

CYTOKINE PROFILE OF KIDNEYS IN RATS WITH EXPERIMENTAL HYPERHOMOCYSTEINEMIA

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Hyperhomocysteinemia (HHcy) is a systemic metabolic disorder known to impair renal function. The kidneys play a crucial role in homocysteine (Hcy) clearance from the bloodstream and represent a key location for Hcy-related metabolic disturbances. A principal mechanism underlying Hcy-induced injury of renal tissue is inflammation, however, the age-related renal cytokine profile under HHcy conditions remains insufficiently characterized. Therefore, the aim of this study was to evaluate the level of pro- and anti-inflammatory cytokines in the kidneys of rats of different ages with experimental HHcy. The study was performed on nonlinear male rats divided into groups of young (one-month-old) and adult (six-month-old) animals with HHcy as well as control animals of the same age. HHcy was induced by the daily intragastric administration of D,L-homocysteine thiolactone (200 mg·kg⁻¹ body weight) for eight weeks. At the end of the experimental period, the kidneys were excised for homogenate preparation. The levels of cytokines IL-1 β , IL-4, IL-6, IL-8, IL-10, TNF- α , and IFN- γ were determined using ELISA. The renal level of the anti-inflammatory cytokine IL-10 was decreased in both age groups of rats with HHcy relative to control values. In young rats with HHcy, increased renal levels of pro-inflammatory cytokines IL-1 β , IL-6 and TNF- α were found, while in adult rats with HHcy, decreased renal levels of IL-6 and TNF- α compared to the age-matched control group were observed. These findings indicate that HHcy induces renal cytokine profile alterations that depend on the age of the animals.

Key words: hyperhomocysteinemia, kidneys, renal homogenate, cytokine profile, interleukins, inflammation.

Homocysteine (Hcy) metabolism is a fundamental biological process that regulates methionine (Met) availability, protein turnover, and DNA methylation, thereby contributing to cellular homeostasis and epigenetic stability [1, 2]. Hcy is a non-proteinogenic, sulfhydryl-containing amino acid generated during Met demethylation reactions. In blood plasma, Hcy is present in several forms: as a free thiol (1%), disulfide-bound to plasma proteins, predominantly albumin (70-80%), and as low-molecular-weight disulfides (20-30%) [2]. In healthy individuals, optimal plasma total homocysteine (tHcy) concentrations range from 5.0 to 15.0 $\mu\text{mol}\cdot\text{l}^{-1}$. Deviations from this physiological range are associated with a wide spectrum of pathological conditions, including cardiovascular, endocrine, neurological, gastrointestinal, oncological disorders, and chronic kidney disease, making Hcy an important biomarker of disturbances in

amino acid metabolism and protein homeostasis [3-6]. An increase in tHcy above 15 $\mu\text{mol}\cdot\text{l}^{-1}$ defines hyperhomocysteinemia (HHcy), a systemic metabolic disorder commonly classified as mild (16-30 $\mu\text{mol}\cdot\text{l}^{-1}$), moderate (31-100 $\mu\text{mol}\cdot\text{l}^{-1}$), or severe (above 100 $\mu\text{mol}\cdot\text{l}^{-1}$) [2, 5]. The accumulation of Hcy primarily results from impaired regulation of its metabolic pathways, which may arise from genetic polymorphisms in key enzymes involved in Hcy metabolism, including methylenetetrahydrofolate reductase (MTHFR; EC 1.5.1.20), methionine synthase (MS; EC 2.1.1.13), cystathionine β -synthase (CBS; EC 4.2.1.22), and cystathionine γ -lyase (CSE; EC 4.4.1.1). Additionally, nutritional deficiencies in folate, vitamins B₆ and B₁₂, or excessive intake of methionine- and cysteine-rich proteins, and exposure to certain pharmacological agents, significantly contribute to the development of HHcy [7, 8].

Although Hcy is synthesized in all tissues, its detoxification via the transsulfuration pathway occurs predominantly in the liver, pancreas and kidneys. Among these organs, the kidney plays a pivotal role in Hcy clearance and sulfur-containing amino acid metabolism, rendering renal tissue particularly vulnerable to Hcy-induced injury. Clinical and experimental evidence indicates that HHcy is both a contributor to and a consequence of impaired renal function, correlating with reduced glomerular filtration rate [9]. Increased plasma Hcy concentrations have been associated with oxidative stress, endothelial dysfunction, increased thrombogenicity, and structural remodeling of renal tissue, processes that collectively promote the progression of kidney disease [10-13].

