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Short Communication

Effect of Low-Molecular-Weight Heparin Calcium on Senile Diabetes Nephropathy

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HL and DL conducted the experiments in this study. YX and XG contributed to the design and interpretation of the current study and wrote the article. All authors read, revised, and approved the final manuscript.

Key words
Low molecular weight, Heparin calcium, Old age, Diabetes nephropathy, Application effect

ABSTRACT

The objective of this study was to explore the impact of calcium-based low-molecular-weight heparin on nephropathy in elderly individuals with diabetes. We selected ninety elderly patients diagnosed and treated at our hospital from March 2020 to October 2022. The sample was randomly assigned to a control group (CG) (n=45) and an observation group (OG) (n=45). The CG received a standard treatment, while the OG received a low-molecular-weight heparin calcium in addition to the standard treatment. The OG showed a higher overall treatment effectiveness. After the treatment, the OG displayed significantly lower levels of blood urea nitrogen, serum creatinine, fibrinogen and platelet count and higher levels of creatinine clearance. Calcium-based low-molecular-weight heparin demonstrates favorable clinical outcomes in the management of elderly patients with diabetes-related nephropathy. This treatment offers benefits such as improved renal function, the elimination of protein-bound toxins, enhanced blood coagulation parameters, and better glycemic and lipid metabolism indicators, all contributing to the reduction of micro-inflammatory reactions in the body.

Diabetic nephropathy (DN) is the most common cause of end-stage renal disease (ESRD) in patients with diabetes (Din *et al*., 2023). It refers to the systemic microvascular disease in diabetic patients, leading to inadequate arterial perfusion, microcirculatory disorders, subsequent impairment of glomerular filtration function, and ultimately causing renal failure (Dong *et al*., 2022). It is also a factors contributing to disability and mortality in diabetes patients (Bejoy *et al*., 2022). Symptoms commonly seen in diabetic kidney disease encompass issues like protein leakage in urine, elevated blood pressure, swelling, and a range of kidney function abnormalities. As the condition worsens, severe renal dysfunction gradually develops, leading to disturbances in protein, amino acid, lipid, and glucose metabolism, as well as water, electrolyte, and acid-base balance, ultimately affecting the functions of various body systems. Therefore, the prevention and treatment of DN have become a hot topic in the clinical management of diabetes complications (Liu *et al*., 2022).

The pathogenesis is not yet fully understood, but as a chronic inflammatory and immune-related disease, vascular inflammation and thrombosis are important factors contributing to the onset of DN patients (Goo *et al*., 2022). Clinical research has shown that bleeding, thrombosis, and embolism issues often arise during the treatment of DN patients, affecting treatment outcomes and leading to poor prognosis (Jiang *et al*., 2021). Low molecular weight heparin calcium is a small molecule antithrombotic agent widely used in clinical practice. Studies have shown that low-molecular-weight heparin calcium not only possesses strong anticoagulant properties but also has effects on reducing renal basement membrane thickening (Niu *et al*., 2020). This study aims to explore

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the application of low molecular weight heparin calcium in elderly DN patients, with the hope of providing clinical guidance for diagnosis and treatment.

Materials and methods

The sample for this study consisted ninety consecutively admitted patients with DN at our hospital between March 2020 and October 2022. Patients in both groups were eligible to join the study if they were clinically diagnosed with DN based on symptoms, signs, and laboratory tests (Zhou *et al*., 2021), had good adherence to treatment, had complete clinical records, and were aged 65 years or older. The patients with severe diabetes complications, such as ketoacidosis, with other endocrine disorders, with severe heart or liver dysfunction, with malignant tumors, with known allergies to the medications used in this study, and with other urinary system disorders were excluded from the study.

Two groups, control group (CG) and observation group (OG), each of 45 patients were recruited. The CG received a routine treatment, including dietary adjustment, essential amino acid, high-quality low-protein diet, and adequate caloric intake; infection was controlled and anemia was corrected, blood pressure, diuretic, and glycemic control, correction of water, electrolyte, and acid-base balance disorders, and active treatment of the primary disease, among others. The OG group received subcutaneous injection of low-molecular-weight heparin calcium (National Drug Approval Number H19990077, Changshan Biochemical Pharmaceutical Co., Ltd., Jiangsu), 5000 U per injection, once daily, continuously for 8 weeks.

