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ABSTRACT

of the dissertasion for the degree Doctor of Philosophy

CHARACTERISTICS OF PATHOMORPHOLOGICAL CHANGES IN KIDNEY TUBES UNDER THE INFLUENCE OF ENDOTOXIN

Specialization: 3242.01 – Pathological anatomy

Field of science: Medicine

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GENERAL DESCRIPTION OF THE WORK

The urgency and extent of the problem. The study of the damaging effects of various toxic substances on the vital organs of the human body, including the morphofunctional characteristics of the kidneys, is one of the priority issues of nephrology and clinical morphology. Despite the recent innovative development of resuscitation measures, endotoxemia during sepsis causes multiple organ damage and organ failure. In acute kidney injury, acute renal failure and acute tubule injury caused by ischemia and multiple toxic agents continue to be at the forefront. Despite the development of medical innovations and the emergence of new diagnostic methods, in cases of sepsis, endotoxemia is considered the main cause of death in patients in the acute period. Despite the development of medical innovations and the emergence of new diagnostic methods, endotoxemia remains the primary cause of mortality in patients during the acute phase of sepsis. Sepsis has been reported as one of the top ten causes of death in clinical settings in the United States. Research indicates that globally, 30 million patients suffer from sepsis-related complications each year, with 6 million losing their lives due to these complications[1]. Acute kidney injury, which arises following sepsis complications, significantly complicates the treatment of many hospitalized patients and exacerbates the progression of severe outcomes[2]. Statistical data analysis in numerous studies shows that ischemia-induced acute tubular injury occurs in 50% of cases, while acute tubular injury caused by toxic substances accounts for 35%[3]. Many researchers have experimentally introduced various endotoxins into animals to study the effects of endotoxin exposure on the human

[1] Acedillo R.R, Mcarhur Wald E., Characteristics and outcomes of patinents discharged home from an emergency department with AKI // Clinical Journal of the American Sociyety of Nephrology 12: 1215-1225.

[2] John M., Reinhart K., et all. International Sepsis Forum, “Biomarkers of sepsis,” // critical Care Medicine , 2009.vol 37, no 7, p. 2290-2298.

[3] Linlong S., Zou Y., et all. Comparision of two different models of sepsis induced by cecal ligation and puncture in rats” // The Jornal of Surgical Research, 2018. vol. 229, p 277-282.

body, particularly on the kidney tubules, yielding diverse microscopic findings[4]. Currently, researchers such as Lange, Genberg, Bellomo, and their colleagues are investigating acute tubular injuries resulting from endotoxin exposure[5].

The creation of an experimental endotoxemia model allows for the study of results and underlying causes that cannot be achieved in clinical settings by replicating them in animal models. In some scientific studies, the effects of endotoxemia on nerve tissue and its various structures have been investigated, leading to the publication of numerous scientific articles. The ultrastructural characteristics of pathological changes caused by endotoxins have been studied by the staff of the Department of Normal Histology, Histology, and Embryology at Azerbaijan Medical University[6,7,8].

The aim of the study:

The primary objective of this research is to compare the pathological changes observed in the renal tubules following the creation of an experimental endotoxemia model with the biopsy and laboratory findings obtained from clinical cases of kidney damage

Objectives of the study:

1. To determine pathomorphological changes in tubules in kidney biopsies taken 6 hours after creating an experimental endotoxemia model and kidney biopsy samples taken from

[4] Kidney international supplements/ KDIGO 2012. Clinical Practice Guidline for the evaluation and manacment of cronic kidney disease.Volume 3/ issue 1/ January (1) 2013. p.142.

[5] Langenberg C., Wan L., et all: Renal blood Flow in experimental septic acute renal failure // Kidney Int 69:1996. p. 2002-2006.

[6] Anderson G.G, Goller C.C.,: Polysccharide capsule and sialic acid mediated requlation prmote biofilm-like intracellular bacterial 390 communities during cystitis // Infect Immun 2010, 78: p. 963-975.

[7] Qasımov E.K. Aliyarbayova A.A Structural changes of capsular.Glial and neural elements of the spinal ganglia in acute experimental edema. Light and electron mikroskopic studies // national J. Of Neurology (Scientific-practical journal). Baku, 2015, №1 (7). p. 31-39.

[8] Qasımov E.K., Ayyubova G. M., et all. Structuctural changes in microvascular endotelial cells of brain sheath and spinal ganglia in experimental endotoxemia. Light and electron microscopic studies Международный журнал «Вестник морфологии», Украина, г, Винница, 2016, т,22, №1, стр, 7-16.

patients with tubulo-interstitial nephritis, using histochemical and immunohistochemical staining methods.

2. To determine cells with a normal nucleus in the tubules, undergoing apoptosis, karyopyknosis, karyorrhexis and karyolysis changes in the nucleus in the kidney pieces taken 6 hours after creating the endotoxemia model.
3. To determine the pathomorphological changes in the basement membranes in the kidney slices obtained from the experimental endotoxemia model and in the kidney biopsy samples obtained from patients with tubulo-interstitial nephritis at the ultrastructural level.
4. To study the edema formed in the intertubular structure in the experimental endotoxemia model at the histochemical and ultrastructural level.
5. In biopsies obtained from patients with acute and chronic tubulo-interstitial nephritis, to determine the changes in normal nuclei, apoptotic, karyopyknosis, karyorrhexis and karyolysis in the cells that make up the proximal and distal tubules of the kidneys.