Inflammation is considered one of the key mechanisms underlying Hcy-mediated tissue damage [14, 15]. Hcy can induce oxidative stress and activate intracellular signaling pathways, including the nuclear factor kappa B (NF- κ B), resulting in dysregulated cytokine expression. Pro-inflammatory cytokines promote leukocyte recruitment and vascular dysfunction, whereas anti-inflammatory mediators counteract excessive immune activation and support tissue repair and homeostasis. A persistent imbalance between these regulatory pathways contributes to chronic inflammation and progressive organ injury [16]. Despite accumulating evidence linking HHcy to inflammatory activation, data on renal cytokines regulation under HHcy conditions remain limited and inconsistent [17, 18]. Most studies focus on specific experimental models or individual cytokines, leaving the coordinated regulation of pro- and anti-inflammatory cytokines in renal tissue insufficiently characterized, particularly in different age groups [14, 19]. Given the age-related increase in HHcy prevalence, characterization of age-dependent renal cytokine profiles may provide important mechanistic insights into Hcy-associated kidney injury. Therefore, the present study aimed to evaluate the levels of pro- and anti-inflammatory cytokine in kidney homogenates of young and adult rats under conditions of experimental HHcy.

Materials and Methods

Bioethical statement. All experimental procedures involving animals were conducted in accordance with internationally accepted bioethical standards and the principles of the European Convention for the Protection of Vertebrate Animals

Used for Experimental and Other Scientific Purposes (Strasbourg, 1986). All efforts were made to minimize animal suffering and to use the minimum number of animals necessary to achieve scientific validity. The experimental protocol was reviewed and approved by the Ethical Committee of the Educational and Scientific Center "Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv (protocol No. 8 approved 19.12.2025).

Animals and experimental design. The study was performed on non-linear male laboratory rats in two age groups: young (one-month-old) and adult (six-months-old). Animals were housed in polypropylene cages under controlled environmental conditions (temperature $22 \pm 3^{\circ}\text{C}$, relative humidity $60 \pm 5\%$, and a 12 h light/12 h dark cycle) with free access to standard laboratory chow and water ad libitum. The animals were divided into four experimental groups ($n = 10$ per group): young control rats (Young_Control), young rats with HHcy (Young_HHcy), adult control rats (Adult_Control), and adult rats with HHcy (Adult_HHcy).

Experimental HHcy was induced by daily intragastric administration of D,L-homocysteine thiolactone (Acros Organics, Belgium), dissolved in a 1% starch solution, at a dose of $200 \text{ mg}\cdot\text{kg}^{-1}$ body weight for eight consecutive weeks [20]. Control animals received an equivalent volume of a 1% starch solution according to the same schedule. The development of HHcy was confirmed by increased plasma Hcy concentration (above $15 \mu\text{mol}\cdot\text{l}^{-1}$), determined by enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (Homocysteine EIA, Axis-Shield Diagnostics Ltd., UK).

Tissue collection and preparation of renal homogenates. At the end of the experimental period, animals were euthanized in accordance with ethical guidelines: young rats (Young_Control and Young_HHcy groups) by cervical dislocation and adult rats (Adult_Control and Adult_HHcy groups) by decapitation. Kidneys were rapidly excised, rinsed in ice-cold physiological saline, and processed for homogenate preparation using standard laboratory procedures. The obtained renal homogenates were used for subsequent biochemical analyses. Total protein concentration was determined by the Bradford method [21].

