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REPRESENTATION OF KIDNEY DAMAGE AT THE MOLECULAR LEVEL IN PATIENTS WITH UROLITHIASIS BASED ON THE STUDY OF ENZYMATIC TEST INDICATORS

N.V. Avramenko¹, G.V. Bachurin², Yu.S. Kolomoets³, O.A. Nikiforov⁴.

¹Professor of the Head of the Department of Obstetrics, Gynecology and Reproductive Medicine of the Educational and Scientific Institute of Postgraduate Education, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.
²Professor of Urology, Head of the Department of Urology, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.
³Candidate of medicine, Assistant of the Department of Urology, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.
⁴Candidate of medicine, Docent of the Department of Obstetrics, Gynecology and Reproductive Medicine of the Educational and Scientific Institute of Postgraduate Education, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.

Abstract.

Aim: The aim of the work is to improve the results of early diagnosis of acute infectious kidney diseases at the molecular level in urolithiasis (urinary stone disease) through the study of enzymatic test indicators.

Materials and methods: Enzymatic tests (NGAL, IL-1β, β2-microglobulin) were investigated at the molecular level using the IFA method in the urine of patients with urolithiasis. Comparative and prognostic significance of the conducted treatment was established between the groups of patients, and an algorithm was developed based on the results of kidney damage predictors.

Results: It was found that the indicators of general laboratory analysis in patients with urolithiasis within the first 24-48 hours do not reliably indicate the absence of an infectious-inflammatory process in the kidneys and the development of renal failure. It was determined that an increase in the inflammation predictor indicators in more than 50% of patients indicates the development of infectious-inflammatory complications within the first 12-24 hours before the occurrence of general laboratory and clinical changes. The assessment of the effectiveness of conservative therapy in groups IА and II revealed that complications of the inflammatory process in the kidneys were observed five times more frequently in group II (comparison) than in group IА.

Conclusions: The use of enzymatic tests as markers for early kidney damage allows for the classification of two main groups of patients: those requiring conservative treatment and those requiring urgent surgical intervention. This significantly reduces the frequency of inflammatory, purulent-septic complications and improves the treatment outcomes for patients with upper urinary tract obstruction in urolithiasis.

Key words. Urolithiasis, pyelonephritis, kidney damage markers, NGAL, IL-1β, β2-microglobulin, CRP-1.

Introduction.

Despite the progress in the study of urolithiasis (urinary stone disease), the problem of its occurrence remains one of the most challenging and unresolved [1-5]. The course of the disease is accompanied by infectious-inflammatory processes and frequent recurrences in more than 50% of cases, which can lead to complications such as renal failure, disability, and even death [6-9]. In 60-70% of urolithiasis patients, the presence of infection not only exacerbates the disease but also significantly worsens its further prognosis [8,10-14].
activity of tissue enzymes in urine and blood are conducted. However, the activity of enzymes in blood remains almost unchanged in kidney diseases and does not reflect the severity of kidney damage [1,11,16,18,23]. Enzymatic tests provide a better reflection of metabolic processes at the molecular level, even when clinical and laboratory indicators have minimal expression [9,16,21,20,23].

The purpose of this study was to improve the results of early diagnosis of acute infectious kidney diseases at the molecular level in urolithiasis (urinary stone disease) through the study of enzymatic test indicators.

**Materials and Methods.**

The research was conducted at the urology department of the Emergency and Ambulance Hospital in Zaporizhzhia, which serves as the clinical base for the Department of Urology at Zaporizhzhia State Medical University.

The inclusion criteria for the study were individuals of both genders aged 18 years and older with ureteral or urinary stone(s) of various localizations. The exclusion criteria were active concomitant pathologies and the presence of oncological diseases requiring specific therapy.

To achieve the set goal, the results of examination and treatment of 142 patients during the period from 2018 to 2019 were analyzed. Among them, 70 patients were analyzed using the enzyme-linked immunosorbent assay (ELISA) of urine. For the determination of reference values, 30 individuals who were considered clinically healthy were examined. To evaluate the course of pyelonephritis and long-term complications after conservative treatment, a retrospective analysis of the medical histories of 42 patients with urolithiasis was conducted, excluding the results of enzymatic tests.

