



## Ukrainian Journal of Nephrology and Dialysis

Scientific and Practical, Medical Journal

### Founders:

- State Institution «Institute of Nephrology NAMS of Ukraine»
- National Kidney Foundation of Ukraine

ISSN 2304-0238;  
eISSN 2616-7352

Journal homepage: <https://ukrjnd.com.ua>

### Research Article

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doi: 10.31450/ukrjnd.4(64).2019.03

### Apoptosis correction in nephrotic children

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### Citation:

Burlaka IeA, Bagdasarova IV. Apoptosis correction in nephrotic children. Ukr J Nephrol Dial. 2019;4(64):15-19. doi: 10.31450/ukrjnd.4(64).2019.03

**Abstract.** *Immunosuppressive drugs predispose the kidney transplant recipient to reactivation of cytomegalovirus (CMV) infections. Prophylaxis given to these patients is very important for the prevention of opportunistic CMV infections.*

*The objective of this study was to evaluate the short term and standard-dose valganciclovir prophylaxis for CMV infections in living donor kidney transplantation.*

*Methods.* *This study is retrospective one. Between April 2014 and April 2019 100 patients after living donor kidney transplantation with results CMV PCR-DNA and prophylactic treatment were studied retrospectively at Medipol University Medical Faculty Hospital Organ Transplantation Department, Istanbul, Turkey.*

*Results.* *The mean age was 38.3±15.6 years. 68 (68%) patients were males and 32 (32%) patients were females. All patients were treated with 900 mg daily and 90 days valganciclovir prophylaxis. Mean follow-up was 29.1±15 months. There were not detected CMV infections during the follow-up period.*

*Conclusions:* *Short term and standard-dose valganciclovir prophylaxis appears to be successful prevention CMV infections in living donor kidney transplantation.*

**Keywords.** *Living donor kidney transplantation, CMV infections, valganciclovir, prophylaxis.*

**Conflict of interest statement:** all the authors declared no competing interests.

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### Article history:

Received October 25, 2019

Received in revised form

November 11, 2019

Accepted November 14, 2019



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УДК 616.61-0002.3-036.12

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## Корекція апоптозу у дітей з нефротичним синдромом

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**Резюме.** Розлади, пов'язані з гіпоксією, відіграють важливу роль у незворотному ураженні тканин нирок через активізацію певних вторинних процесів, тобто апоптозу.

Метою нашого дослідження було визначити рівні факторів, що контролюють апоптоз та нуклеарного транскрипційний фактор NF-κB, їх взаємозв'язок з порушенням функції нирок; оцінити можливі захисні ефекти традиційної терапії з додаванням антиоксидантного лікарського засобу токоферолу у педіатричних пацієнтів з нефротичним синдромом.

Методи. Проведено обстеження зразків крові 53 дітей у віці від 10 до 15 років з нефротичним синдромом. Обстеження включало рутинне клініко-лабораторне обстеження та імуноблотинг.

Результати. Продемонстровано, що у дітей з нефротичним синдромом визначається високий рівень проапоптотичного маркера Вах, низький рівень антиапоптотичного фактору Bcl-xL та транскрипційного фактору NF-κB (p65). Виявлено їх залежність від порушення функції нирок. Аналіз ефективності лікування із застосуванням токоферолу засвідчило відновлення балансу фактору, що контролює апоптоз та активність NF-κB.

Висновок. У дітей з нефротичним синдромом визначено порушення системи апоптозу, що може бути скориговано застосуванням токоферолу.

**Ключові слова:** лікування, токоферол, нефротичний синдром, HIF-1α, Вах, Bcl-xL, NF-AB, імуноблотинг.

**Introduction.** Nephrotic-range proteinuria is the loss of 3 grams or more per day of protein into the urine or on a single spot urine collection, the presence of 2 g of protein per gram of urine creatinine. Nephrotic syndrome is the combination of nephrotic-range proteinuria with a low serum albumin level and edema.

Nephrotic syndrome has many causes, including primary kidney diseases such as minimal-change nephropathy, focal glomerulosclerosis, and membranous nephropathy. Nephrotic syndrome can also result from systemic diseases that affect other organs in addition to the kidneys, such as diabetes, amyloidosis, and lupus erythematosus [1].

Main pathomorphological outcomes that apply to kidney damage in nephrotic syndrome are glomerulosclerosis, vascular sclerosis, tubule-interstitial fibrosis [2]. Inflammation plays an important role in the development and progression of chronic proteinuric kidney pathologies and is the primary and persistent violation, which underlies the pathogenesis of others. In addition to inflammation, fibrosis has a role in nephrotic syndrome. These disorders are accompanied by activation of the renin-angiotensin-aldosterone system, oxidative

stress, endothelial dysfunction and others [1-3]. All mentioned above pathophysiological violations might be accompanied by apoptosis.