Three milliliters of fasting peripheral venous blood were collected from all study subjects, and serum was separated at 3500 r/min for 10 min and stored at -80°C. Serum levels of tumor necrosisfactor-α (TNF-α), interleukin 6 (IL-6), interleukin 10 (IL-10), serum creatinine (Scr), creatinine clearance (Ccr), blood urea nitrogen (BUN), indoxyl sulfate (IS), p-cresyl sulfate (PCS), chemokines, and adiponectin were determined using enzyme-linked immunosorbent assays (Varioskan LUX fully automatic enzyme immunoassay instrument, Thermo Fisher Scientific, USA). Fibrinogen (FIB), activated partial thromboplastin time (APTT), platelet count (PLT), and partial thrombo plastin time (PT) were measured using an automatic coagulation analyzer (ACL TOP 700, Werfen, USA).

Data were processed using SPSS 20.0. For categorical data, the chi-squared test was applied, while numerical data were presented as mean ± standard deviation ($\bar{x}\pm s$). t-test was applied, with significance of $P<0.05$.

Results and discussion

The gender distribution was 21 males and 24 females, rage from 66 to 82 years with an average age of 70.34±10.62 years in the CG and 22 males and 23 females, aged 65 to 81 years, with an average age of 71.72±11.20 years in the OG. While the duration of illness was 1 to 12 years with a duration of 4.64±1.62 years, it was 1 to 13 years with a duration of 4.50±1.03 years in the OG. There were no statistically significant differences in the general characteristics of the two groups ($P>0.05$), ensuring their comparability. Table I demonstrates that the clinical effectiveness rate in the OG was markedly superior ($P<0.05$).

Table I. Results of clinical efficacy [n (%)].

	OG (n=45)	CG (n=45)	χ^2	P
Apparent effect	19(42.22)	11(24.44)		
Effective	23(51.11)	24(53.33)		
Ineffective	3(6.67)	10(22.22)		
Effective rate	42(93.33)	35(77.78)	4.406	0.036

Prior to therapy, there existed no notable variance in the kidney function metrics among the patient cohorts ($P>0.05$). Following the intervention, both groups displayed markedly reduced BUN and Scr levels compared to their pre-treatment measurements, with the OG exhibiting significantly superior outcomes ($P<0.05$). Additionally, Following the treatment, there was a substantial improvement in CCr levels in both cohorts, with the OG exhibiting a marked superiority over the CG to a significant degree ($P<0.05$) (Table II).

Following the therapeutic interventions, both sets of patients exhibited notably elevated PT and APTT levels in comparison to their pre-treatment values, with the OG surpassing the CG to a significant degree ($P<0.05$). Furthermore, the levels of FIB and PLT markers registered significant reductions from their pre-treatment levels, with the OG displaying a markedly superior outcome ($P<0.05$) (Table II).

Prior to the initiation of treatment, there existed no discernible statistical contrast in the presence of protein-bound toxins between the two patient cohorts ($P>0.05$). However, subsequent to the therapeutic regimen, both groups experienced a substantial reduction in the levels of IS and PCS, with the OG demonstrating a pronounced superiority ($P<0.05$) (Table II).

Prior to the commencement of therapy, no noticeable statistical divergence was evident in inflammatory markers ($P>0.05$). Nevertheless, following the treatment regimen, both groups experienced a marked reduction IL-6 and

Table II. Effect of low molecular weight heparin calcium on various biochemical indicators of nephropathy in patients with diabetes.

Indicators	Control group (n=45)		Observational group (n=45)		t	P
	Pre treatment	Post treatment	Pre treatment	Post treatment		
BUN (mmol/L)	14.02±4.80	10.14±3.63	14.12±4.72	7.76±2.58	-3.585	0.001
Scr (μmol/L)	280.58±73.81	250.70±64.42	283.46±80.92	224.25±58.35	-2.041	0.044
CCr (ml/min)	27.63±8.41	31.84±9.25	27.56±8.35	36.62±10.20	2.207	0.030
PT (s)	8.12±1.48	11.56±2.84	8.24±1.37	13.04±1.56	3.064	0.003
APTT (s)	23.72±3.74	29.46±5.40	24.14±4.85	33.26±3.74	3.881	0.000
FIB (g/L)	4.05±0.97	3.46±0.70	3.98±0.82	2.74±0.55	-5.426	0.000
PLT ($\times 10^9$/L)	308.50±20.87	258.64±32.83	312.62±24.67	205.60±22.74	-8.909	0.000
IS (mg/L)	31.76±10.13	27.23±8.14	32.53±9.23	23.16±7.24	-2.506	0.014
PCS (mg/L)	23.15±7.82	19.85±6.12	22.52±7.21	17.23±5.93	-2.062	0.042
TNF-α (μg/L)	21.82±4.10	15.42±2.85	22.41±3.58	11.38±3.14	6.391	0.000
IL-10 (pg/mL)	4.53±1.24	6.13±1.73	4.30±1.12	7.52±1.82	-3.713	0.000
IL-6 (pg/mL)	43.21±7.25	30.37±5.42	42.56±8.23	23.42±4.37	6.696	0.000
Chemokines (mg/L)	8.92±2.16	5.34±1.72	8.45±2.56	3.72±1.15	5.252	0.000
Adiponectin (mg/L)	3.23±1.25	9.47±3.80	3.34±1.24	12.26±4.33	-3.249	0.002