In this study, patients and healthy individuals were divided into three clinical groups, as described below:

- **Group I:** This group consisted of patients with urolithiasis (urinary stone disease) who underwent enzymatic urine tests. A total of 70 patients were included in this group.
- **Subgroup IA:** This subgroup included patients from Group I whose inflammatory markers were within normal range or showed elevated levels of only one of the three early kidney damage markers. These patients received conservative therapy. The number of patients in this subgroup was 48.
- **Subgroup IB:** In this subgroup, patients from Group I were identified with elevated levels of two or more inflammation markers. These patients received surgical treatment. The number of patients in this subgroup was 22.
- **Group II:** This group consisted of patients for whom a retrospective analysis of their medical histories was conducted, specifically comparing the treatment outcomes of pyelonephritis and long-term complications. There were 42 patients in this group.
- **Group III:** This group comprised 30 individuals who were considered conditionally clinically healthy and served as the control group.

All groups were homogeneous in terms of gender and age (p>0.05).

All patients included in the study underwent a collection of anamnestic data and comprehensive clinical and laboratory examinations. The main criterion for the clinical—diagnostic effectiveness of using inflammation predictors was the detection of early signs of infectious—inflammatory complications in patients with urinary tract dysfunction in groups IA and IB.

The obtained results were entered into a Microsoft Excel 2010 database and underwent statistical processing using a licensed Statistical 13.0 software package (license number JPZ8041382130ARCN10-J).

Conservative therapy (n=48) was based on providing immediate urgent care for renal colic according to the current protocols of medical care.

For patients who showed signs of infectious—inflammatory processes along with urinary tract obstruction and persistent pain syndrome, urgent surgical intervention was performed (n=22). The choice of the surgical method depended on the results of the IFAs of early kidney damage markers, the overall condition of the patient, as well as the results of general laboratory and instrumental examinations. All patients in Group IB, diagnosed with infectious—inflammatory processes, underwent lithotripsy or stone extraction, kidney drainage, and received antibacterial, anti-inflammatory, and infusion therapy (Table 1).

### Results.

According to the findings of the conducted study in patients with urolithiasis who were hospitalized within the first hours, the detection of early preclinical signs of infectious—inflammatory complications was most effective using the Immunoassay (IFA) test for NGAL, IL-1β, and β2-microglobulin in urine. The research revealed that MSR – 1 did not provide a high informative value compared to other predictors, and therefore further analysis of this marker was not carried out.

For β2-microglobulin, the following results were obtained: the cut-off point value was 0.13µg/ml sensitivity was 95.5%, specificity was 66.7%, and accuracy was 75.5%. The area under the ROC – curve (AUC) is 0.707. More detailed data can be found in Table 2 and Figure 1 of the study.

The parameters for IL-1β were as follows: the cut-off point value was 6 pg/ml, sensitivity was 86.4%, specificity was 85.4%, and accuracy was 85.7%. The area under the ROC – curve (AUC) is 0.689 (see Figure 2 and Table 3).

The ROC – analysis with the constructed logistic regression model for NGAL yielded the following results: the cut-off point value was 11 ng/ml, sensitivity was 72.7%, specificity was 100%, and accuracy was 91.4%. The area under the ROC – curve (AUC) is 0.678 (see Figure 3 and Table 4).

During the examination of results of Group IA, which included 48 patients, it was found that the indicators of leukocytes, neutrophils, erythrocyte sedimentation rate (ESR), and creatinine were within normal limits at the time of hospitalization. This indicated the absence of significant signs

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**Table 1. Surgical Treatment of Patients in Group IB, (n/%)**

<table>
<thead>
<tr>
<th>Surgical Methods</th>
<th>Number, (n/%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact Ureterolithotripsy (CUL)</td>
<td>9 (40.9%)</td>
</tr>
<tr>
<td>Ureterolithoextraction</td>
<td>8 (36.4%)</td>
</tr>
<tr>
<td>Extracorporeal Shock Wave Lithotripsy (ESWL)</td>
<td>4 (18.2%)</td>
</tr>
<tr>
<td>Percutaneous Nephrostomy + ESWL</td>
<td>1 (4.5%)</td>
</tr>
</tbody>
</table>