Materials and methods of research:

The study materials included kidney biopsies from rats injected with lipopolysaccharide LPS endotoxin and biopsies and clinical data of 30 patients with tubulointerstitial nephritis (TIN). 30 adult laboratory white rats with a weight of 200-250 grams, bred under special conditions, and kidney tissue taken from them were used as research objects. In the study, the kidney tissue used for the control group was compared with the microscopic results of biopsies of healthy rats and humans with autopsy samples taken from 10 car accident victims. Standardized passported data were used in the KDİGO (Kidney Disease Improving Global Outcomes) system (a global non-profit organization that develops and implements evidence-based clinical practice guidelines for kidney disease) for the normal indicators of ultrastructural examinations. Microscopy analysis of patient biopsies with a high titer of E.Coli in the bacteriological examination of urine was carried out in the referral form to evaluate tubules in examining biopsy pieces of tubulointerstitial nephritis patients. To stain the pieces taken from the biopsy and experimental endotoxemia

model in the study, hematoxylin-eosin, "Masson Trichrome" (MT) and "Periodic Acid Schiffe" (PAS) method, E.Coli LPS (2D7/1) from the histochemical dyes produced by the "Chem Bio" company) immuno-histochemical staining of the contrast medium against 1/100 and ultra-thin sections obtained to examine the changes at the ultrastructural level were studied under a Japanese-made JEM-1400 (JEOL-Japan) transmission electron microscope under a voltage of 80-120 kV and photomicrographs were drawn. Statistical methods were used to determine the integrity of the obtained results. Characterization of groups consisting of units, analysis of variation, mean and average structural indicators were calculated in the description of quantitative indicators by groups. To describe the quality indicators, the frequency of the intensity indicators was calculated for each group, the results of the comparisons were the arithmetic mean value (m) for each group, the mean square deviation of the arithmetic mean value, its standard error (Se) as well as the minimum (min) and maximum (max) of the series) prices have been determined. The obtained results were evaluated by Pearson, U-Mann-Whitmney (Whitmey) statistical integrity of the difference between the indicators of the groups with t-Student-Bonferroni. A non-parametric method that evaluates the difference between indicators to compare quantitative indicators in groups - the Mann-Whitney U test was considered honest if the statistical difference between groups was $p<0.05$. Intra-group and inter-group variances were calculated based on the dispersion of quantitative indicators, and acceptance or rejection of the "0 hypothesis" was given based on the F-Fisher test. Correlation analysis was performed to reveal the dependence between different indicators in the examination groups. For this purpose, the correlation coefficient was calculated and reliable correlation relationships were taken into account in the research to determine the integrity of the obtained coefficient.

Key theses to be defended:

1. 6 hours after introducing E. coli LPS endotoxin into the experimental model, ultrastructural changes at the level of organelles are determined in the renal tubules.
2. In the kidney biopsies obtained from the experimental endotoxemia model and patients with tubulointerstitial nephritis, the indicators of cells with a normal nucleus, undergoing

apoptosis, karyopyknosis, karyorrhexis and karyolysis changes in the nucleus are determined by statistical analysis.

3. In cases of endotoxemia in patients, clinical-laboratory indicators of creatinine, urea, SRP, and protein in urine and the specificity of changes in the results of cultivation of E. coli in urine are confirmed by pathohistological analysis of biopsies.

Scientific novelty of the study:

The scientific novelty of the research work: As a result of our research, for the first time, changes occurring in the epithelial cells of the proximal and distal tubules of the kidneys were discovered 6 hours after the creation of E.Coli endotoxin. model of endotoxemia was comprehensively defined and studied by immunohistochemical staining.

6 hours after the creation of the endotoxemia model, the characteristics of damage areas between the epithelial cells of the proximal and distal tubules of the kidneys were clarified at the ultrastructural level. For the first time, after the creation of the experimental endotoxemia model and in the kidney biopsy samples obtained from patients with tubulointerstitial nephritis, the characteristics of cells with a normal nucleus in the tubules, as well as karyopyknosis, karyorrhexis, karyolysis changes and apoptotic cells were determined at the pathohistological and ultrastructural level in the nucleus. At the same time, for the first time, a comparative correlation was made between the pathomorphological results of the biopsy of patients with tubulo-interstitial nephritis and the results of laboratory analyzes of the kidney pieces obtained from the experimental endotoxemia model.

Theoretical and Practical Significance of the Study

The scientific significance of the study is that the changes occurring in the renal tubules and surrounding structures in acute kidney injury have been comprehensively studied. "During the experimental and clinically oriented research work carried out at the Azerbaijan Medical University", which won the comprehensive research programs (EIF-KETPL-2015-1(25)) presented within the framework of the main grant competition of the Science Development Foundation under the President of the Republic of Azerbaijan in 2015

within the framework of the project "Provision of molecular genetic assessment of pathological changes", as well as as a tool in the pathology of kidney diseases, the obtained preparations and images as a result of changes caused by endotoxins in the renal tubules were used in the educational work of the Department of Pathological Anatomy. After the creation of the acute endotoxemia model, the main practical significance in the pathomorphological diagnosis of kidney diseases and, as a consequence, the changes detected in the epithelial cells that make up the tubules at the ultrastructural level using histochemical, immunohistochemical staining and electron microscopic examination are of primary importance. as a result, in the choice of new treatment principles in the future.

Approbation of dissertation.

The materials and individual fragments of the research work were discussed at many scientific meetings and conferences.