BUN, blood urea nitrogen; Scr, serum creatinine; CCr, creatinine clearance; PT, partial-thromboplastin time; APTT, activated partial-thromboplastin time; FIB, fibrinogen; PLT, platelet count; IS, indoxyl sulfate; PCS, p-cresyl sulfate; TNF-α, tumour necrosis factor; IL, interleukin. Notable Outcome: Marked reduction in Scr and BUN values by ≥ 20%, accompanied by amelioration in clinical indications and manifestations; Moderate Outcome: Scr and BUN levels reduced by 10% to 20%, along with alleviation of clinical symptoms and signs; Unsuccessful Outcome: Scr and BUN levels remain unchanged, and clinical symptoms and signs show no improvement.

TNF-α, as evidenced by a significant decrease (P<0.05), with the OG displaying a notably superior outcome (P<0.05). Additionally, the IL-10 levels saw a significant elevation compared to their pre-treatment values (P<0.05), with the OG surpassing the CG to a significant extent (P<0.05) (Table II).

Prior to the commencement of therapy, there was a lack of statistically notable variance in the serum concentrations of chemokines and adiponectin between the two sets of individuals (P>0.05). Subsequent to the treatment regimen, both groups experienced a marked reduction in serum chemokine levels compared to their pre-treatment values, with the OG displaying a significantly superior outcome relative to the CG (P<0.05). Furthermore, serum adiponectin levels exhibited a significant increase in both groups (P<0.05), with the OG surpassing to a marked degree (P<0.05) (Table II).

The use of low molecular weight heparin calcium in DN patients has a favorable clinical effect, which is beneficial for improving coagulation parameters and renal function. Scr is a metabolic product of muscle in the body, primarily excreted through glomerular filtration into urine (Mikhailov *et al*., 2022). When patients experience renal parenchymal damage, glomerular filtration rate decreases, leading to elevated BUN and Scr, as well as decreased CCr. Therefore, urinary Scr, BUN, and CCr are important indicators for diagnosing renal dysfunction and assessing treatment efficacy. PCS and IS are gut-derived protein-bound toxins known to have clear toxic effects on multiple organs. When renal function is normal, these toxins are excreted from the body via the kidneys. However, in cases of severe renal impairment in DN patients, renal clearance rate decreases, leading to the accumulation of IS and PCS in the body. This not only accelerates renal fibrosis but also increases the risk of cardiovascular complications (Noori *et al*., 2022). Low molecular weight heparin calcium, prepared by depolymerizing regular heparin, has been found to not only regulate renal hemodynamics and reduce renal hypercoagulability but also act as a negatively charged molecule similar in structure to heparan sulfate on the glomerular basement membrane. It can help restore the anionic charge barrier of the damaged capillary basement membrane, effectively reducing albuminuria (Cassinelli *et al*., 2020). Negatively charged low molecular weight heparin calcium can also bind to endothelial cells, improve coagulation factor activity, inhibit the formation of microthrombi within glomeruli, ensure unobstructed renal blood flow, and thus effectively improve renal function. It also promotes the clearance of protein-bound toxins within the body, reducing levels of blood creatinine and urea nitrogen and slowing down the progression of renal failure (von Zur-Mühlen *et al*., 2019).

It has been suggested that chemokines also function as adipokines, and studies have found that serum chemokine levels may be related to oxidative stress associated with renal function impairment in DN patients (Zhao *et al*.,

2020). This study's findings suggest that post-treatment, the OG experienced substantial reductions in the levels of IL-6 and TNF-α compared to their respective pre-treatment levels. Simultaneously, IL-10 exhibited a marked increase relative to. Furthermore, following the treatment regimen, the OG displayed notable declines in serum chemokine levels when contrasted with their pre-treatment levels and those of the CG. Additionally, the serum adiponectin levels in the OG significantly escalated post-treatment in comparison to their pre-treatment values. The use of low-molecular-weight heparin calcium in of DN patients is beneficial for improving metabolic parameters, reducing micro-inflammatory responses, and regulating adipokines. Studies have found that low molecular weight heparin calcium can improve lipid metabolism. Furthermore, it can reduce the expression of nuclear factors and pro-inflammatory factors caused by endotoxins, exerting anti-inflammatory effects (Zou *et al*., 2019).

