ORIGINAL PAPER

BIOCHEMICAL CHANGES IN THE EVOLUTION OF CHRONIC KIDNEY DISEASE ASSOCIATED WITH URINARY TRACT INFECTIONS

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Abstract. *The interrelationship of pathogenicity between urinary tract infections and chronic kidney disease is an incompletely debated issue. In chronic kidney disease, a variety of quite different substances (uremic toxins, betaine, amino acids, creatinine, urea, glucose) influence the microbial environment by biochemical changes. The study was performed in one year period on a group of 105 patients admitted in a Nephrology Department, with urinary tract infection in the presence or absence of CKD. In the group with UTI and CKD, predominated females in the postmenopausal period, especially in CKD stages with higher severity. Analyzing the recurrent UTIs and deaths we have found the presence of advanced age, female gender, pre-existing kidney disease, diabetes mellitus, hemodialysis and a large number of multi-resistant urinary microbial strains. The use of mathematical relationships allowed us to make some correlations between CKD evolution with or without UTI. However, having a small group of patients over one year period with less few subgroups of demographic, causal, comorbidity and biological factors, risk factors for reinfection or death has sometimes made it impossible to interpret mathematically the data obtained from the retrospective observational study.*

Keywords: *urinary tract infections, chronic kidney disease, incidence, risk factors, recurrent UTI.*

Abbreviations: *UTI - urinary tract infection; CKD - chronic kidney disease; UPEC - uropathogenic E. coli; GFR - glomerular filtration rate; ESKD - end-stage kidney disease; DM - diabetes mellitus; HT - hypertension; vs – versus.*

1. INTRODUCTION

Urinary tract infections (UTIs) remains one of the most common bacterial infectious diseases in the human population [1-6]. It is estimated to affect 150 million people each year worldwide, with an annual incidence of 12.6% in women and 3% in men [7, 8]. UTIs

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represent more than 8 million office visits and 1 million emergency department visits each year in the United States, eventually resulting in approximately 100,000 hospitalizations [9], with a cost more than 3 billion dollars annually [10].

UTIs refer to the presence of microbial pathogens within the urinary tract and it is usually classified by the site of infection as the bladder (cystitis), kidney (pyelonephritis) or urine (bacteriuria) [11]. UTI can be caused by Gram-negative bacteria such as *Escherichia coli*, *Klebsiella* species, *Enterobacter* species, *Proteus* species and gram-positive bacteria like *Enterococcus* species, and *Staphylococcus saprophyticus* [12]. Strains of uropathogenic *E. coli* (UPEC) are the primary cause of urinary tract infections, including both cystitis and pyelonephritis [13]. These bacteria have evolved a multitude of virulence factors and strategies that facilitate bacterial growth and persistence within the adverse settings of the host urinary tract [13]. UTIs are a significant cause of morbidity in infant boys, older men and females of all ages [14]. Serious squeals include frequent recurrences, pyelonephritis with sepsis, renal damage in young children, and complications caused by frequent antimicrobial use, such as high-level antibiotic resistance [13]. Complicated UTIs are defined as UTIs associated with factors that compromise the urinary tract or host defense, including urinary obstruction, urinary retention caused by neurological disease, renal failure, renal transplantation, immunosuppression, pregnancy and the presence of foreign bodies such as stones, indwelling catheters or other drainage devices [14]. For complicated UTIs, the order of prevalence for causative agents, following *UPEC* as most common, is *Enterococcus* spp., *K. pneumoniae*, *Candida* spp., *S. aureus*, *P. mirabilis*, *P. aeruginosa* and group B *Streptococcus* [14].

Chronic kidney disease (CKD) is a general term for heterogeneous disorders affecting the structure and function of the kidney [15]. CKD is an important public health problem characterized by poor health outcomes and very high healthcare costs [16]. It is estimated that 10–13% of adults in the USA suffer from some degree of CKD [17].

The underlying reasons for the higher risk of UTI in patients with chronic kidney disease are thought to be an altered host reaction and anatomic and functional disorders of the urinary tract [17]. The alteration in host protective functions is thought to be due to: loss of antibacterial properties of the urine; mild immunosuppression in uremia; and inhibition of protective mucosa production in the urothelium [18]. Evidence of a correlation between chronic kidney disease and higher UTI risk is most solid for autosomal dominant polycystic kidney disease and CKD associated with stone disease [18]. It has long been speculated that genetic modifiers may play a role in influencing host susceptibility to recurrent UTIs. For instance, the female relatives of women with recurrent UTIs are more prone to UTIs than the general population [19].