Turaz Academy, Turkey-Azerbaijan 1st International Congress of Forensic Medical Expertise and Pathology. October 13-16, 2016, Baku. Azerbaijan.

Proceedings of the 5th Congress of the Russian Society of Pathologists. Chelyabinsk, June 1-4, 2017

6th International Symposium-Cum-Training Course on Molecular Medicine and Drug Research (MMDR-6). November 2017.

Scientific and practical conference "Actual problems of modern nephrology" dedicated to the 14th World Kidney Day. March 14, 2019. Baku, Azerbaijan.

International scientific-practical conference dedicated to the 100th anniversary of the establishment of the Faculty of Medicine. April 18-19, 2019. Baku, Azerbaijan.

One health: Problems and solutions. 24.05.2019-25.05.2019. Baku. Azerbaijan (One health. Problems and solutions. 24.05.2019-25.05.2019. Baku, Azerbaijan).

Joint scientific meeting of employees of the Scientific Research Center of Azerbaijan Medical University. 08 July 2019. Baku, Azerbaijan..

36th National Nephrology Congress, 29th National Nephrology Nursing Congress. Antalya. Turkey. November 16-20, 2019 (36th

National Congress of Nephrologists, 29th National Congress of Nephrology Nurses. Antalya, Turkey. November 16-20, 2019).

- ISN (International Society of Nephrology) World Congress of Nephrology Abu Dhabi 2020, UAE. Kidney International Reports (2020) 5, p. 34. March 01.2020.
- 57th ERA (European Renal Association) – EDTA (European Association for Dialysis and Transplantation) Congress Abstracts. Nephrology Dialysis Transplantation p-419. 06-09 June 2020
- Joint scientific meeting of the staff of the Department of Pathological Anatomy on January 25, 2022. Baku. Azerbaijan.
- On June 10, 2024, at the scientific seminar of the BED 4.20 Dissertation Council operating under the Azerbaijan Medical University of the Higher Attestation Commission under the President of Azerbaijan.

Organization where the Dissertation was conducted. The dissertation work was performed at the Department of Pathological Anatomy of the Azerbaijan Medical University.

Application of research work to practice. The dissertation work and its fragments were conducted at the Department of Pathological Anatomy of the Azerbaijan Medical University, the Scientific Research Center, the Laboratory of Electron Microscopy and the Bureau of Pathological Anatomy of the Ministry of Health "Forensic Medical Expertise and Pathological Anatomy" SCR Union.

The connection of the research with the planned scientific work at the Azerbaijan Medical University. The dissertation work and its fragments were conducted at the Department of Pathological Anatomy of the Azerbaijan Medical University, the Scientific Research Center, the Laboratory of Electron Microscopy and the Bureau of Pathological Anatomy of the Ministry of Health "Forensic Medical Expertise and Pathological Anatomy" SCR Union.

The scope and structure of the dissertation. Dissertation is written in classical style on 152 pages (171496 characters) compiled on a computer and annotated in "Introduction" (volume: 20528 characters), "Conclusion" (volume: 17157 characters), "Results" (volume: 1975 characters), "Practical recommendations" (volume:

1038), "List of References" consists of structural sections. The "main content of the dissertation" section is divided into 5 chapters. Chapter I "Summary of literature" (volume: 34777 marks), Chapter II: (volume: 19813 marks), Chapter III: (volume: 26729 marks), Chapter IV (volume: 7843 marks), Chapter V (volume: 41562 marks).

RESULTS OF INDIVIDUAL RESEARCH

Statistical Analysis of Clinical and Morphological Findings in Patients with Acute and Chronic Tubulointerstitial Nephritis

It was found that 12 (40%) patients included in the study had acute TIN, and 18 (60%) had chronic TIN. Fourteen patients were men (46.7%) and 16 (53.3%) were women. Table 1 shows that biopsies of patients with acute and chronic TIN were compared with kidney samples taken from 10 (100%) cadavers who died as a result of accidents and were considered healthy. For the purpose of pathomorphological comparative study of biopsies of patients with acute and chronic TIN, the results of selective blood tests, the amount of protein in the urine, and the determination of the E.Coli bacterial culture were statistically analyzed. In the intergroup examination, the mean SC amount in statistical evaluation was 37.5±0.08 mg/dL in CTIN and 32.6±1.4 mg/dL in CTIN. Fisher's exact test was determined based on the analysis of variance (Pf=0.010). P>0.005 in nonparametric Fisher's test. The mean creatinine amount was 2.268±0.093 mg/dL in ATIN and 1.78±0.090 mg/dL in CTIN. The values of Pf<0.001 and Pu<0.005 were statistically significant. In the intergroup comparison of CRP results, it was 9.75±0.72 mg/dL in CTIN (n=12) and 13.56±0.80 mg/dL in CTIN (n=18). The Pf=0.003, Pu<0.005 indices were statistically significant.

In the intergroup statistical examination of the results of E.Coli cultivation in urine, comparing the results of E.Coli with the control group, the average number of E.Coli in patients with CTIN was 115.9±2.8 CFU/ml, and the average number in patients with CTIN was 118.9±2.0 CFU/ml. According to Fisher, Pf=0.037 and Pu>0.005 were statistically significant (Table 1).