---
The study revealed that the absence of pronounced leukocytosis, left shift in the leukocyte count, elevated ESR (erythrocyte sedimentation rate), and creatinine levels within the first 24-48 hours after hospitalization cannot fully confirm the absence of an infectious-inflammatory process in the kidneys and the development of renal insufficiency. This means that only CBC (complete blood count) and biochemical analysis of blood are not sufficient for early diagnosis of infectious-inflammatory processes in the kidneys in patients with urinary tract dysfunction.

In the further analysis of the group of patients with IA, after 7 days of therapy, spontaneous passage of the kidney stone was observed in 34 patients (70.8%). In 11 patients (27.9%), there was no spontaneous passage of the stone, and these patients underwent planned surgical intervention. Additionally, in 3 patients (6.3%), exacerbation of obstructive symptoms occurred on the 5th day of conservative treatment, and they were transferred to group IB for urgent surgical management. After performing the urgent surgical intervention in group IB, normalization of leukocyte and creatinine levels was observed on the 10th day post-surgery. Detailed results of these observations can be found in Tables 5 and Figure 4 of the study.

The restoration of kidney microcirculation, as assessed by color Doppler mapping (CDM), was diagnosed in groups IA and IB on the 10th day after the treatment was administered (see Table 6).

In the conducted retrospective analysis of 42 medical case histories, a comparative assessment of the effectiveness of conservative treatment was carried out in patients who did not undergo instrumental functional analysis (IFA) of urine with the determination of predictor levels. Based on the obtained data, patients in groups IA and IB, who underwent IFA of urine, showed a consistently positive trend in laboratory indicators on the 10th day of treatment and on the 30th day after therapy. However, in group II, which was used for comparison, a significant negative trend in the administered treatment was observed, leading to the development of purulent-septic complications and, consequently, necessitating urgent surgical intervention. The proposed information is presented in tabular format in Table 7.

**Discussion.**

During the analysis of the nearest complications that occurred within the first 10-14 days of treatment, it was found that the frequency of complications in group II was 5 times higher compared to group IA. In the comparative assessment of complications one month after conservative treatment, it was revealed that the frequency of complications in group II was 3 times higher than in group IA. This information is presented in tabular format in Table 8.

An algorithm has been developed for the examination and selection of treatment methods in patients with urodynamic disorders, which includes the following steps. During hospitalization, data on complaints and a detailed history of past and concomitant somatic diseases are collected. The next step in early diagnosis is the performance of instrumental functional analysis (IFA) of urine with the determination of various markers such as NGAL, IL-1β, β2-microglobulin. This helps to detect signs of infectious-inflammatory processes and the development of renal insufficiency. Subsequently, a study of general laboratory indicators is conducted as an additional guide to determine the presence of an inflammatory process and

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**Table 2. Results of assessment of β2-microglobulin in groups IA and IB.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Value β2-microglobulin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;0,13 μg/ml</td>
</tr>
<tr>
<td>Conservative treatment (IA), (n=48)</td>
<td>32</td>
</tr>
<tr>
<td>Operative treatment (IB), (n=22)</td>
<td>1</td>
</tr>
<tr>
<td>In total</td>
<td>33</td>
</tr>
</tbody>
</table>

**Table 3. Results of assessment of IL-1β in groups IA and IB.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Value IL-1β</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;6 pg/ml</td>
</tr>
<tr>
<td>Conservative treatment (IA), (n=48)</td>
<td>41</td>
</tr>
<tr>
<td>Operative treatment (IB), (n=22)</td>
<td>3</td>
</tr>
<tr>
<td>In total</td>
<td>44</td>
</tr>
</tbody>
</table>