Cytokines immunoassay. The levels of pro-inflammatory (IL-1 β , IL-6, IL-8, TNF- α and IFN- γ) and anti-inflammatory (IL-4 and IL-10) cytokines were determined using ELISA according to the

manufacturers' instructions [22]. Microtiter plates were coated overnight at 4°C with renal homogenate samples diluted in 50 mM Tris-buffered saline (pH 7.4) to a final protein concentration of 10 µg·ml⁻¹. After washing, nonspecific binding sites were blocked with 5% nonfat dry milk for 1 h at 37°C. Samples were then incubated with cytokine-specific primary antibodies, followed by incubation with horseradish peroxidase-conjugated secondary antibodies for 1 h at 37°C. After thorough washing, color development was achieved using o-phenylenediamine and hydrogen peroxide as substrates. The enzymatic reaction was stopped by adding of 2.5 N sulfuric acid, and absorbance was measured at 492 nm using a microplate reader (mQuant™, BioTek Instruments, Inc.). Cytokine levels were calculated based on optical density values and expressed relative to total protein content.

Statistical analysis. Statistical analysis was performed using Statistica 12.0 (StatSoft Inc., USA) and Microsoft Excel 2016 software. Data are presented as mean ± standard error of the mean (SEM) for each experimental group ($n = 10$). The normality of data distribution was assessed using the Shapiro – Wilk test. For normally distributed data, differences between groups were evaluated using Student's *t*-test, whereas the Mann – Whitney U test was applied for non-normally distributed variables. Differences were considered statistically significant at $P < 0.05$.

Results and Discussion

Analysis of the renal cytokine profile in rats with experimentally induced HHcy revealed pronounced age-dependent alterations in inflammatory and anti-inflammatory signaling pathways.

In young animals, HHcy was associated with a distinct shift toward a pro-inflammatory cytokine profile (Fig. 1). Specifically, renal levels of IL-1β, IL-6 and TNF-α were increased by 15, 20, and 18%, respectively, compared with age-matched control animals ($P < 0.05$). In contrast, IL-8 and IFN-γ levels demonstrated only a tendency toward elevation and did not reach statistical significance. Importantly, this enhanced pro-inflammatory response was accompanied by impaired anti-inflammatory regulation, as evidenced by a 25.0% decrease in renal IL-10 content compared to the Young_Control group ($P < 0.05$), whereas IL-4 level remained statistically unchanged. Collectively, these findings indicate that HHcy in young animals promotes the formation of a pro-inflammatory renal microenvironment through

concurrent activation of classical inflammatory cytokines and suppression of IL-10-mediated immune control.

In adult rats, the renal cytokine response to HHcy differed markedly from that observed in young animals (Fig. 2). In the rats of Adult_HHcy group, renal levels of IL-6 and TNF-α were decreased by 19.7% and 19.6%, respectively, compared with Adult_Control animals ($P < 0.05$), whereas IL-1β and IL-8 and IFN-γ levels remained unchanged. Despite this apparent attenuated of pro-inflammatory signaling, anti-inflammatory regulation was also compromised, as renal IL-10 content was decreased by 23.8% relative to control values ($P < 0.05$), with no significant changes observed in IL-4 level. Notably, the decrease in IL-10 levels observed across both age groups may suggest that impaired anti-inflammatory control represents a common and largely age-independent feature of HHcy-associated renal alterations.

The divergent cytokine patterns observed between young and adult animals reflect fundamental differences in the mechanisms of immune response to excess Hcy. In young rats, HHcy acts as a potent trigger of renal inflammation, characterized by a coordinated upregulation of the classical pro-inflammatory triad IL-1β, IL-6, and TNF-α. Such a cytokine profile is indicative of acute innate immune activation and is consistent with rapid engagement of NF-κB-dependent signaling pathways, resulting in pro-inflammatory gene induction [23].

These observations are in agreement with reports demonstrating that Hcy-induced oxidative stress and endothelial dysfunction are mechanistically linked to NF-κB activation and subsequent upregulation of pro-inflammatory cytokine expression in experimental models of HHcy [15, 18]. In support of this mechanism, our previous studies [24] revealed pronounced oxidative stress and disruption of antioxidant defenses in the kidneys of one-month-old rats with HHcy, exceeding the magnitude of changes observed in sexually mature animals. Given the well-established interplay between redox imbalance and inflammatory signaling, it is likely that reactive oxygen species generated under HHcy conditions act as upstream mediators of cytokine overproduction in the kidneys of young animals.