Table 1

Laboratory Characteristics in Blood and Urine of Patients with Acute and Chronic Tubulointerstitial Nephritis

Göstəricilər		N	M	±m	Min	Max	Pf	Pu
Urea mq/dl	Cronic	18	32.6	1.4	24.4	41.2	0,010	P>0,005
	Acute	12	37.5	0.8	31.8	41.4		
Creatinin mq/dl	Cronic	18	1.758	0.090	1.29	2.61	0,001	P<0,005
	Acute	12	2.268	0.093	1.68	2.61		
CRP	Cronic	18	13.56	0.80	8	21	0,003	P<0,005
	Acute	12	9.75	0.72	7	15		
Protein in urine g/l	Cronic	18	0.254	0.090	0.021	1.206	0,146	P>0,005
	Acute	12	0.087	0.021	0.01	0.303		
E.Coli in urine CFU/ml	Cronic	18	118.9	2.0	107	134	0,037	P>0,005
	Acute	12	115.9	2.8	103	134		

Note: The number of observations (N) refers to the total cases included in each group. Minimum and maximum values indicate the range of measurements recorded.The p-values (PF) assess statistical significance, while U-statistic (PU) represents results from the non-parametric tests.

Histological characteristics of renal tubules of animals included in the control and experimental endotoxemia groups.

Before euthanizing the healthy rats included in the control group of the experimental endotoxemia model, the temperature of the animals was measured, and the fur coat was noted to be smooth and dense.

Before the kidney removal of the rats in the control group, blood was collected from the tail vein for biochemical tests, then the abdominal cavity was opened, and after the kidney removal of the rats, the kidneys were visually examined macroscopically. Thus, the kidneys are normal in size and have a natural reddish color. It is characterized by the presence of a thin and smooth capsule. The density of nephrons in the cortex is normal when stained with the hematoxylin-eosin method in the light microscope examination of preparations made from pieces taken from both kidney tissues.

Bowman's capsule of some nephrons is stained according to the light norm. Exudation and lympho-leukocyte infiltration were not noticeable in the shell and brain layer of the kidneys.

The experimental endotoxemia model was created after the preparations made from pieces taken from the kidneys of rats were stained with hematoxylin-eosin dye for examination with a light microscope. 6 hours after the injection of LPS E.Coli endotoxin, edema and necrobiotic changes in the tubules and interstitial area were observed. During the examination, reversible and irreversible pathomorphological changes were found in the proximal and distal tubules of the kidneys. Inflammation and edema were found in Bowman's cavity due to inflammation caused by endotoxemia in many glomeruli, and destructive changes due to edema were observed in glomeruli located in the cortex and brain substance of kidney tissue. Thus, 6 hours after the experimental model of endotoxemia, a decrease in the number of nephron apparatus in the cortex layer of the kidneys (Figure 1A) and destruction of nephrons in some areas was observed. Histological characteristics of renal tubules in animals included in the control and experimental endotoxemia groups

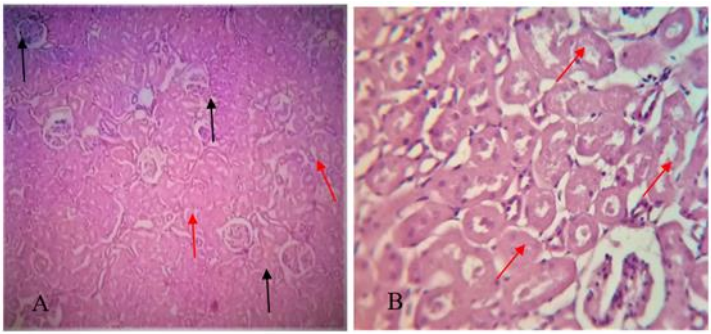


Figure 1. Experimental endotoxemia model: Pathomorphological changes in renal tissue. A: Glomerulus, B: Proximal and distal tubules. Stained with hematoxylin and eosin, magnification: x 200.

As can be seen in Figure 1B, in addition to the damage due to acute endotoxemia, edema, and delamination of capillary loops are observed in

other visual fields. Basal membranes of proximal tubules are slightly thickened as a result of swelling due to edema, and hydropic dystrophy in the form of vacuoles in the cytoplasm is observed in the cells, and the loss of the normal epithelial structure is manifested. In some areas of the proximal tubules, "membrane tearing" membranorrhexis is observed due to the effect of LPS endotoxin. Individual apoptotic bodies were detected in the cells. Karyopyknosis, karyorrhexis, and karyolysis occurred in the nuclei due to the changes due to the effect of LPS endotoxin, and the micropiles located in the apical part of the cells were lost. As a result of this, the opening of the ducts is slightly narrowed. In some tubules, areas of focal necrosis of the tubules due to karyolysis were noted. The apical surface of the cells located in the basal membrane of the distal tubules was damaged, the membrane lost its integrity, the opening of the tubules was narrowed due to cell edema, and sharp destructive changes were observed in some areas. Membranorrhexis, destruction of cells and organelles, and focal necrobiosis areas are found in the basal membranes of tubules, and dystrophic changes in the cytoplasm of epithelial cells are found in some tubules. The apical surface of the cells of the distal tubules was damaged, and focal destructive changes were observed.

Characteristics of immunohistochemical staining of the pathomorphological changes of antibodies against E. coli LPS (2D7/1) endotoxin in the proximal and distal tubules of the kidneys in the experimental endotoxemia model.