Apoptosis is programmed cell death that occurs when kidney disease has a place and plays an important role in their physiology. Harmful effects of apoptosis are in fact a source of a large number of kidney cells lost during and/or renal inflammation, scarring, loss of kidney function. The process of apoptosis is of fundamental importance to biological pathways ranging from embryogenesis to aging and normal tissue homeostasis to the stress response. The most common form of NF-κB is RelA(P65)/p50 heterodimers, which are generally retained inert in the cytoplasm by the inhibitor protein, I-κappaB (IκB). Following stimulation of the cell by a variety of agents, IκB is degraded, allowing NF-κB to translocate to the nucleus and bind to the promoter regions of its multiple target genes [4, 5]. The molecular mechanisms underlying irreversible renal damage in children with nephrotic syndrome depending on apoptosis activation might be a potential therapeutic issue its treatment.

The main tasks in nephrotic syndrome treatment are renal insufficiency compensation, complications prevention and lowering the rate of the disease progression. Basic medicines for nephrotic syndrome treatment are immunoregulative drugs (corticosteroids, immunosuppressive agents), ACEi, angiotensin receptors blockers. The pharmacologic manipulations that reduce urinary protein excretion in humans also limit the progressive decline in renal function.

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Antihypertensive drugs have been used in humans to slow the progression of renal disease in diabetic as well as non-diabetic glomerulopathies. ACE inhibitors, for the same level of blood pressure control, are more renoprotective than other antihypertensives also used in human nephropathies, and this appears to be linked to their property of lowering urinary proteins to a greater extent than conventional drugs [6]. However, there are no specific non-toxic drugs regulating apoptosis and apoptosis-dependent disorders in this cohort of patients.

**Materials and methods. Patients.** An examination of renal biopsies of 53 patients (aged 10 to 15 years) with nephrotic syndrome hospitalized in the Pediatric Nephrology Unit of the Children Clinical Hospital №7 (Kyiv, Ukraine) was done. All patients were treated accordingly to the local protocols. Among all patients 24 (45.28%) were with hormone-sensitive type of nephrotic syndrome, others – 29 (54.72%) represented the hormone-dependent type of nephrotic syndrome. Complex examination other than conventional methods (inspection, monitoring blood pressure, general and biochemical blood tests, determination of daily proteinuria, urinary sediment study and concentration ability of the kidneys, ultrasound of the abdomen, etc.), immunohistochemical assessment of apoptosis-dependent glomerular and tubule-interstitial damage were done.

The level of kidney function impairment (stage of Chronic Kidney Disease, CKD) was assessed by the value of glomerular filtration rate (GFR). GFR was calculated by the Schwartz formula.

All patients were treated accordingly to local protocols which include corticosteroids (prednisolone), immunosuppressive drugs (CellCept, Leikeran), ACEi, diuretics (furosemide). An antioxidant (Vitamin E - tocopherol) besides mentioned above drugs in dose 100

mg/day was administered to patients from the experimental group for 1 month.

**Immunoblotting for detection of Bax, Bcl-xL, NF-κB.** Proteins solubilized in Laemmli sample buffer were resolved in polyacrylamide gels by SDS-PAGE and transferred to a polyvinylidene difluoride membrane. Membranes were then blocked in 5% non-fat milk in TBS-T (136 mM NaCl, 10 mM Tris, 0.05% Tween 20) and immunoblotted using the Bax, Bcl-xL, NF-κB and HIF-1α Ab (Cell Signaling Technology, Danvers, MA USA) and actin mouse mAb (BD, Lexington KY, USA) for 1 hour at room temperature. The actin mouse mAb was used as a loading control. After three washes with TBS-T, the membranes were incubated with secondary anti-rabbit or anti-mouse antibodies labeled with horseradish peroxidase for 1 hour at room temperature. Membranes were washed three times with TBS-T. The protein bands were visualized by chemiluminescent substrate ECL. Quantification of the protein content was done by densitometric analysis.

**Statistics.** Statistical analysis was done using the method of variation statistics (STATISTICA 6.0) and nonparametric statistical approaches (Mann-Whitney test). Results are presented as Mean ± SEM.  $P < 0.05$  was considered as statistically significant.