In contrast, the cytokine profile observed in adult rats suggests a transition from acute inflammatory activation to a state of attenuated or maladaptive immune responsiveness. The reduction in

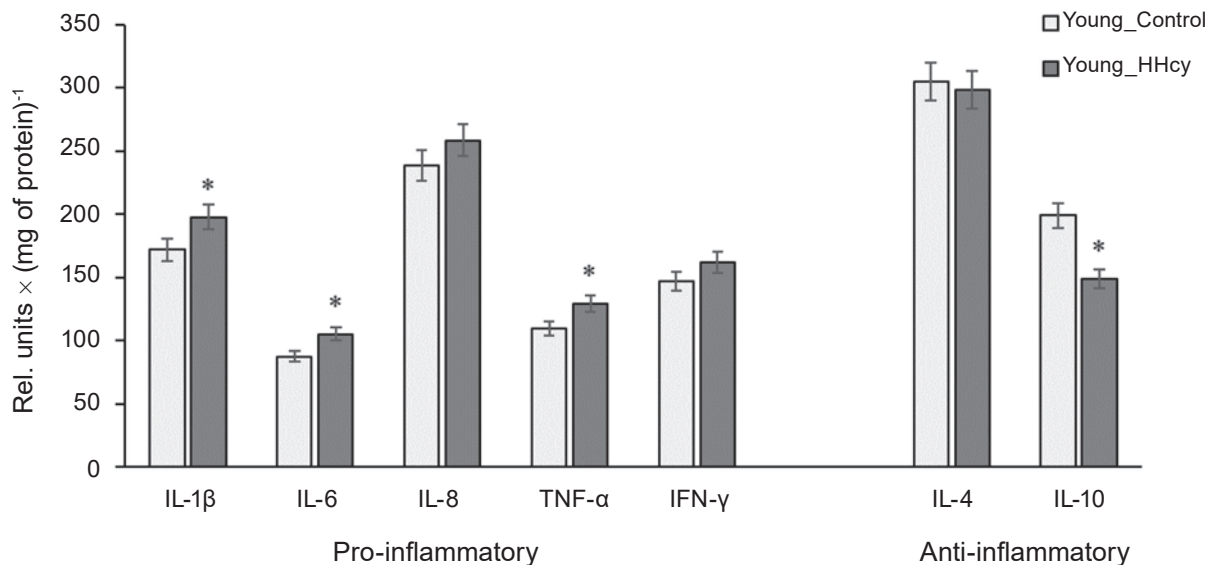


Fig. 1. Levels of pro- and anti-inflammatory cytokines in the kidneys of young rats with hyperhomocysteinemia (HHcy), relative units per mg of protein. Data are presented as mean ± SEM (n = 10). *P < 0.05 compared with the Young_Control group