When we analyzed the staining property of E. Coli LPS (2D7/1) immunohistochemical dye to detect antibodies against E. Coli LPS endotoxin in the proximal and distal tubules 6 hours after the creation of the experimental endotoxemia model, in the control group, in the proximal and distal tubule cells within the group, proximal (n =10) and distal tubule (n=10) cells staining was recorded. Mean staining number, minimum, and maximum of 3 (+++) positive staining, 2 positive staining (++), and 1 positive (+) staining in proximal and distal tubules to evaluate E. coli LPS (2D7/1) immunohistochemical staining indicators were calculated. The diagram is illustrated graphically in Figure 1 A and B.

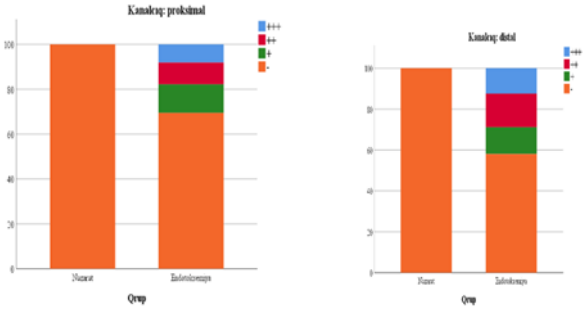
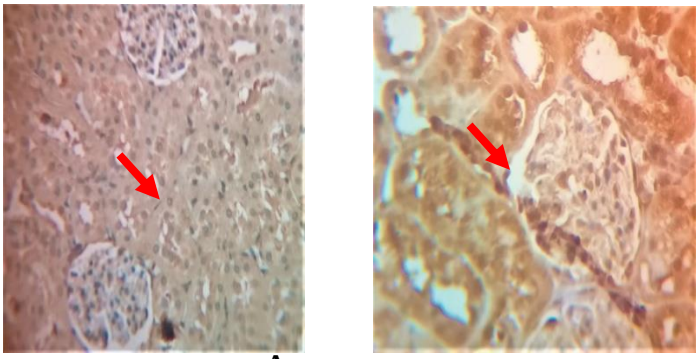


Diagram 1. Six hours after the administration of Escherichia coli LPS endotoxin, chemical staining characteristics of proximal and distal tubular cells in the kidneys were identified, highlighting the presence of E. Coli LPS (2D7/1) anty-bodies.

Within-group Fisher's Pf<0.001 and Wilcoxon's Pu=1.000. LPS (2D7/1) immunohistochemical staining in proximal tubular epithelial cells (n=20), average number of 3 positive (+++) stained cells was 8.10±0.73, minimum 4 cells and maximum 14 cells, distal tubular cells (n =20), 12.25±0.99 cells, a minimum of 7, a maximum of 25 cells and Fisher's Pf<0.001 and Pu<0.001 for intragroup comparison were statistically honest. E. coli LPS (2D7/1) immunohistochemical staining occurred in proximal tubule cells (n=20), the average number of 2 positive (++) stained cells (9.70±0.79) cells, minimum 4 cells and maximum 20 cells, in distal tubule cells (n=20), 16.45±0.88, minimum 10, maximum 22 cells and according to Fisher intragroup comparison, Pf<0.001 and Pu<0.001. According to the inclusion criteria of the within-group study, changes in proximal and distal ductal epithelial cells were statistically significant. E. coli LPS (2D7/1) immunohistochemical staining in proximal tubule epithelial cells, the average number of 1 positive (+) stained cells 12.75±0.69 cells, a minimum of 8 cells, and a maximum of 20 cells, in distal tubule cells, 13.10 ±0.77, minimum 8, maximum 20 cells and within-group Fisher's Pf=0.737 and Pu=0.837 showed statistical integrity (Figure 2).



A B

Figure 2. Model of experimental endotoxemia. Proximal (A) and distal tubules (B). Immunohistochemical staining of E. coli LPS (2D7/1). Magnification x 200

Changes in proximal and distal tubule cells were not statistically significant according to the inclusion criteria in the within-group study. The average number of unstained cells (-) in proximal tubule epithelial cells unstained by E. coli LPS (2D7/1) immunohistochemical dye was 69.45±0.92 cells, minimum of 61 cells and a maximum of 76 cells, in distal tubule epithelial cells 58.05 ±1.17, minimum 45, maximum 65 cells and according to within-group Fisher, Pf<0.001 and Pu<0.001 are honest. Thus, according to intragroup studies, the results obtained using E.Coli LPS (2D7/1) immunohistochemical staining in proximal and distal tubular epithelial cells were statistically honest.

In the experimental endotoxemia model, let's look at the statistical characteristics of E.Coli LPS (2D7/1) immunohistochemical staining in proximal tubule cells between groups, in the control group, 3 positive (+++), 2 with E.Coli LPS (2D7/) immunohistochemical staining. According to the staining characteristics of the cells marked as positive (++) and 1 positive (+), the average staining number was 0, and the minimum and maximum values were 0, in preparations obtained from E.Coli LPS (2D7/1) immunohistochemical staining of kidney pieces 6 hours after the endotoxemia model, In 3 positive stainings, the mean number of staining was 8.10±0.73, minimum 4 cells and maximum 14 cells, and between groups Pf<0.001 and Pu<0.001 according to Fisher and gave statistically honest results.

In 2 positive stainings (++) of proximal tubules with E. coli LPS (2D7/1) immunohistochemical staining, the mean staining number was 9.70 ± 0.79, with a minimum of 4 cells and a maximum of 20 cells, and between-group Fisher's Pf<0.001 and Pu<0.001 and a statistically honest result was obtained. The mean number of proximal tubules, 1 positive (+) stained cells with E. coli LPS (2D7/1) immunohistochemical dye was 12.75±0.69, minimum 8, maximum 20 cells and intergroup Fisher's Pf<0.001 and Pu<0.001 and a statistically honest result was obtained. The average number of cells not stained with E.coli LPS (2D7/1) immunohistochemical dye was 69.45±0.92, minimum 61, maximum 76 cells, and Pf<0.001 and Pu<0.001 according to intergroup Fisher, and a statistically honest result was obtained.