The risk of developing chronic renal insufficiency due to a UTI without other risk factors is low. The pathogenicity and virulence of the infective microorganisms as well as the efficiency of local or systemic defense mechanisms determine the course and severity of the disease [20]. Virulence properties (adhesins, toxins, capsule, iron uptake) are encoded by genomic structures and the determination of virulence is influenced by the host situation. In renal insufficiency, a variety of quite different substances (uremic toxins, betaine, amino acids, creatinine, urea, glucose) influence the microbial environment [20]. Defense factors (Tamm-Horsfall protein, defensin, phagocytic activity of granulocytes) and underlying anatomical lesions, as well as pre-existing renal disease, determine the severity of UTI and the prognosis of renal insufficiency [1,20]. UTI can be dangerous if treated late or incompletely because of late diagnosis. Equally so, resultant chronic kidney failure cannot be cured, but with effective and timely treatment, the progression of kidney disease can be stopped and end-stage renal failure can be avoided effectively [11].

2. MATERIALS AND METHOD

This retrospective observational study contain data collected from the hospital electronic database involving patients hospitalized in the Nephrology Department from Clinical Emergency Hospital of Craiova, Romania, between July 1, 2016 and June 30, 2017. In this period, 712 patients (359 females and 353 males) were hospitalized in this department (Table 1) and a number of 512 urine samples were collected (275 from females and 237 from males). A descriptive study was conducted including 105 strains isolated from urine cultures obtained from patients.

Table 1. Distribution of patients hospitalized in the Nephrology Department between July 1, 2016 and June 30, 2017

	Total admissions	Patients without UTI	Patients with UTI
Total [no. (%)]	712 (100%)	607 (85.25%)	105 (14.75%)
Female [no. (%)]	359 (50.42%)	297 (41.71%)	62 (8.71%)
Male [no. (%)]	353 (49.58%)	310 (43.54%)	43 (6.03%)

For all positive samples were collected data about demographic characteristics of the group (age, gender, and origin), making or not hemodialysis, kidney insufficiency degree, co-morbidities. Exclusion criteria of patients from the study: kidney transplant, patients with kidney-specific infections such as kidney tuberculosis, renal tumors, pregnancy, allergy to the tested antibiotics. The Ethics Committee of Clinical County Emergency Hospital of Craiova, Romania approved the access to the database and all the patients in the moment of admission in hospital signed a written consent by which they agree with the using of all medical data in different studies [21]. Not only were all data securely protected (by delinking identifying information from the main data sets) and made available only to investigators, but they were also analyzed anonymously.

2.1. MICROBIOLOGICAL METHODS

The urine collection was done by midstream jet method (or clean catch urine sample). Those urine samples were exanimated from a microbiological point of view within 2 hours from the collection moment. This is necessary because urine is a culture medium suitable for germ growth, being sufficient evens the presence of some germs from the urethra, which cannot be removed by any method we apply. All the bacterial species involved in urinary tract infections were isolated from urine samples by inoculating them on Columbia blood agar plate, *MacConkey* and *Sabouraud* media (all from *bioMérieux* SA) with incubation at 37˚C for 24 h. The identification of isolated microorganisms was performed using cultural, morphological and biochemical characters. The antibiotic susceptibility testing was done by the disk diffusion method according to the guidelines of the current Clinical Laboratory Standards Institute (CLSI). The antibiotics tested were from different classes and the interpretation of the results was as susceptible, intermediate resistant or resistant, with an important clinical value.

2.2. STATISTICAL METHODS

Continuous data were expressed as mean ± standard deviation. Categorical data were expressed as the number of events and percentages. The patients with UTI with and without CKD were compared for their demographic and clinical characteristics using Chi-square test for categorical variables and t-test for continuous variables (normally distributed) or Mann-Whitney (not normally distributed) [22]**.** Each variable was assessed for the proportion of missing data, and the subjects with complete and incomplete data were compared for other variables, in order to determine whether data imputation was necessary. To illustrate the differences, we used non-parametric Wilcoxon and Whitney-U tests with a significance threshold of 0.05, and Binomial, set for 50% proportions. To highlight the correlation between factors, the χ^2 test (significance threshold of 0.05). Survival was analyzed using the Kaplan-Meier test. Time series analysis was used to evaluate trends in the reinfection rates and mortality rates. We performed a univariate analysis of the variables of interest by constructing Kaplan-Meier survival curves.