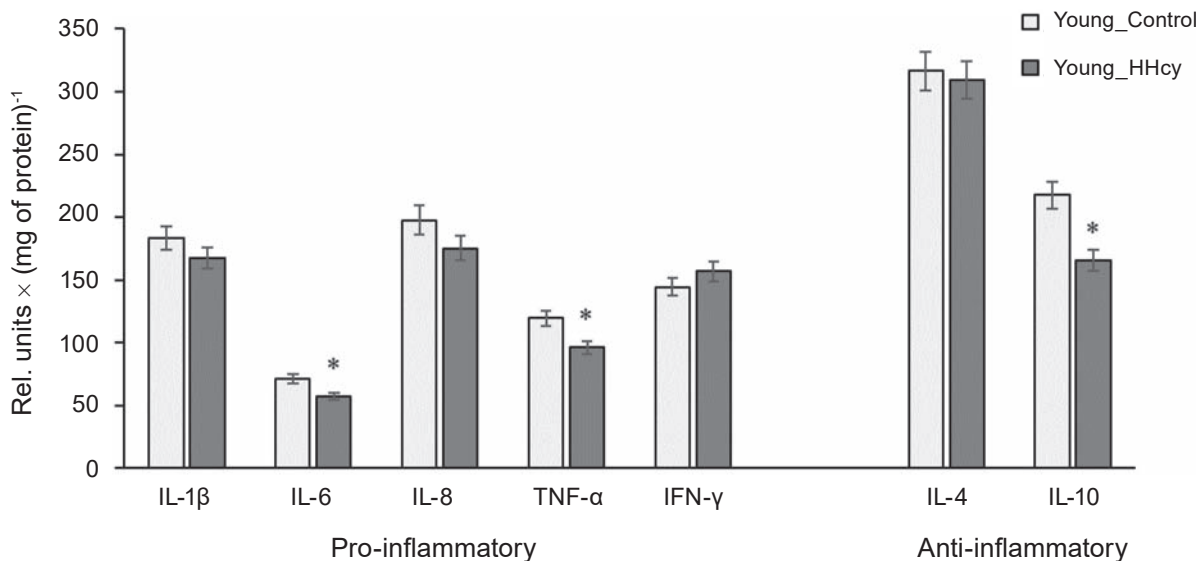


Fig. 2. Levels of pro- and anti-inflammatory cytokines in the kidneys of adult rats with hyperhomocysteinemia (HHcy), relative units per mg of protein. Data are presented as mean ± SEM (n = 10). *P < 0.05 compared with the Adult_Control group

IL-6 and TNF-α levels under sustained HHcy may reflect immune exhaustion or impaired inflammatory adaptability associated with chronic metabolic stress. Prolonged HHcy has been shown to induce epigenetic modifications that alter cytokine gene expression [25-27]. These effects are largely attributed to homocysteine thiolactone, a highly reactive cyclic thioester formed during errors in aminoacyl-tRNA synthesis, which links metabolic imbalance

to protein modifications [28, 29]. Homocysteine thiolactone can modify proteins through N-homocysteinylolation, thereby altering the structure and function of regulatory proteins, including histones and enzymes involved in methylation processes. In addition, elevated intracellular Hcy levels disrupt the S-adenosylmethionine/S-adenosylhomocysteine (SAM/SAH) ratio, leading to inhibition of DNA and histone methyltransferases. This results in

global DNA hypomethylation and altered promoter methylation patterns of genes involved in inflammatory regulation. These mechanisms may contribute to long-term transcriptional reprogramming of cytokine genes under HHcy conditions. Therefore, in adult animals, suppression of pro-inflammatory cytokine production may not signify resolution of inflammation but rather reflect epigenetically mediated loss of appropriate immune responsiveness due to impaired methylation capacity and protein N-homocysteinylation.

Despite these age-specific differences in pro-inflammatory signaling, the consistent suppression of IL-10 across both age groups emerges as a key pathogenic feature of HHcy-associated renal dysfunction. IL-10 is an important anti-inflammatory cytokine that plays a critical role in limits excessive immune activation, restricting leukocyte infiltration, and protecting renal tissue from oxidative damage and fibrotic remodeling. Sustained IL-10 deficiency promotes a permissive environment for chronic inflammation, thereby facilitating progressive renal injury and loss of kidney function [30]. The observation that IL-10 levels are decreased irrespective of age underscores the fundamental role of impaired anti-inflammatory regulation in the renal response to Hcy excess. Notably, epigenetic dysregulation of IL-10 expression has also been linked to altered promoter methylation under conditions of methylation stress, which may further explain the uniform reduction of this cytokine in both young and adult animals with HHcy.

Comparison with other organs further highlights the kidney's unique vulnerability to HHcy-induced cytokine dysregulation [20, 31-33]. In cardiac tissue, aging is associated with elevated basal cytokine levels even in the absence of additional pathological stimuli, reflecting an age-related pro-inflammatory shift that predisposes the myocardium to inflammatory remodeling [31]. Moreover, in several experimental models of HHcy, the heart exhibits compensatory increases in IL-4 and IL-10 levels, which may partially mitigate inflammatory damage. In contrast, the kidney appears to lack such effective compensatory mechanisms, particularly with respect to IL-10, rendering it more susceptible to sustained HHcy-induced injury. This heightened renal sensitivity is consistent with high metabolic demand and mitochondrial activity of renal tubular cells, which increase their vulnerability to sulfur-containing metabolites such as Hcy and may lead to early exhaustion of protective cytokine pathways [34].