If we look at the statistics of immunohistochemical staining to detect E.Coli LPS (2D7/1) antibodies in the distal tubule cells in the intergroup examination in the experimental endotoxemia model, in the control group there were 3 positive (+) with E.Coli LPS (2D7/1) immunohistochemical staining. ++), 2 positive (++), and 1 positive (+), according to the staining characteristics of the cells, the average staining number, minimum and maximum indicators were 0 (-). In preparations obtained from E.Coli LPS (2D/71) immunohistochemical staining of kidney slices taken 6 hours after the endotoxemia model, the mean number of staining in 3 (+++) positive stainings was 12.40±0.99, with a minimum of 7 cells and a maximum of 25 cells and between groups According to Fisher, Pf<0.001 and Pu<0.001 were obtained and a statistically honest result was obtained. In 2 (++) positive staining, the average number of staining was 16.45±0.88, a minimum of 10 cells and a maximum of 22 cells, and according to intergroup Fisher, Pf<0.001 and Pu<0.001, and a statistically honest result was obtained. The mean number of cells stained 1 positive (+) with E. coli LPS (2D/1) immunohistochemical stain of proximal tubules was 13.10±0.77, minimum 8, maximum 20 cells and between groups according to Fisher, Pf<0.001 and Pu<0.001 and a statistically honest result was obtained. The mean number of cells not stained by the immunohistochemical method to detect E. coli LPS (2D7/1) antibodies was 58.05±1.17, minimum 45, maximum 65 cells, and according to between-group Fisher, Pf<0.001 and Pu<0.001, and the honest result received.

STATISTICAL CHARACTERISTICS OF THE RESULTS OF KIDNEY BIOPSY OF PATIENTS WITH TUBULOINTERSTITIAL Nephritis

For the study of kidney biopsies obtained from patients with acute and chronic TIN and positively stained with an immunohistochemical dye for the detection of E. coli antibodies to LPS (2D7/1), in the laboratory of patients with elevated titers of creatinine, urea, CRP, and urine culture results were used. Thus, a comparative analysis of intragroup and intergroup indicators was conducted in the statistical analysis of patients with acute (n=12) and chronic (n=18) TIN. Statistical reliability was checked relative to the control group. The following results were obtained during the pathomorphological examination of biopsies of patients with acute (n=12) and chronic (n=18) TIN and the statistical analysis of damage to the cells of the proximal and distal tubules. When analyzing intergroup results, statistical integrity was determined by comparing the cells of the proximal tubules with the control group.

Table 2 shows that pathohistological changes in the proximal tubules of the kidneys in biopsies of patients with acute and chronic TIN compared to the control group were statistically significant. Pieces of the autopsy of the kidneys obtained from the control group were stained with the usual method of staining with hematoxylin-eosin. If we look at the statistical results of the changes obtained in the cells of the proximal and distal canals of the control group and patients with TIN, normal cells in the control group (n=10) were 96.3±0.4, minimum 94, maximum 98 cells, in patients with acute and chronic TIN (n=30), average value 67.5±0.3, minimum 64, maximum 70 cells, intergroup Pf Fisher <0.001 and Pu<0.001 were statistically significant. The average amount of apoptotic cells in the control group was 1.5±0.3, minimum 1, maximum 4 cells, and in patients with acute and chronic TIN (n=30), the average amount was 11.6±0.3, minimum 9, maximum 16 cells. Comparisons between groups were statistically fair: Pf<0.001 according to Fisher and Pu<0.001 according to Wilcoxon.

The average number of cells with karyorexic changes in the nuclei was 0, the minimum was 0, the maximum was 2 cells in the

control group, the average number of cells in patients with acute and chronic TIN was 4.67±0.23, the minimum was 3, the maximum was 8 cells and according to Fisher's intergroup data, Pf<0.001 and Pu<0.001 are statistically significant. The average number of cells with karyolysis changes in the nuclei was 0.300±0.153, the minimum - 0 cells, the maximum - 1 cell, the average number of cells in patients with acute and chronic TIN - 4.667±0.422, the minimum - 1, the maximum was 8 cells, and Pf<0.001 and Pu<0.001 according to Fisher's intergroup statistic. Cell changes were statistically reliable in the control group according to the criteria included in the study.

Table 1

Comparative characterization of changes in the nuclei of epithelial cells in the proximal tubules of renal biopsies of the control group, acute and chronic tubulointerstitial nephritis patients

The parameters		N	M	M	Min	Max	Pf	Pu
Normal cells (pr)	Control	10	96.3	0.4	94	98	<0,001	<0,001
	TIN	30	67.5	0.3	64	70		
Apoptotic cells (pr)	Nəzarət	10	1.5	0.3	1	4	<0,001	<0,001
	TIN	30	11.6	0.3	9	16		
Cells with karyopyknosis changes in the nuclei (pr)	Nəzarət	10	1.0	0.211	0	2	<0,001	<0,001
	TIN	30	11.5	0.274	9	14		
Cells with karyorrhexis changes in the nuclei (pr)	Nəzarət	10	0.90	0.18	0	2	<0,001	<0,001
	TIN	30	4.67	0.23	3	8		
Karyolysis in nuclei cells with changes (pr)	Nəzarət	10	0.3	0.153	0	1	<0,001	<0,001
	TIN	30	4.67	0.422	1	8		