3. RESULTS AND DISCUSSION

3.1. DEMOGRAPHIC CHARACTERISTICS

From a total of 712 patients hospitalized in the Nephrology Department over a year (July 1, 2016 and June 30, 2017), 85.25% were patients without UTI and 14.75% patients with UTI (Table 1). Of the 105 UTI patients, 58.04% were women and 40.96% men (Table 2). The female/male ratio in the group of patients without UTI was slightly in favor of males, but the difference is insignificant (p = 0.62). In the case of patients with UTI the ratio was slightly higher in favor of females, but also insignificant (8.71% *vs* 6.04%, p = 0.07, Table 1). The test Mann-Whitney U and Wilcoxon, calculating score Z = -1.91 (p = 0.056) shows that the ratio female/male with and without UTI does not differ significantly. Within the 105 microbial strains involved in UTI on patients admitted in the Nephrology Department, there were 101 (95.34%) bacterial strains and 4 (3.81%) yeasts strains (*Candida albicans*) with significant difference (p < 0.001) between the two groups. From 105 patients with UTI, 97 (96.19%) have been diagnosed with CKD (Table 2). In the CKD group 93 patients (95.77%) had UTI with bacteria and 4 patients (4.23%) fungal urinary infections. The most common bacterial species isolated from the 105 patients were *uropathogenic Escherichia coli* (*UPEC*) (48 strains – 45.71%) and *Klebsiella spp.* (30 strains – 28.57%). Also, were isolated strains of *Proteus spp*. and *Enterobacter spp*. (each with 7 strains – 6.67%), and *Enterococcus spp*. (4 strains – 3.81%). In a very small number were isolated *P. aeruginosa* (2 strains – 1.91%) and other *Gram-negative non-fermenting bacilli*, *Staphylococcus albus, Citrobacter spp.* with one strain each (0.95%, Table 2). UTI with UPEC was more significant in the case of females (33.33% *vs* 12.38%, p=0.002), but *Klebsiella spp* was more isolated from males (16.19% *vs* 12.38%, p=0.585, statistically insignificant, Table 2).

Table 2. Bacterial species involved in UTI in patients admitted in the Nephrology Department between July 1, 2016 and June 30, 2017

Bacterial species	Number of patients [no. (%)]	Female [no. (%)]	Male [no. (%)]
Escherichia coli	48 (45.71%)	35 (33.33%)	13 (12.38%)
Klebsiella spp.	30 (28.57%)	13 (12.38%)	17 (16.19%)
Proteus spp.	7 (6.67%)	1 (0.95%)	6 (5.72%)
Enterobacter spp.	7 (6.67%)	4 (3.81%)	3 (2.86%)
Enterococcus spp.	4 (3.81%)	3 (2.86%)	1 (0.95%)
Pseudomonas aeruginosa	2 (1.91%)	2 (1.91%)	0 (0%)
Other *Gram negative non-fermenting bacilli*	1 (0.95%)	1 (0.95%)	0 (0%)
Staphylococcus albus	1 (0.95%)	1 (0.95%)	0 (0%)
Citrobacter spp.	1 (0.95%)	1 (0.95%)	0 (0%)
Candida albicans	4 (3.81%)	1 (0.95%)	3 (2.86%)
Total	105 (100%)	62 (58.04%)	43(40.96%)

Proteus spp. prevailed significantly in males ($p < 0.001$) and *Enterobacter spp.* was insignificant in females ($p > 0.5$). The other bacterial strains, although reduced in number, were in a higher proportion in females, some of them being absent in males (*P. aeruginosa, GNNF bacillus, Staphylococcus albus* and *Citrobacter spp*., Tables 2 and 3). *Candida albicans* instead was isolated especially from males (2.86% *vs* 0.95% $p = 0.002$) (Tables 2 and 3). The average age of all patients with UTI was 67.19±11.09 years, in females 67.65±11.56 years and in males 66.53±10.47 years. In the case of UTI without CKD the average age was for females 48.5±18.44 years and for males 69.75±6.7 years. In patients with UTI without CKD, males are older: $Z = -2.3$, $p = 0.021$.The average age of females with UTI and CKD was 68.97±9.88 years and 66.21±10.79 years for males. In patients with UTI and CKD, there are no age differences between females and males ($Z = -1.41$, $p = 0.15$). The average age of females with and without CKD differs significantly (48.5 *vs* 68.97): $Z = -2.71$, $p = 0.007$, being higher in females with UTI and CKD.