**Results. Apoptosis regulation in nephrotic children.** We have detected a decrease of anti-apoptotic factor Bcl-xL levels. Detailed analysis shows that levels of Bcl-xL decrease gradually depending on kidney function impairment. In nephrotic children with preserved kidney function, Bcl-xL levels were decreased to  $75.1 \pm 2.2\%$  in comparison to control values ( $p < 0.01$ ). In patients with CKD II-III decrease in Bcl-xL documented as  $60.1 \pm 1.8\%$  of control value ( $\tau a p < 0.001$ ) (Fig. 1).

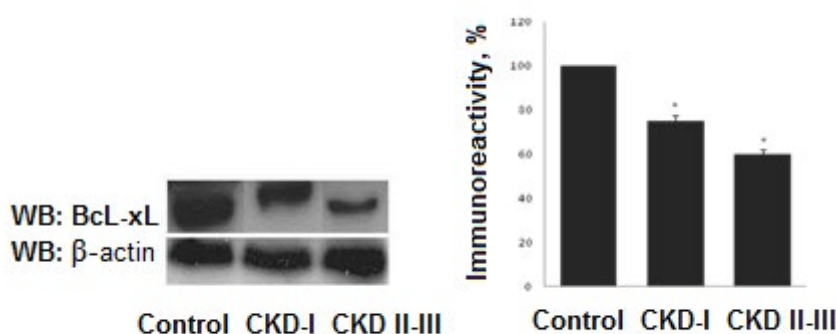


Fig. 1. Bcl-xL levels in children with nephrotic syndrome.\* –  $p < 0.05$ .

**NF-κB levels in nephrotic children.** We performed a measurement of NF-κB (p65 subunit) that is responsible for protection against apoptosis. A pronounced decrease in its activity detected in all nephrotic children. In nephrotic patients with CKD I the level of

NF-κB decreased by  $15.2 \pm 5.9\%$  as compared to control ( $P < 0.01$ ) and by  $20.6 \pm 6.4\%$  in nephrotic patients with CKD II-III ( $P < 0.001$ ). A comparison between CKD I group and CKD II-III group didn't show any statistical difference (Fig. 2).

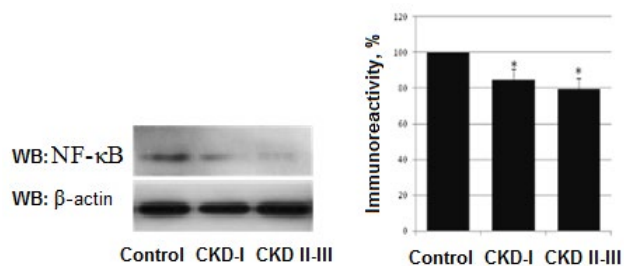


Fig. 2. Levels of NF-κB in nephrotic children. \*- P<0.05.

*Therapeutic correction of the apoptotic process in children with nephrotic syndrome.* To evaluate the effectiveness of treatment in children with nephrotic syndrome all patients were randomized and divided into three groups. The first group (main group) were children with nephrotic syndrome who received conventional basic therapy. The second (experimental group) were children with nephrotic syndrome who received conventional basic therapy and tocopherol. The third group (control) included 45 healthy children matched by age and sex.

In our previous study, we found that after the course of therapy all children investigated for serum level marker of chronic hypoxia HIF-1α show the decreased level as well as Bax levels [7].

Here we performed the measurement of serum levels of anti-apoptotic factor BcL-xL. In children included in the main group, BcL-xL concentration was decreased to  $81.7\% \pm 1.9\%$  ( $p < 0.001$ ) as compared to control. The control group value was taken as 100%. After the course of treatment BcL-xL value was increased to  $94.4\% \pm 1.0\%$  ( $p < 0.01$ ) as compared to level before treatment. Children from the experimental group exhibited BcL-xL before treatment at level  $81.2 \pm 1.8\%$  ( $p < 0.001$ ) as compared to the control group. After the course of treatment, BcL-xL was up-regulated to  $87.25 \pm 1.6\%$  ( $p < 0.01$ ) in comparison to the level before treatment (Fig. 3).

After the course of treatment, all patients were analyzed for NF-κB levels. In patients of the main group the level NF-κB before treatment was down-regulated to  $74.2 \pm 1.06\%$  in comparison to the control group ( $p < 0.001$ ). Control group value we set as 100%. After the course of treatment, the level of NF-κB increased up to  $90.8 \pm 2.51\%$  ( $p < 0.01$ ). In children from the experimental group, the level of NF-κB was down-regulated to  $75.1 \pm 1.98\%$  ( $p < 0.001$ ) as compared to the control group. After the treatment, we found a slight increase in NF-κB up to  $76.7 \pm 2.17\%$  ( $p > 0.05$ ).

