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Effectiveness of glycosaminoglycan in patients with glomerulonephritis: A prospective longitudinal cohort study

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Abstract. *Our study aimed to evaluate the effectiveness of Sulodexide in the treatment of patients with glomerulonephritis(GN).*

Methods. We conducted a prospective longitudinal cohort study involving 105 patients with CKD, who were hospitalized at the Ivano-Frankivsk Regional Clinical Hospital (Ukraine) from 2021-2022. Only patients with proteinuria of 0.3 g – 3.5 g/day and chronic kidney disease (CKD) stages 1-3 and the diagnosis of GN were included in the study. The clinical diagnosis was determined based on standard examination methods according to the Classification of Kidney Diseases and protocols of management of CKD patients

All patients were randomly assigned into 2 groups. Group 1 received basic therapy, and Group 2 received Sulodexide in oral capsules containing 250 lipasemic units (LSU) twice daily in addition to basic therapy. The duration of treatment was 6 months.

Results. Our study showed that the additional use of sulodexide for 6 months significantly reduced the level of proteinuria in patients with GN, so in the second group, it was possible to achieve a significantly lower level of proteinuria 567 (356; 745) mg/day, compared to the first group 956 (765; 1233) mg/day (p 0.05).

As the glomerular filtration rate remained stable or even increased, proteinuria reduction cannot be explained by alteration of filtration capacity. We also noted a significant decrease in the level of D-dimers in the group of patients who additionally received Sulodexide for 6 months, which may indicate the prevention of thromboembolic and cardiovascular events in this population of patients. However, this hypothesis needs further research.

Conclusions. The additional use of Sulodexide in patients with GN statistically significantly reduced proteinuria levels preventing CKD progression rate.

Keywords. *chronic kidney disease, glomerulonephritis, treatment, glycosaminoglycans, sulodexide.*

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Ефективність застосування глікозаміноглікану у хворих на гломерулонефрит: проспективне поздовжнє когортне дослідження

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Резюме. *Метою нашого дослідження було оцінити ефективність сулодексиду в лікуванні хронічної хвороби нирок: гломерулонефриту.*

Методи. Ми провели проспективне поздовжнє когортне дослідження за участю 105 пацієнтів із хронічною хворобою нирок, які перебували на стаціонарному лікуванні в Івано-Франківській обласній клінічній лікарні (Україна) протягом 2021-2022 років. У наше дослідження включені лише пацієнти з протеїнурією 0,3 г – 3,5 г/добу та хронічною хворобою нирок (ХХН) I-III стадій з діагнозом гломерулонефрит (ГН). Клінічний діагноз встановлено на основі загальноприйнятих методів обстеження відповідно до класифікації хвороб нирок та протоколів ведення хворих із ХХН.

Усі пацієнти були випадковим чином розподілені на 2 групи. Перша група отримувала базисну терапію, а друга група додатково до базисної терапії отримувала сулодексид в капсулах по 250 ліпопротеїнліпазних одиниць двічі на день. Тривалість лікування становила 6 місяців.

Результати. Наше дослідження показало, що додаткове застосування сулодексиду протягом 6 місяців значно зменшувало рівень протеїнурії у пацієнтів з ГН, так у другій групі вдалось досягнути достовірно нижчого рівня протеїнурії 567 (356; 745) mg/day, в порівнянні з першою групою 956 (765; 1233) mg/day (p 0,05).

Оскільки швидкість клубочкової фільтрації залишалася стабільною або навіть зростала, зниження протеїнурії не можна пояснити зміною фільтраційної здатності. Також ми відзначили достовірне зниження рівня Д-димерів у групі пацієнтів, які додатково отримували сулодексид протягом 6 місяців, що може свідчити про зниження ризику виникнення тромбоемболічних та серцево-судинних подій у даної категорії пацієнтів. Проте дану гіпотезу потрібно перевірити в подальших дослідженнях.

Висновки. Додаткове застосування сулодексиду в лікуванні гломерулонефриту статистично значуще знижує рівень протеїнурії, що сприяє зниженню швидкості прогресування ХХН.

Ключові слова. *Хронічна хвороба нирок, гломерулонефрит, лікування, глікозаміноглікани, сулодексид.*

Introduction. Chronic kidney disease (CKD) is a global health care problem [1-3]. During the last decades, the development of safe and effective kidney-protective interventions to slow or stop the progression of CKD is an important strategy in reducing the incidence of end-stage kidney disease [1]. Thus, it is to be understood why many studies have focused on the investigation of molecules that could contribute to reducing proteinuria and slowing down the progression of CKD [2-4].