The average age of patients with UTI for the group 36-45 years is 44±1.41; for group 46-55 years is 51.09±2.5; for group 56-65 years is 61.67±2.89; for group 66-75 years is 69.3±2.58, while for the group over 75 years is 80.25±3.8.

Related to the incidence of cases by age groups in CKD stages to patients with UTI it is found that stages 2 and 3 have a greater incidence in group 56-65 years (42.9% from all cases with CKD), and stages 4 and 5 have an incidence directly proportional with age (0% for ages below 25 years and grows to 34.2% for group 66-75 years, Table 4). Chi-square test - 18,62, p=0.017, indicates a significant correlation between the incidence of CDK stages in age groups. *Eta* suggests that the severity state of CDK is dependent and proportionate to the advancing in age ($\eta = 0.338$). The groups 66-75 years and over 75 years included the most cases of CKD stages 4 and 5 and UTI (65.8% of the two groups, Table 4).

Female gender predominates in the stages 3 and 5, but significant differences in the ratio female/male are just in stage 5 (63% *vs* 37%, $p = 0.038$, Tables 5 and 6; Fig. 1).

Table 3. Distribution of germs (bacteria and yeasts) by age, gender and CKD degree

AGE/ GERMS	Without CKD	CKD stage 1	CKD stage 2	CKD stage 3	CKD stage 4	CKD stage 5
<25 years						
E. coli	1F	0	0	0	0	0
26-35 years						
-	0	0	0	0	0	0
36-45 years						
E.coli	0	0	0	1F	0	0
Proteus spp.	0	0	0	0	1M	0
46-55 years						
E. coli	0	0	0	2(1F+1M)	0	4 (2F+2M)
Klebsiella spp.	1F	0	0	1M	0	2M
Enterococcus spp.	0	0	0	0	0	1F
56-65 years						
E.coli	2(F+M)	0	1	4(2F+2M)	0	5 (4F+1M)
Kebsiella spp.	1	0	0	2M	0	4(2F+2M)
Proteus spp.	0	0	0	1M	0	3(1F+2M)
Enterococcus spp.	0	0	0	0	0	1F
Enterobacter spp.	0	0	0	0	0	3(2F+1M)
66-75 years						
E. coli	1M	0	1F	1F	1F	12(10F+2M)
Klebsiella spp.	0	0	0	3F	1M	5 (2F+3M)
Proteus spp.	1M	0	0	0	0	0
Enterococcus spp.	0	0	0	0	1M	1M
Enterobacter spp.	0	0	0	0	1M	1M
Pseudomonas aeruginosa	0	0	0	0	0	2F
GNNF bacilli	0	0	0	0	0	1F
Staph. Albus	0	0	0	0	0	1F
>75 years						
E.coli	1M	0	0	2(1F+1M)	1F	8 (6F+2M)
Klebsiella spp.	0	0	0	0	2M	8 (4F+4M)
Proteus spp.	0	0	0	1M	0	0
Enterococcus spp.	0	0	0	0	0	1F
Enterobacter spp.	0	0	0	0	0	2F
Citrobacter spp.	0	0	0	0	1F	0
Candida albicans						
56-65 years	0	0	0	0	0	2(1F+1M)
66-75 years	0	0	0	0	0	1M
>75 years	0	0	0	0	0	1M

F-female; M-men

Table 4. Statistical description CKD of age groups for the all 105 patients with UTI

Age groups* CDK 2-3 and 4-5 Crosstabulation

			-CKD	CKD 2-3 and 4-5		Total
				CKD stg 2-3	CKD Stg 4-5	
Age Groups	<25y	Count	1	0	0	1
		% within in Age Groups	100.0%	.0%	.0%	100.0%
		% within CKD 2-3 and 4-5	12.5%	.0%	.0%	1.0%
	35 – 45 y	Count	0	1	1	2
		% within in Age Groups	.0%	50.%	50.%	100.0%
		% within CKD 2-3 and 4-5	.0%	4.8%	1.3%	1.9%
	46 – 55 y	Count	1	3	7	11
		% within in Age Groups	9.1%	27.3%	63.6%	100.0%
		% within CKD 2-3 and 4-5	12.5%	14.3%	9.2%	10.5%