**Table 4. Results of assessment of NGAL in groups IA and IB.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Value NGAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;11 ng/ml</td>
</tr>
<tr>
<td>Conservative treatment (IA), (n=48)</td>
<td>48</td>
</tr>
<tr>
<td>Operative treatment (IB), (n=22)</td>
<td>6</td>
</tr>
<tr>
<td>In total</td>
<td>54</td>
</tr>
</tbody>
</table>

**Table 5. Methods of removing stones of the upper urinary tract, (n=70).**

<table>
<thead>
<tr>
<th>Conservative treatment, (IA), (n=48)</th>
<th>Operative treatment, (IB), (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n/%</td>
<td>n/%</td>
</tr>
<tr>
<td>Independent removal of calculi</td>
<td>Percutaneous nephrostomy + remote lithotripsy</td>
</tr>
<tr>
<td>34 (70.8%)</td>
<td>3 (12.0%)</td>
</tr>
<tr>
<td>Transferred to group IB</td>
<td>Contact ureterolithotripsy</td>
</tr>
<tr>
<td>3 (6.3%)</td>
<td>10 (40.0%)</td>
</tr>
<tr>
<td>Planned surgical treatment:</td>
<td>Ureterolithoextraction</td>
</tr>
<tr>
<td>– remote lithotripsy;</td>
<td>11 (22.9%)</td>
</tr>
<tr>
<td>8 (72.7%)</td>
<td>8 (32.0%)</td>
</tr>
<tr>
<td>– contact ureterolithotripsy</td>
<td>Remote lithotripsy</td>
</tr>
<tr>
<td>3 (27.3%)</td>
<td>4 (16.0%)</td>
</tr>
</tbody>
</table>

**Table 6. Indicators of kidney dopplerography in patients of group I (n=70) on the 10th day after the treatment, M±m.**

<table>
<thead>
<tr>
<th>Indexes</th>
<th>Conservative treatment (IA), (n=48)</th>
<th>Operative treatment (IB), (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V_max, m/s</td>
<td>0.68±0.09</td>
<td>0.66±0.09</td>
</tr>
<tr>
<td>V_min, m/s</td>
<td>0.19±0.02</td>
<td>0.17±0.02</td>
</tr>
<tr>
<td>RI</td>
<td>0.65±0.04</td>
<td>0.78±0.04</td>
</tr>
<tr>
<td>PI</td>
<td>0.96±0.06</td>
<td>1.02±0.02</td>
</tr>
<tr>
<td>S/D</td>
<td>0.31±0.03</td>
<td>0.35±0.03</td>
</tr>
</tbody>
</table>
### Table 7. Dynamics of indicators of the nitrogen excreting function of the kidneys (average indicators) in patients of all experimental groups (n=142), M±m.

<table>
<thead>
<tr>
<th>Indexes</th>
<th>IA group, n=48</th>
<th>IB group, n=22</th>
<th>II group (comparison) n=42</th>
<th>III group (contr.) n=30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day 10 day 30 day</td>
<td>1 day 10 day 30 day</td>
<td>1 day 10 day 30 day</td>
<td>1 day 10 day 30 day</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>92,3±6,8* 89,0±6,4* 74,0±4,2*</td>
<td>138,0±16,1* 108,2±12,4* 103,0±14,3*</td>
<td>105,0±16,3* 123,0±14,5* 110,3±14,2*</td>
<td>95,0±6,8*</td>
</tr>
<tr>
<td>Leukocytosis, 10^9/L</td>
<td>8,6±0,54* 8,5±0,7* 7,9±0,44*</td>
<td>11,7±0,68* 9,7±0,42* 8,0±0,52*</td>
<td>9,0±0,75* 12,0±14,5* 12,0±14,5*</td>
<td>6,5±0,42*</td>
</tr>
<tr>
<td>ESR, mm/hour</td>
<td>9,4±1,9* 10,3±3,20* 7,3±3,4*</td>
<td>19,6±1,13* 18,1±3,6* 15,3±3,2*</td>
<td>16,4±3,3* 26,4±1,03* 19,1±6,9*</td>
<td>8,4±2,3*</td>
</tr>
<tr>
<td>Rod nuclear, %</td>
<td>6,0±1,2* 6,75±1,13* 6,01±0,84*</td>
<td>9,45±1,4* 8,05±1,2* 6,01±0,72*</td>
<td>9,0±1,4* 11,45±1,47* 9,01±2,3*</td>
<td>5,01±0,74*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Note: * - the difference in indicators regarding treatment is likely, P≤0,05.</td>
</tr>
</tbody>
</table>