Conclusion

The use of low molecular weight heparin calcium in the treatment of elderly diabetic nephropathy patients helps improve renal function, clear protein-bound toxins from the body, enhance coagulation parameters and metabolic indicators, and mitigate micro-inflammatory responses within the body.

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Ethical approval

The study was carried out in compliance with guidelines issued by ethical review board committee of Shaanxi Kangfu Hospital, China. The official letter would be available on fair request to corresponding author.

IRB approval

This study was approved by the Advanced Studies Research Board of Shaanxi Kangfu Hospital, Shaanxi Province, China.

Statement of conflict of interest

The authors have declared no conflict of interest.

References

Bejoy, J., Farry, J.M., Peek, J.L., Cabatu, M.C., Williams, F.M., Welch, R.C., Qian, E.S. and Woodard, L.E., 2022. *Stem. Cell. Res. Ther.,* **13**: 355. https://doi.org/10.1186/s13287-022-03040-6

Cassinelli, G., Torri, G. and Naggi, A., 2020. *Adv. Exp. Med. Biol.,* **1221**: 493-522. https://doi.org/10.1007/978-3-030-34521-1_20

Dong, S., Li, N., Zhang, J. and Wang, T., 2022. *Pakistan J. Zool.*, **54**: 1591-1597. https://doi.org/10.17582/journal.pjz/20201110141129

Goo, Y.J., Song, S.H., Kwon, O.I., Kim, M., Suh, S.H., Oh, T.R., Choi, H.S., Bae, E.H., Ma, S.K. and Kim, S.W., 2022. *Annls Palliat. Med.,* **11**: 2756-2760. https://doi.org/10.21037/apm-21-2025

Jiang, L.Q., Zhou, J.Y., Zhang, L., Du, Y.F., Jiang, M.M., Xie, L.Q., Ma, Z.N. and Chen, F.L., 2021. *Glycoconj. J.,* **38**: 697-707. https://doi.org/10.1007/s10719-021-10035-7

Kuo, C.W., Chen, H.L., Tu, M.Y. and Chen, C.M., 2019. *Am. J. Physiol. Renal. Physiol.,* **316**: F32-F41. https://doi.org/10.1152/ajprenal.00401.2017

Liu, F., Zhang, H., Wu, H., Yang, S.K., Liu, J. and Wang, J.W., 2022. *J. Pharmacol. exp. Ther.,* **384**: 296-305. https://doi.org/10.1124/jpet.122.001138

Mikhailov, A.V., Liu, Y., Cheng, H.J., Lin, J.J. and Cheng, C.P., 2022. *Heliyon,* **8**: e11653. https://doi.org/10.1016/j.heliyon.2022.e11653

Niu, Q.Y., Yang, S., Gan, L.Y., Zhao, H.P. and Zuo, L., 2020. *Nephrology,* (Carlton), **25**: 551-558. https://doi.org/10.1111/nep.13632

Noori, T., Sureda, A. and Shirooie, S., 2022. *Fundam. clin. Pharmacol.,* **22**: 1-19.

Din, U.A., Khan, W.A., Shahzad, K., Ali, B., Hussain, M. and Awan, F.R., 2023. *Pakistan J. Zool.*, **55**: 1537-1544. https://doi.org/10.17582/journal.pjz/20220628070655

von Zur-Mühlen, B., Lundgren, T., Bayman, L., Berne, C., Bridges, N., Eggerman, T., Foss, A., Goldstein, J., Jenssen, T., Jorns, C., Morrison, Y., Ryden, M., Schwieger, T., Tufveson, G., Nilsson, B. and Korsgren, O., 2019. *Transplantation,* **103**: 630-637. https://doi.org/10.1097/TP.0000000000002425

Zhao, K., Ding, W.Y., Zhang, Y., Ma, K.H., Wang, D., Hu, C.H., Liu, J.J. and Zhang, X.D., 2020. *Int. J. Biol. Macromol.,* **165**: 1574-1580. https://doi.org/10.1016/j.ijbiomac.2020.10.030

Zhou, X.F., Zhou, W.E., Liu, W.J., Luo, M.J., Wu, X.Q., Wang, Y., Liu, P., Wen, Y.M., Li, J.L. and Zhao, T.T., 2021. *J. Transl. Int. Med.,* **9**: 98-113. https://doi.org/10.2478/jtim-2021-0020

Zou, P.M., Li, H., Cai, J.F., Li, C., Chen, Z.J. and Li, X.W., 2019. *Ren. Fail.,* **41**: 623-628. https://doi.org/10.1080/0886022X.2019.1635030