Table 4. (*continued*)

		-CKD	CKD 2-3 and 4-5		Total
			CKD stg 2-3	CKD Stg 4-5	
56 – 65 y	Count	3	9	18	30
	% within in Age Groups	10.0%	30.0%	60.0%	100.0%
	% within CKD 2-3 and 4-5	37.5%	42.9%	23.7%	28.6%
66 – 75 y	Count	2	5	26	33
	% within in Age Groups	6.1%	15.2%	78.8%	100.0%
	% within CKD 2-3 and 4-5	25.0%	23.8%	34.2%	31.4%
>75y	Count	1	3	24	28
	% within in Age Groups	3.6%	10.7%	85.7%	100.0%
	% within CKD 2-3 and 4-5	12.5%	14.3%	31.6%	26.7%
Total	Count	8	21	76	105
	% within in Age Groups	7.6%	20.0%	72.4%	100.0%
	% within CKD 2-3 and 4-5	100.0%	100.0%	100.0%	100.0%

Table 5. Incidence of CKD stages related to sex in patients with UTI and CKD.

Chronic kidney disease			Category	N	Observed Prop.	Test Prop.	Asymp. Sig. (2-tailed)
STG 5	Gender	2	Female	43	0.63	0.50	0.038 [a]
		Group	Male	25	0.37		
		Total		68	1.00		

a - Based on Z approximation

Table 6. Incidence of CKD stage related to gender.

Chronic kidney disease			Frequency	Percent	Valid Precent	Cumulative Percent
No	Valid	Male	4	50.0	50.0	50.0
		Female	4	50.0	50.0	
		Total	8	100.0	100.0	100.0
STG 2	Valid	Female	2	100.0	100.0	100.0
STG 3	Valid	Male	9	47.4	47.4	47.4
		Female	10	52.6	52.6	
		Total	19	100.0	100.0	100.0
STG 4	Valid	Male	5	62.5	62.5	62.5
		Female	3	37.5	37.5	
		Total	8	100.0	100.0	100.0
STG 5	Valid	Male	25	36.8	36.8	36.8
		Female	43	63.2	63.2	
		Total	68	100.0	100.0	100.0

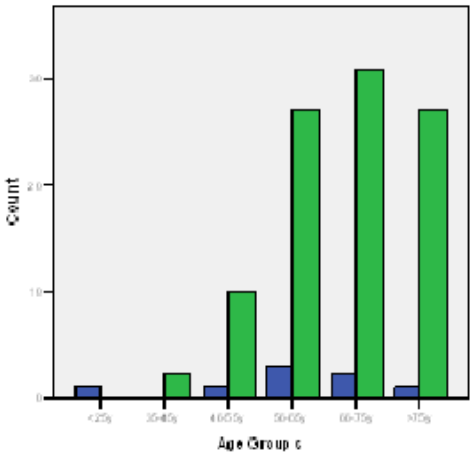

Figure 1. The incidence of CKD by age groups in patients with UTI.

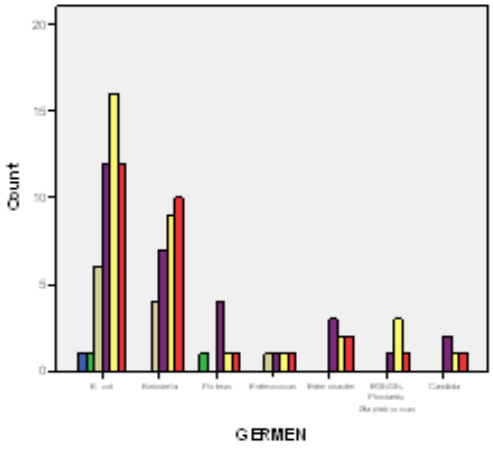

Figure 2. Incidence of bacterial species in patients urine by age groups.

There is no significant correlation between the type of microbe and the age groups (X^2 18.41, p = 0.95). However, the diversity of germs from urine increases to the final stages of CKD, which are present especially in older ages. Thus, a number of eight types of germs are present in stage 5 compared to 4 types in stage 3 (Tables 7 and 8; Figs. 2 and 3).