Significant steps in this direction were made by introducing angiotensin-converting enzyme inhibitors (ACEIs) in 1993 and angiotensin receptor blockers (ARBs) in 2001, while other molecules like glycosaminoglycans (GAGs), despite promising data in experimental studies, are still gathering evidence of clinical applicability [5].

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One of the pathogenic mechanisms leading to proteinuria in GN involves an alteration in heparin sulfate expression in the glomerular basement membrane (GBM). Heparin sulfate is a member of the family of GAGs that is generally bound to a core protein to form a proteoglycan [6]. Alterations in heparin sulfate expression in the GBM had been reported in many proteinuric renal diseases which include diabetic nephropathy, minimal change nephropathy, and membranous GN [7]. The decrease of heparin sulfate content causes a reduction in the permselectivity to negatively charged macromolecules such as albumin thus allowing protein to leak into the urinary space [8].

Sulodexide (SDX) belongs to a class of GAGs. Experimental and clinical studies identified several mechanisms of action of Sulodexide which can explain its potential benefit in renal diseases [9]. Thus, SDX can replace lost endogenous heparan sulfate and restore the anionic charges, both at vascular endothelial and glomerular cell levels [10].

Restoration of heparan sulfate in mesangial cells inhibits their proliferation, and in podocytes contributes to the restoration of GBM permeability to albumin [8]. At the extracellular level, SDX precludes the extracellular matrix expansion and type III and type

IV collagen deposition, by inhibiting of the expression and bioactivity of transforming growth factor TGF-β1. Also, SDX inhibits heparanase-1, preventing epithelial-mesenchymal transition and renal interstitial fibrosis induced via fibroblast growth factor-2 [11]. Most studies of the antiproteinuric effect of SDX have been carried out in patients with type 1 or type 2 diabetes mellitus. The antiproteinuric effect of sulodexide in GN is less well studied [12].

The **present study aimed** to evaluate the effectiveness of SDX in the treatment of patients with GN.

Materials and Methods. We conducted a prospective longitudinal cohort study involving 105 patients with GN admitted to the Ivano-Frankivsk Regional Clinical Hospital (Ukraine) in 2021-2022. The research was performed in accordance with international standards for the coordinated participation of respondents, the ethical component of research and biomaterial collection (WMA Declaration of Helsinki – "Ethical principles for medical research involving human subjects" and "Universal Declaration on Bioethics and Human Rights" (UNESCO)). The research protocol was approved by the local ethics committee of the Ivano-Frankivsk National Medical University. All patients signed a written informed consent to participate in the study.

Among the patients, there were 92 men (87.6 %; 95% CI 79.8-93.2) and 13 women (12.4 %; 95% CI 6.8-20.2). The average age of the patients was 46 (41; 49) years. The criteria for including patients in the study were: age over 18 years, presence of proteinuria 0.3-3.5 g/day, glomerular filtration rate (GFR) >30 ml/min/1.73m^2. Exclusion criteria were: patient's refusal to participate in the study, age <18 years, proteinuria > 3.5 g/day, systemic connective tissue diseases, systemic vasculitis, type 1 and type 2 diabetes, thromboembolic and cardiovascular events in history, chronic heart failure III-IV functional class (according to the NYHA classification), information about acute infectious processes of any etiology, oncological diseases, acute and chronic liver failure, mental disorders.

All patients have been receiving treatment with an ACEI or an ARB. 32 patients (30.5%; 95% CI 21.9-40.2) received stable maintenance glucocorticosteroid therapy (methylprednisolone 4 mg every other day). The effectiveness of treatment was assessed by the level of proteinuria, GFR, and D-dimers and the frequency of thromboembolic and cardiovascular events that occurred during the observation period.

The starting point of patient observation was the date of signing the informed consent. The primary endpoint of the study was death from any cause, and surrogate endpoints were the occurrence of cardiovascular and thromboembolic events.

CKD stage 1 was diagnosed in 33 patients (31.4%; 95% CI 22.7-41.2), CKD stage 2 – in 21 patients (20.0%; 95% CI 12.8-28.9), CKD stage 3a – in 22 patients (21.0%; 95% CI 13.6-30.0), and CKD stage 3b – in 29 patients (27.6%; 95% CI 19.3-37.2).

In 65 patients (61.9%; 95% CI 51.9-71.2) the diagnosis of GN was confirmed morphologically as follows 24 patients (36.9%; 95% CI 25.3-49.8) were diagnosed with mesangioproliferative GN, 13 patients (20.0%; 95% CI 11.1-31.8) had membranous nephropathy, 12 patients (18.5%; 95% CI 9.9-30.0) had focalsegmental glomerulosclerosis, 10 patients (15.4%; 95% CI 7.6-26.5) had nephropathy with minimal changes, and 6 patients (9.2%; 95% CI 3.5-19.0) was confurmed membrane proliferative (mesangiocapillary) GN.