Experimental evidence from other tissues also indicated that HHcy does not uniformly induce cytokine-driven inflammation [32]. In systemic immune organs, such as the spleen, disturbances in proteolytic balance may occur in the absence of pronounced cytokine activation. Similarly, in the brain, thyroid gland, lungs, heart, and liver, enhanced proteolytic activity under HHcy conditions has been reported to occur independently of overt inflammatory cytokine responses, with protease activation evident despite minimal changes in pro-inflammatory markers [20, 31, 33]. These observations support the concept that HHcy induces a complex, organ-specific pattern of immune and metabolic adaptation, in which oxidative stress-associated proteolytic remodeling may predominate over classical cytokine-mediated inflammation in certain tissues, while the kidney remains particularly susceptible to cytokine imbalance.

This study has several limitations that should be considered when interpreting the results. First, a specific HHcy model based on homocysteine thio-lactone administration was used, which may not fully reproduce all metabolic features of clinical HHcy. Second, the study was conducted exclusively on male animals; therefore, potential sex-dependent differences in renal cytokine responses were not assessed. Third, cytokine levels were analyzed in whole renal homogenates without cellular localization. In addition, only two age groups were examined, limiting detailed characterization of gradual age-related changes in immune responsiveness. Finally, direct evaluation of epigenetic parameters (DNA methylation, SAM/SAH ratio, and protein N-homocysteinylation) was beyond the scope of this study and represents a promising direction for future research.

Conclusion. Overall, the results of the present study demonstrate that experimental HHcy is associated with marked disturbances in renal immune homeostasis, with cytokine regulation being strongly modulated by age. The kidney emerges as a particularly vulnerable target organ under conditions of Hcy excess, exhibiting complex and non-uniform alterations in inflammatory signaling rather than a uniform pro-inflammatory response. Age-dependent differences in pro-inflammatory cytokine profiles reflect distinct adaptive and maladaptive immune mechanisms, while the persistent suppression of IL-10 across both age groups identifies impaired anti-inflammatory control as a central and largely

age-independent feature of Hcy-associated renal injury. These findings highlight the importance of considering age-specific immune regulation in the pathogenesis of Hcy-related kidney dysfunction and suggest that modulation of anti-inflammatory pathways, particularly those mediated by IL-10, may represent a promising direction for limiting Hcy-induced renal damage.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ЦИТОКІНОВИЙ ПРОФІЛЬ НИРОК У ЩУРІВ З ЕКСПЕРИМЕНТАЛЬНОЮ ГІПЕРГОМОЦИСТЕЇНЕМІЄЮ

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Гіпергомоцистеїнемія (ГГЦ) є системним метаболічним розладом, який супроводжується порушенням функції нирок. Нирки відіграють ключову роль у кліренсі гомоцистеїну (ГЦ) із системного кровотоку та є однією з основних мішеней метаболічних порушень, асоційованих із підвищеним рівнем ГЦ. Запалення розглядається як один із провідних механізмів ушкодження ниркової тканини, індукованого ГЦ, проте вікові особливості цитокінового профілю нирок за умов ГГЦ залишаються недостатньо вивченими. Метою дослідження було оцінити рівень про- та проти-запальних цитокінів у нирках щурів різного віку за умов експериментальної ГГЦ. У дослідженні використовували нелінійних щурів-самців, розділених на групи молодих (один місяць) та дорослих (шість місяців) тварин із ГГЦ, а також групи контрольних тварин відповідні віку. ГГЦ індукували щоденним внутрішньошлунковим введенням D,L-гомоцистеїнтіолактону (200 мг·кг⁻¹ маси тіла) впродовж восьми тижнів. Наприкінці експериментального періоду нирки вилучали для подальшої гомогенізації. Рівні цитокінів IL-1 β , IL-4, IL-6, IL-8, IL-10, TNF- α

та IFN- γ визначали за допомогою ELISA. Встановлено, що рівень протизапального цитокіну IL-10 у нирках знижувався в обох вікових групах тварин із ГГЦ порівняно з контрольними значеннями. У молодих щурів із ГГЦ виявлено підвищеним рівень прозапальних цитокінів IL-1 β , IL-6 та TNF- α , тоді як у дорослих щурів із ГГЦ спостерігалось зниження рівня IL-6 та TNF- α порівняно з контрольною групою тварин того ж віку. Отримані результати свідчать, що ГГЦ індукує зміни профілю цитокінів у нирках, які залежать від віку тварин.

Ключові слова: гіпергомоцистеїнемія, нирки, гомогенат нирок, цитокіновий профіль, інтерлейкіни, запалення.

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