Table 7. Frequency of etiological agents in patients with UTI in stage 3 of CKD

Chronic kidney disease			Frequency	Percent	Valid Percent	Cumulative Percent
STG3	*Valid*	*E.coli*	10	52.6	52.6	52.6
		Klebsiella	6	31.6	31.6	84.2
		Proteus	2	10.5	10.5	94.7
		Enterococus	1	5.3	5.3	100.0
		Total	19	100.0	100.0	

3.2. CKD WITH UTI AND HEMODIALYSIS RELATIONSHIP

CKD stage 5 is strongly correlated with hemodialysis: Chi-test 46.3; p < 0,001. Hemodialysis depends on CKD stage (*eta* 0.664). It is noted the use of hemodialysis in stage 5 when the kidney purification function is much diminished (Fig. 4).

Table 8. Frequency of etiological agents in patients with UTI in stage 5 of CKD

Chronic kidney disease			Frequency	Percent	Valid Percent	Cumulative Percent
STG 5	Valid	*E. coli*	29	42.6	42.6	42.6
		Klebsiella	19	27.9	27.9	70.6
		Proteus	3	4.4	4.4	75.0
		Enterococcus	3	4.4	4.4	79.4
		Enterobacter	6	8.8	8.8	88.2
		BGNGN				
		Pseudomonas	4	5.9	5.9	94.1
		Staphilococcus	4	5.9	5.9	100
		Candida	68	100	100	
		Total				

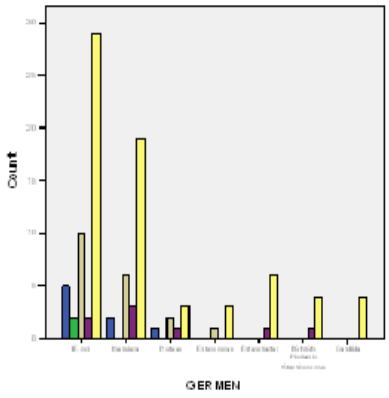

Figure 3. Incidence of bacterial species in patients urine by stage CKD groups

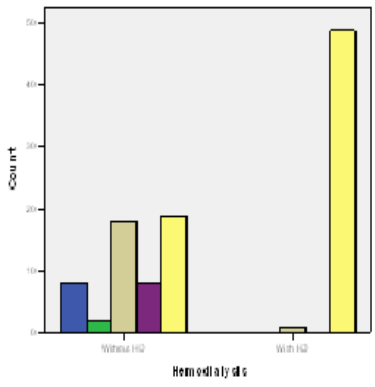

Figure 4. Incidence of hemodialysis in patients with CKD and UTI

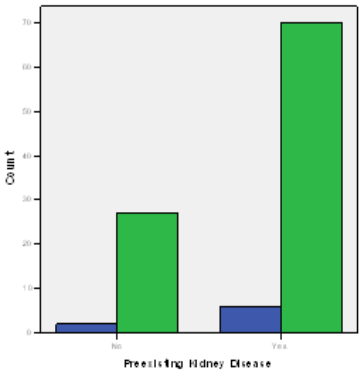

Figure 5. Correlations of pre-existing kidney diseases and CKD with UTI

3.3. CAUSAL KIDNEY DISEASES OF CKD AND UTI

Considering all renal disorders in a single batch and naming them causal kidney diseases from the CKD history (pyelonephritis, glomerulonephritis, kidney stones, kidney cysts, hydronephrosis and ureterohydronephrosis and more general nephropathy), the significance test shows some net differences between the CKD group and the preexisting kidney pathology and frequency of CKD patients without preexisting pathology: 72% *vs* 28%, $p < 0.0001$ (Fig. 5). If we refer to each condition in part because of the small number of patients, they do not correlate with CKD ($p > 0.05$), except the presence of kidney stones and urinary catheter, ureterohydronephrosis.