The clinical diagnosis was determined based on standard examination methods according to the Classification of Kidney Diseases and protocols of management of CKD patients. During the study, all patients underwent a standard examination, which included general clinical, biochemical and instrumental research methods. Biochemical tests and enzyme-linked immunosorbent assays (ELISAs) were performed in the laboratory of Ivano-Frankivsk Regional Clinical Hospital.

The GFR was determined using a CKD-EPI calculator. Daily protein excretion (DPE) was determined by the colorimetric method (Dialab, Wiener Neudorf, Austria).

D-dimer was determined in serum quantitatively by ELISA using a set of reagents (Getein Biotech, Nanjing, China).

STATISTICA 8 software (StatSoft, Serial STA862D175437Q) was used for statistical analysis. The frequency of qualitative indicators was presented in absolute (n) and relative (%) frequencies with the indication of the 95% confidence interval (CI) in the form of "n (%; 95% CI)". When analyzing quantitative data, it was necessary to determine the nature of the distribution of indicator values using Shapiro-Wilk's test. For quantitative data with a normal distribution, the results were represented as a mean value and the standard deviation (M±SD). For quantitative data with an abnormal distribution, the median and 25-75 quartiles (Me (Q25-Q75) were used. Quantitative indicators with the normal distribution of values in 2 independent groups were compared using the Student's criterion. The abnormally distributed data were compared using the Mann-Whitney test. A comparison of 2 independent groups for the qualitative indicator was carried out according to the exact Fisher criterion.

Results. All patients were randomly assigned into 2 groups. Group 1 received basic therapy, and group 2 in addition to basic therapy received SDX in oral capsules of 250 lipasemic units equivalent to 25 mg twice daily. The duration of treatment was 6 months.

Baseline demographics, and clinical and laboratory characteristics were tabulated in Table 1.

Table 1

Baseline characteristics of the studied groups

	I group (n=55)	II group (n=50)	p-value
Age, years Me (Q25-Q75)	42 (36; 48)	48 (42; 51)	0.871
Disease duration, years (Me (Q25-Q75)	10 (7; 14)	12 (8; 16)	0.644
Mesangioprolifera-tive GN (%; 95% CI)	21.8 (11.8-35.0)	24.0 (13.1-38.2)	0.812
Focalsegmental glomerulosclerosis (%; 95% CI)	10.9 (4.1-22.2)	12.0 (4.5-24.3)	1.000
Membranous GN (%; 95% CI)	12.7 (5.3-24.5)	12.0 (4.5-24.3)	1.000
GN with minimal changes (%; 95% CI)	9.1 (3.0-20.0)	10.0 (3.3-21.8)	1.000
Mesangiocapillary GN (%; 95% CI)	5.5 (1.1-15.1)	6.0 (1.3-16.5)	1.000
Creatinine μmol/L Me (Q25-Q75)	128.5 (91.4; 175.3)	168.2 (125.2; 212.8)	0.433
Urea, mmol/L Me (Q25-Q75)	11.4 (7.8; 13.3)	14.8 (11.5; 16.3)	0.642
GFR ml / min. / 1.73 m^3 Me (Q25-Q75)	62 (37; 92)	66 (43; 94)	0.761
DPE mg / day Me (Q25-Q75)	1832 (1123; 2545)	2242 (1987; 2624)	0.124
D-dimer, mg / l Me (Q25-Q75)	0.98 (0.26; 1.64)	1.23 (0.45; 2.15)	0.453
Treatment of glucocorticosteroids (%; 95% CI)	29.1 (17.6- 42.9)	32.0 (19.5-46.7)	0.832
Treatment of ACEIs (%; 95% CI)	58.2 (44.1-71.3)	58.0 (43.2-71.8)	1.000
Treatment of ARBs (%; 95% CI)	41.8 (28.7-55.9)	42.0 (28.2-56.8)	1.000

Abbreviations: ACEIs – angiotensin-converting enzyme inhibitors; ARBs – angiotensin receptor blockers; CI – confidence interval; DPE – daily protein excretion; GFR – glomerular filtration rate; Me (Q25-Q75)– median and quartiles.

As shown in Table 1, there were no significant differences in baseline demographic and clinical values between the two groups studied.

Following 6 months of treatment with low-dose SDX, we observed a significant decrease in proteinuria levels in patients with GN (Table 2).