**Discussion.** Progression to irreversible renal parenchymal damage and end-stage renal disease is the final common pathway of chronic proteinuric kidney disease and is relatively independent of the type of initial insult. Previously, the number of proteins found in the urine, taken as an indicator of the underlying abnormality in glomerular permeability, was considered by most ne-

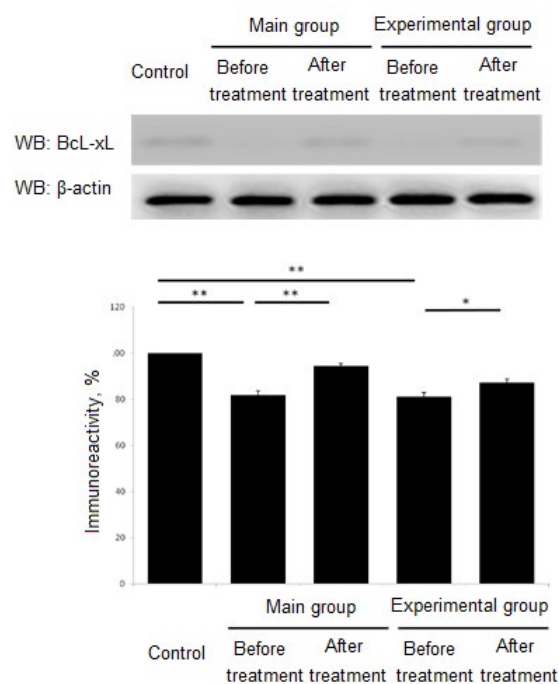


Fig. 3. Treatment effect of anti-apoptotic factor BcL-xL. \*- P<0.01. \*\*- P<0.001.

phrologists simply as a marker of the severity of renal lesions. Today the results of many studies indicate that proteins filtered through the glomerular capillary may have intrinsic renal toxicity, which together with other independent risk factors such as hypertension, can play a contributory role in the progression of renal damage [1, 5]. Indeed, the secondary process of reabsorption of filtered proteins can contribute substantially to renal interstitial injury by activating intracellular events, including up-regulation of vasoactive and inflammatory genes, apoptosis activation. The corresponding molecules formed in excessive amounts by the renal tubules cause an interstitial inflammatory reaction that normally precedes renal scarring and correlates with declining renal function.

A reduction in proteinuria is associated with a slower decline in GFR. Enhanced albuminuria leads to secondary pathological processes – inflammation, hypoxia, fibrosis [8]. Ischemia as a result of peritubular capillary loss or hypoperfusion is also considered a major factor for the progression of tubulointerstitial damage, which is closely associated with impairment of renal function. Renal tissue hypoxia induces profibrogenic responses and tubulointerstitial injury, which includes degeneration, dedifferentiation, cell death [9-11]. Microenvironmental changes, such as hypoxia, strongly affect inflammatory cell recruitment and function. Moreover, hypoxia has been shown to induce apoptosis, where HIF-1 plays a complex role. It has also been demonstrated that the expression of HIF-1α significantly correlated with apoptosis and the pro-apoptotic factors, such as caspase-3, Fas, and Fas

ligand. This finding has been shown in in vitro models [12].

Tocopherol is a fat-soluble antioxidant that is able to intercept free radicals in the plasma membrane, which helps to prevent the oxidative damage to lipids. It is known that patients with CKD have reduced levels of tocopherol in plasma, which is a prerequisite for this use in therapies [13]. Previous studies have shown that the administration of tocopherol in patients with CKD helped to reduce the risk of cardiovascular complications, increased activity of endogenous antioxidant systems - GPX, catalase [14]. In our previous study [7] we have shown that treatment with an antioxidant lowered the hypoxia-induced cell damage as well as the elevation of HIF-1alpha in vitro [15]. Here we demonstrate

that tocopherol administration has a potent effect on hypoxia-induced apoptosis development in children with nephrotic syndrome and subsequent restoration of the proapoptotic factor Bax, Bcl-xL and NF- $\kappa$ B activation. We suppose that this therapeutical intervention may be a new non-toxic approach in apoptosis prevention in chronic albuminuric kidney pathologies.

**Acknowledgements.** This work has been done with support from nephrology department of Children Clinical Hospital №7 (Kyiv, Ukraine).

#### Author contributions.

**Ie. Burlaka:** collected, analyzed, interpreted the data, and wrote the manuscript;

**I. Bagdasarova:** control and article redaction.

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