- Pyelonephritis is not correlated with CKD stages: X^2 0.77, $p = 0.94$.
- We have no corelation between glomerulonephritis - CKD: X^2 1.1; $p = 0.89$.
- Significant differences are in case of urinary catheters or kidney stones presence in patients with CKD *vs* patients without CKD (32 patients *vs* 5 patients), patients with urinary catheter or kidney stones, representing 86% and 14% from the total of 37 patients ($p < 0,001$). Were considered together kidney stones and urinary catheters, both as foreign bodies in the urinary tract, which can favor urinary tract infections. Urinary catheter is not correlated with CKD stages and UTI: X^2 2.31; $p = 0.67$
- Kidney stones are not correlated with CKD stages: X^2 5.15, $p = 0.27$
- No correlation between kidney cysts and CKD: X^2 0.65; $p = 0.95$
- Hydronephrosis has no relation with CKD stages: X^2 3.78, $p = 0.436$
- Ureterohydronephrosis can be correlated with CKD stages: X^2 11.29, $p = 0.023$. Ureterohydronephrosis seems to favor CKD and UTI: $\eta = 0,485$ (in 48.5% of cases).

3.4. COMORBIDITIES OF PATIENTS FROM NEPHROLOGY DEPARTMENT - RISK FACTORS FOR CKD AND UTI

Analyzing the relation diabetes mellitus – CKD in patients with UTI, in which CKD has all stages, any significant relation could not be highlighted. If we have analyzed by groups (without CKD and CKD stages 2-4 and 5), the relation with diabetes become significant: X^2 6.47, $p = 0.039$. *Eta* suggests that diabetes is correlated with CKD stage (diabetes incidence tends to increase as CKD stage is higher): $\eta = 0.248$. So the association with diabetes occurs especially in stage 5, which shows that the evolution to the final stages of CKD is increased if the patient has diabetes. Still the incidence of diabetes is insignificant in stages 2-4 ($p = 0.136$) and in stage 5 ($p = 0.812$). Wasn't found a significant correlation between the increase of HbA1c (glycated hemoglobin) over the normal values and CKD with UTI (Chi-square test 7.624, $p = 0.471$) and neither between the increased, normal and decreased values of glycaemia and CKD stages (Chi-square test 4.563 $p = 0.803$). Noteworthy that all 4 UTIs with *Candida albicans* are from patients with CKD stage 5 and diabetes. Most of these patients were male, between 62 to 76 years old, with pre-existing kidney diseases (nephropathy, kidney stones or kidney cyst). There was no correlation between HT (Hypertension) and CKD stages with UTI (Chi test 4.87, $p = 0.3$). Isolated in stage 5 of CKD, the incidence of HT is significant: without HT 10% *vs* 90% with HT, $p < 0.001$. CVC (central venous catheter) is correlated with UTI: X^2 14.29, $p = 0.006$. *Eta* indicates that the presence of CVC depends by the stage of disease ($\eta = 0,546$).

3.5. CORRELATIONS BETWEEN CKD WITH UTI AND BIOLOGICAL PARAMETERS

In Table 9 we have analyzed the biological parameters modified by CKD and UTI (kidney function markers, hematological parameters, ionogram). Overall, we do not have significant causal relationship anemia - CKD with UTI: Chi-test 7.64; $p = 0.1$. However, isolate in CKD stage 5, a significantly higher incidence of anemia was observed: 83% of patients in stage 5 also had anemia ($p = 0.001$) (Table 9). Serum potassium levels are correlated with CKD stages, but levels of serum sodium are not (Table 9).

3.6. ANALYSIS OF RECURRENCES AND REINFECTIONS IN UTI DURING JULY 2016-JUNE 2017

In the analyzed period from the 105 positive urine samples, 6 samples were obtained from patients with recurrent UTI or with urinary reinfection with other germs and CKD. They represent 6.18% of the 97 samples isolated from patients with CKD. The average age of patients was 55.16 years, most of them being female (4 females and 2 males) and coming from rural area (3 females and 1 male), respectively 1 female and 1 male from urban area. From the 6 patients with recurrent UTI, 5 were in CKD stage 5 (3 females and 2 males), thus with a reduced immune responses, and a female patient in stage 3. All patients had a history of kidney disease that altered renal function, with the exception of a patient who only had type 2 diabetes. From all 6 cases, 3 had diabetes as a comorbidity. Relapses were in 3 cases with *E. coli* and a case with *Klebsiella spp.* Reinfections were from *Enterococcus spp*. to *Klebsiella spp*. and from *Enterobacter spp.* to *P. aeruginosa.* All patients had an alteration of renal function with high levels of urea, creatinine and proteinuria present. From all 6 patients, 2 died at second admission in hospital. Except for one strain of *Klebsiella,* all other strains were multidrug-resistant from 3 to 8 antibiotics.