As can be seen from Table 3, in the intragroup comparison of patients with acute and chronic TIN, the average number of normal cells in acute TIN is 67.8±0.4, minimum of 66, a maximum of 70 cells, the average number of cells is average number of cells in chronic TIN

is 67.4±0.4, minimum 64, maximum 70 cells and intergroup comparisons according to Fisher, Pf=0.501, Pu=0.662 were obtained, and statistical integrity was not achieved. Statistically different results were obtained for the average number of apoptotic cells in biopsies in patients with acute and chronic TIN, compared within the group. 11.4±0.4, minimum 9, maximum 13 cells in acute TIN, 11.8±0.4, minimum 9, maximum 16 cells in chronic TIN and intragroup comparison according to Fisher, Pf=0.545, Pu=0.692 and the statistical result was not fair.

Table 3

Statistical indicators of within-group characteristics of nuclear changes in proximal tubule epithelial cells in kidney biopsies of patients with acute and chronic tubulo-interstitial nephritis

Göstəricilər		**N**	**M**	**±m**	**Min**	**Max**	**Pf**	**Pu**
Normal cells (pr)	Cronic	18	67.4	0.4	64	70	0,501	0,662
	Acute	12	67.8	0.4	66	70		
Apoptotic cells (pr)	Cronic	18	11.8	0.4	9	16	0,545	0,692
	Acute	12	11.4	0.4	9	13		
Cells with karyopyknosis changes in the nuclei (pr)	Cronic	18	11.611	0.363	9	14	0,628	0,662
	Acute	12	11.333	0.432	9	14		
Cells with karyorrhexis changes in the nuclei (pr)	Cronic	18	4.83	0.32	3	8	0,377	0,491
	Acute	12	4.42	0.31	3	6		
Karyolysis in nuclei cells with changes (pr)	Cronic	18	4.389	0.537	1	8	0,429	0.465
	Acute	12	5.083	0.690	1	8		

According to the study, the average number of cells with karyopyknotic changes in the nucleus was 11,333±0.363, a minimum of 9, a maximum of 14 in patients with acute TIN, 11,611±0.432 in patients

with chronic TIN, a minimum number of cells was 9, maximum 14 cells Pf=0.628 and Pu=0.662 statistically fair results were not obtained. The average number of cells with karyorrhexis changes in the nuclei in patients with acute TIN is 4.42±0.31, minimum 3, maximum 6 cells, in patients with chronic TIN 4.83±0.32, minimum 3, maximum 8 cells, according to Fisher, Pf=0.377, Pu=0.491 statistically different and unfair. The average number of characteristic cells with karyolytic changes in the nuclei of the proximal tubules was 5.083±0.690, minimum 1, maximum 8, according to Fisher, Pf=0.429 and Pu=0.465 in patients with acute TIN, which is statistically unfair.

When comparing within the group, the average number of normal cells in the distal tubules was 73.1±0.5, minimum 51, maximum 58 cells in patients with acute TIN, the average number of cells in patients with chronic TIN was 54.2±0.5, minimum 51, maximum 58 cells, and according to Fisher within the group Pf<0.001, Pu<0.001, which is statistically unfair.

In biopsies, the mean number of apoptotic cells in the distal tubules was 12.3±0.3, minimum 11, maximum 14 cells in patients with acute TIN, 27.8±0.4, minimum 25, maximum 31 cells in patients with chronic TIN, and intragroup comparison according to Fisher, Pf=0.577, Pu=0.491, did not show a statistically fair result. The mean number of cells with karyopyknotic changes in the distal tubules was 11,083±0.484 in patients with acute TIN, minimum 8, maximum 13, and the mean number of cells in patients with chronic TIN was 10,389±0.304. The minimum number of cells was 8, maximum 13 cells, according to Fisher, Pf=0.628, and Pu=0.662, and this was not statistically fair. The average number of cells with karyolytic changes in the distal tubules in patients with acute TIN was 3.00±0.30, minimum 2, maximum 5 cells, in patients with chronic TIN the average number of cells was 3.89±0.40, minimum 2, maximum 8 cells, according to Fisher, Pf=0.114 and Pu=0.158 are statistically different and are not honest. The average number of cells with karyolytic changes in the nuclei of the distal tubules was 1.667±0.310, minimum 2, maximum 7, according to Fisher, Pf<0.001 Pu<0.001 in