### Table 8. Frequency of complications during conservative treatment of patients with ureteral stones, n=90.

<table>
<thead>
<tr>
<th>Complication</th>
<th>IA group (conservative treatment), n=48</th>
<th>II (comparison) group, n=42</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10-14 days</td>
<td>1 month</td>
</tr>
<tr>
<td>Exacerbation of hr. pyelonephritis</td>
<td>3</td>
<td>6,25%</td>
</tr>
<tr>
<td>Acute pyelonephritis</td>
<td>3</td>
<td>6,25%</td>
</tr>
<tr>
<td>Kidney carbuncle</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Persistent leukocyturia</td>
<td>6</td>
<td>12,5%</td>
</tr>
<tr>
<td>Increasing the level of azotemia</td>
<td>2</td>
<td>4,2%</td>
</tr>
<tr>
<td>In total</td>
<td>14</td>
<td>5</td>
</tr>
</tbody>
</table>

**Figure 1.** ROC-curve for β2-microglobulin.

**Figure 2.** ROC-curve for IL – 1β.
Figure 3. ROC curve for NGAL.

Figure 4. Results of ELISA and general laboratory studies of patients included in the IA group.

Figure 5. Results of ELISA and general laboratory studies of patients included in the IB group.
Based on the conducted research and obtained results, risk factors have been identified, and criteria for selecting further treatment tactics have been developed. The main recommendations are as follows:

- Upon hospitalization of the patient and detection of an elevation in the level of one of the three early kidney injury markers, it is recommended to initiate comprehensive conservative therapy with intensified diagnostic monitoring over a period of 5-7 days. If the kidney stone does not pass on its own, scheduled surgical intervention is recommended. In cases of exacerbation of obstructive symptoms, urgent surgical treatment is necessary. These guidelines aim to address the risk factors and tailor the treatment approach to each patient’s specific condition for better outcomes.

- In cases where the levels of two or more inflammatory markers are elevated upon hospitalization, regardless of the results of general laboratory indicators indicating the presence or absence of an infectious – inflammatory process and renal insufficiency, immediate surgical intervention is recommended. This intervention should involve draining the affected kidney and removing the kidney stone, as well as administering antibiotic, anti – inflammatory, and infusion therapy.

These recommendations help establish criteria for selecting the optimal treatment approach based on the results of diagnostic studies and the risks of kidney damage and complication development. By promptly addressing multiple elevated inflammatory markers, healthcare providers can effectively manage and treat the condition to minimize potential adverse outcomes.

Conclusion.

1. General laboratory analysis indicators in patients with upper urinary tract calculi (Urinary stone disease) within the first 24-48 hours cannot definitively indicate the absence of an infectious-inflammatory process in the kidneys or the development of renal insufficiency.

2. The elevation of inflammatory predictor markers in more than 50% of cases indicates the development of infectious-inflammatory complications 12-24 hours before clinical and general laboratory changes occur.

3. The comparative analysis of the effectiveness of conservative treatment in groups IA and II showed that complications of the inflammatory process in the kidneys occurred 5 times more frequently in group II (comparison group) compared to group IA.

4. The use of enzymatic tests for early kidney injury markers allows distinguishing two main treatment directions - conservative and urgent surgical, significantly reducing the frequency of inflammatory, purulent-septic complications, and improving the treatment outcomes of patients with upper urinary tract obstruction in urinary stone disease.

**Conflict of interest:** None.

These conclusions highlight the importance of using early diagnostic markers for kidney injury and implementing appropriate treatment strategies to manage complications effectively in patients with upper urinary tract calculi. The absence of conflicts of interest ensures the impartiality of the research and recommendations provided.

**REFERENCES**