Table 2

Baseline and post-treatment laboratory parameters of the studied groups

Treatment group	Baseline	1 month	6 month
DPE mg/day Me (Q25-Q75)			
I group (n=55)	1832 (1123; 2545)	1343 (986; 1678) p=0.125	956 (765; 1233) p=0.032 p_1=0.041
II group (n=50)	2242 (1987; 2624)	879 (657; 1158) p=0.016	567 (356; 745) p=0.001 p_1=0.027 p_2=0.024

Continuation of Table 2

Treatment group	Baseline	1 month	6 month
GFR ml/min./1.73 m^3 Me (Q25-Q75)			
I group (n=55)	62 (37; 92)	64 (39; 97) p=0.872	59 (32; 89) p=0.891 p_1=0.845
II group (n=50)	66 (43; 94)	69 (45; 96) p=0.916	73 (50; 102) p=0.652 p_1=0.774 p_2=0.081
D-dimer, mg/l Me (Q25-Q75)			
I group (n=55)	0.98 (0.26; 1.64)	1.12 (0.67; 1.89) p=0.647	1.24 (0.78; 1.92) p=0.472 p_1=0.721
II group (n=50)	1.23 (0.55; 2.15)	0.68 (0.35; 1.05) p=0.027	0.45 (0.21; 0.63) p=0.012 p_1=0.041 p_2=0.018

Abbreviations: GFR – glomerular filtration rate; DPE – daily protein excretion; Me (Q25-Q75) – median and quartiles.

Note: p - reliability of the difference in indicators before treatment and 1 and 6 months after treatment;
p_1 - the reliability of the difference in indicators after 1 month and after 6 months after treatment;
p_2 - the reliability of the difference in indicators 6 months after treatment in the II group in comparison with the I group.

Since GFR remained stable or even increased, proteinuria reduction cannot be explained by alteration of filtration capacity. We also noted a significant decrease in serum D-dimers level in group 2 patients, which may indicate the prevention of thromboembolic and cardiovascular events in this population of patients.

12 (24.0 %; 95 % CI 13.1-38.2) patients reported at least one adverse event associated with taking SDX. Nausea occurred in 6 patients (12.0 %; 95 % CI 4.5-24.3), vomiting - 3 (6.0 %; 95 % CI 1.3-16.5), diarrhea – in 3 3 (6.0 %; 95 % CI 1.3-16.5) patients, dizziness – 2 (4.0 %; 95 % CI 0.5-13.7), skin rash – 1 (2.0%; 95% CI 0.1-10.6). The observed adverse events were mild in severity and did not require discontinuation of the drug.

During the observation period, we did not note any deaths among the patients included in the study. We noted 3 (5.5 %; 95 % CI 1.1-15.1) cases of deep vein thrombosis of the lower extremities in patients of Group I. In Group II, we did not note any cardiovascular and thromboembolic events, however, the difference between the groups was not statistically significant (p=0.153).

Discussion. A large number of studies advocated the potential role of SDX as an antiproteinuric agent in type 1 and 2 diabetes patients [6]. In the DiNAS trial, Gambaro et al. demonstrated that a 4 months course of oral SDX can significantly improve either micro- or macroalbuminuria in both type 1 and type 2 diabetes mellitus, with approximately linear dose-response. The maximum dose (200 mg/day) produced the greatest decrease in albuminuria. This effect persisted after the cessation of the treatment [8]. More recently, the DAVET study presented evidence for the efficacy of low-dose 50 mg/day SDX, which was able to induce a significant reduction in albuminuria when continued therapy over 12 months [6].

Nowadays, few studies have described the use of SDX in non-diabetic nephropathies with conflicting results. On the one hand, Rozita et al. reported a significant response after 3 and 6 months of SDX as rescue therapy in patients with chronic GN unresponsive to conventional therapies [12]. On the other hand, Bang et al. did not find a significant difference between placebo vs SDX 75 mg/day and 150 mg/day administered in patients with IgA nephropathy, although the highest dose significantly reduced proteinuria [7].

SDX apparently alters glomerular permeability and effectively reduces proteinuria by a non-blood pressure, non-renin angiotensin aldosterone system (RAAS) related mechanism [13]. As a result, SDX could add to the therapeutic options for GN patients who fail to respond to RAAS inhibition. Moreover, it may further reduce proteinuria in patients who show a partial response to RAAS inhibition. Such additive effects are very important.

In addition, a decrease in the level of D-dimers, such as a biological marker of hypercoagulation, may indicate the prevention of thromboembolic and car-

diovascular events, the risk of which is increased in the population of patients with CKD [14]. However, this hypothesis needs further research.

Limitations. The sample size presented in the study is small, of course, more participants and long-term clinical trials are needed for more valid conclusions.

Conclusions. The additional use of SDX in patients with GN statistically significantly reduced proteinuria levels preventing CKD progression rate.

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Information about the contribution of the authors.

I. Mykhaloiko: literature search, study design planning, data analysis, manuscript writing and submission;

R. Yatsyshyn: concept and management of the work;

I. Dudar: concept and management of the paper.

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