hs-CRP is a sensitive marker of chronic inflammation [36], and studies have shown that there is an independent association between hs-CRP and UAE in patients with diabetes [35, 37, 38]. It has been known for several years that ACEIs and ARBs reduce UAE through blocking the renin–angiotensin–aldosterone system (RAAS) and thus have a slowing effect on the progression of diabetic nephropathy [39]. The use of ACEI or ARB is currently the first step in the strategy to reduce proteinuria in patients with type 1 and type 2 diabetes [20, 40]. The linkage between diabetes and inflammation has generated interest in anti-inflammatory therapies to slow diabetes and the associated nephropathy progression [41]. Pentoxifylline is a non-specific phosphodiesterase inhibitor with known anti-inflammatory and antifibrotic effects [42]. Potential mechanisms of pentoxifylline action on diabetic nephropathy are manifold. Phosphodiesterase (PDE) is the main enzyme in the synthesis of pro-inflammatory cytokines [43]. Therefore, the inhibitory effect of PTX on PDEs results in the reduction of inflammatory markers such as TNFα, hs-CRP, IL-6, and interferon-α [44]. Several studies have shown that PTX has protective effects on renal function and reduces UAE rates in patients with diabetes [45–49], but there are only a few studies that evaluate the effect of PTX on serum markers of diabetic nephropathy and glycemic indices.

Alidadi et al. conducted a randomized clinical trial in patients on hemodialysis who received either PTX or placebo [50]. They found a significant decrease in serum CRP levels compared to the placebo. Similarly, we found a superior reduction in hs-CRP in the PTX arm. Rabizadeh et al. conducted a randomized trial and assessed the effect of PTX on NT-pro BNP, hs-CRP, and UAE in patients with type 2 diabetes and nephropathy. In agreement with our results, they showed that the PTX arm had a larger reduction in hs-CRP and UAE [51].

Contrary to our expectations, we failed to observe a significant reduction in serum TNFα in the PTX group. Similarly, Han et al. also failed to show any significant changes in serum TNFα in patients with diabetic nephropathy in a randomized clinical trial [19]. A meta-analysis of the use of PTX for treating nonalcoholic fatty liver disease also failed to show significant changes in serum TNFα levels compared with placebo [52]. Another study by Chen et al. showed no significant changes in TNFα during the administration of PTX 800 mg daily in patients with proteinuric primary glomerular diseases [53]. Moreover, Akbari et al. showed that use of PTX in inflammatory processes was a double-edged sword and increased the expression of inflammatory genes in the rat hippocampus as a result of its vasodilatory effects [54].

CONCLUSION

Treatment with PTX add-on losartan in comparison with high-dose losartan monotherapy was more effective in reducing hs-CRP and UAE. In addition, high-dose losartan monotherapy significantly reduced TNFα, SBP, and DBP. In conclusion, add-on PTX to losartan may be a more effective approach to reduce residual albuminuria and inflammation compared to high-dose losartan alone in the management of diabetic nephropathy, although further studies are needed to evaluate the effect of add-on PTX on serum markers of diabetic nephropathy.

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Compliance with Ethics Guidelines. The study protocol is available in the Iranian Registry of Clinical Trials (IRCT 20121104011356N10) (https://en.irct.ir/trial/46758) and use of human data was in accordance with guidelines of the Helsinki Declaration of 1964 and its later amendments. Written informed consent was obtained from each subject regarding the privacy and anonymity of data collection. All individuals' experiments were approved by the National Institute for Medical Research Development (NIMAD) ethical committee (IR.NIMAD.REC.1398.193).

Data Availability. The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

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