patients with acute TIN, a statistically honest result was obtained. Statistical analysis of the staining characteristics of the immunohistochemical dye for the detection of E. coli LPS counterbodies (2D7/1) used for research purposes in distal tubular epithelial cells in sections taken from kidney samples of patients with TIN showed that no staining was recorded in the control group. The average number of 3 positive (+++) cells stained with immunohistochemical dye E.Coli LPS (2D7/1) in the distal tubules of patients with TIN was 3.87±0.24, minimum 1, maximum 6 cells, E.Coli LPS (2D7/1)) the average number of 2 positive (+++) cells stained with immunohistochemical dye was 10.60±0.41, minimum 6, maximum 15, the average number of 1 positive (+) cell stained with immunohistochemical dye E.Coli LPS (2D7/1) was 18.37±0.44, minimum 14, maximum 24, the average number of distal cells not stained with immunohistochemical dye E.Coli LPS (2D7/1) was 67.17±0.41, minimum 62, maximum 71. According to the intergroup Statistically significant results were obtained according to Fisher, $Pf<0.001$ and $Pu<0.001$. When analyzing the features of immunohistochemical staining of the E.Coli LPS (2D7/1) antibody used for research purposes in the epithelial cells of the proximal tubules in sections prepared from kidney biopsies of the control group and kidney biopsies of patients with TIN, no staining was registered in the control group. The immunohistochemical method determined 3 (+++) positive staining indices, 2 positive staining indices (++), and 1 positive (+) staining index of the E.Coli LPS (2D7/1) counterbody in the proximal tubules, the minimum and maximum number, as well as the average number of stained cells. Immunohistochemical staining of E.Coli LPS (2D7/1) contrast material E.Coli LPS (2D7/1) in the proximal tubules of patients with TIN, the average number of 3 positively stained cells was 10.03±0.21, minimum 7, maximum 12 cells, E.Coli LPS (2D7/1) immunohistochemical average number of positively stained cells by method 2 is 10.17±0.37, minimum 4, maximum 15, the average number of cells positively stained by immunohistochemical method 1 when determining E.Coli LPS (2D7/1) antibodies is 10.17±0.37, minimum 7,

maximum was 14. The number of proximal cells not stained by immunohistochemical staining of E. coli LPS (2D7/1) was 70.03±0.51, minimum 64, maximum 79. Pf<0.001 and Pu<0.001 according to the intergroup Fisher criterion, statistically fair results were obtained.

CONCLUSİONS

1. E. Coli LPS antibody (2D7/1) was detected by immunohistochemical stain in the experimental model, and 2 positive stains were predominant in the proximal and distal tubular epithelial cells of TIN patients. [12, 15].
2. At 6 hours after the establishment of the experimental endotoxemia model, the average number of apoptotic cells in the proximal tubules of the kidneys was Pk14.20±0.82, Dk 12.80±0.68, cells with karyopyknosis changes in the nucleus Pk 16.40±0.87, Dk 1.40±0.79, cells with karyorrhexis changes in the nucleus Pk 5.50±0.47, Dk 4.55±0.43 and cells with karyolytic changes in the nucleus Pk 0.40±0.16, Dk 2.85±0.32, P<0.005 [5, 13].
3. In the endotoxemia model, destructive changes in E.Coli endotoxin were noted in the proximal and distal tubules of the kidneys, at the ultrastructural level in the microvilli covering the apical part of the cells, mitochondria, lysosomes, endoplasmic reticulum, and nucleus. If patients with CTIN showed thickening of the basement membranes due to edema, then patients with CTIN showed thickening of the basement membranes of the tubules due to an increase in connective tissue due to chronic inflammation [2, 4, 13, 16].
4. Six hours after the creation of the experimental model of endotoxemia, an increase in the intertubular structure, proximal and distal intertubular area is due to interstitial edema. The disruption of these structures was confirmed by the method of histochemical staining with PAS and Masson's trichrome. At the ultrastructural level, the connective tissue elements surrounding the tubules are destroyed and lose their integrity [1, 2, 3, 7].

5. Kidney biopsy in patients with acute and CTIN revealed damage to apoptotic cells, karyopyknosis, karyorrhexis, and karyolytic changes, as well as damage to the micropile covering the apical part of the tubules, as a result of toxic effects. As a result of staining, diffuse microvessels are found in the proximal tubules, and focal lesions in the distal tubules. Positive staining of contrast bodies proves that these changes are an indicator of endotoxin action. Detection of cell damage and determination of structural changes by the immunohistochemical method provide grounds for coming to this conclusion [6, 7, 8, 17].

PRACTICAL RECOMMENDATIONS

1. Based on the results obtained in the experimental model of endotoxemia, it can be said that it is important to perform a biopsy in patients with CTIN and CTIN of the kidneys. For this purpose, taking into account the etiological factor, it can be recommended to include these results in the biopsy guide.
2. The use of new endotoxin biomarkers can help determine the cause of acute kidney injury and early diagnosis by detection.
3. Pathomorphological diagnosis of the disease can be considered necessary in cases where laboratory diagnostics are uninformative in the diagnosis of patients with CTIN and CTIN, and the number of E. coli cultivations in the urine is large. Pathomorphological results can become the basis for a faster therapeutic effect in the treatment of nephrologists in the clinic.
4. Final results of the work When studying the cause of kidney damage in toxicosis at the Department of Forensic Medicine, images obtained from microscopic preparations can be used in theoretical and practical classes when teaching the pathomorphological features of kidney changes in patients with CTIN and CTIN at the Department of Pathological Anatomy.

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LİST OF ABBREVIATIONS

ACTH	– adrenocorticotropic hormone
BM	– basement membrane thickness
E. Coli	– Escherichia coli
EDTA	– Ethylenediaminetetraacetic acid
CTIN	– Chronic tubulointerstitial nephritis
IHC	– immunohistochemistry
IL1	– interleukin 1
AKI	– acute kidney injury
APP	– acute phase protein
ATIN	– acute tubulo-interstitial nephritis
LPS	– lipopolysaccharide
NPAR	– non-parametric
PAS	– periodic acid schiff
P-	– Pearson
TIN	– tubulointerstitial nephritis
TLR4	– toll-like receptor 4
TEM	– transmission electron microscope
CRP	– C-reactive protein

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