Table 9. Statistical significance of modified biological parameters in CKD with UTI

Lab parameters	**Statistic test**	**Statistical values**	**Clinical significance**	**Observations**
Kidney function markers				
Urea serum	Pearson chi-square	X^2 32.17, $p<0.001$	Urea serum level correlated with CKD stages.	42% from CKD stage 5 have urea increased.
Serum creatinine	Pearson chi-square	X^2 29.28, $p<0.001$	*Eta* suggests that the disease stage depends of creatinine level.	η =54.6%.
Uric acid	Pearson chi-square	X^2 1.161, p=0.88	Uric acid is not correlated with CKD stages.	
Proteinuria	Pearson chi-square	X^2 0.36, p=0.54	Frequency of proteinuria is higher in patients with CKD than in non-CKD patients: p <0.001.	
Proteinuria + diabetes mellitus	Pearson chi-square	X^2 6.373, p=0.173	No significant correlations in patients with CKD +/- diabetes.	In CKD stage 5 - Diabetes, incidence of proteinuria was 79% *vs* 21%.

Table 9. (*continued*)

Lab parameters	Statistic test	Statistical values	Clinical significance	Observations
Kidney function markers				
Proteinuria – diabetes mellitus	Pearson chi-square	X^2 3.282, p=0.512		In CKD stage 5 + diabetes, incidence of proteinuria was 83% *vs* 17%.
Hematological parameters				
Hemoglobin (Hb)	Pearson chi-square	X^2 7.45, p=0.024	Relation Hb-CKD is significant regroup patients with CKD in 3 sets: without CKD, CKD stages 2-3 and CKD stages 4-5.	
Hematocrit (Ht)	Pearson chi-square	X^2 11.52, p=0.021	96.6% of patients with CKD stage 5, had a decreased level of Ht.	
Number of red blood cells	Pearson chi-square	$X^2$17.43, p=0.026	Significant correlation between the number of RBC and CKD stages.	
Number of white blood cells	Pearson chi-square	X^2 3.71, p=0.88	Levels of WBC are not correlated with CKD stages and UTI.	
Platelet count	Pearson chi-square	X^2 1.010, p=0.908	Thrombocytopenia is not correlated with CKD stages.	
Ionogram				
Serum sodium	Pearson chi-square	X^2 4.53, p=0.325	In stage 5 frequency of hyponatremia is significantly higher than normal natrium level: 68% *vs* 32%, p=0.028	
Serum potassium	Pearson chi-square	X^2 18.27, p=0.019	Levels of serum potassium is correlated with CKD stages.	

3.7. ANALYSIS OF DEATHS BETWEEN JULY 2016 AND JUNE 2017

In the analyzed period there have been 5 deaths in majority female (4 females and 1 male) and from rural area (4 from rural area and 1 from urban area), with the average age 69.6±10.45 years and limits between 56 and 82 years. All of them had CKD stage 5 (5.15%).

Bacterial strains isolated were *Escherichia coli* (2 strains), *Klebsiella spp*. (2 strains) and one patient had two bacteria, the first one was *Enterobacter spp*. and the second one *Pseudomonas aeruginosa*. All strains were multi-drug resistant (from 3 to 8 antibiotics) with the exception of one strain of *Escherichia coli* without antibiotic resistance in a female with type 2 diabetes. With the same exception the other 4 patients had pre-existing kidney impairment (nephropathy, kidney stones). All patients had high levels of urea, creatinine, uric acid and proteinuria is present. From them one patient had a relapse of UTI with *Klebsiella spp*. and another with *Pseudomonas aeruginosa*.

The Kaplan-Meier Survival function brings details about the recurrence of one or more events in the time unit. We used this test for predicting the survival of patients in the batch during the study period. The average survival (no event) is 11.6 months (CI 95%: 11.24-12.036 months), from the time the case is highlighted (in our case, the entry is obviously equivalent to the beginning of the study).

The "Survival function" graph is plotted by the percentage of patients remaining in the study (continuous line) after each occurrence of the event (death) over the duration of the study (Fig. 6). The graphics with the hazard function is centred on the occurrence frequency

of the event over time. It is noticed that death occurs at a low frequency in the first months of the study, and then tends to no longer appear (linear plateau) until close to 11-12 months.

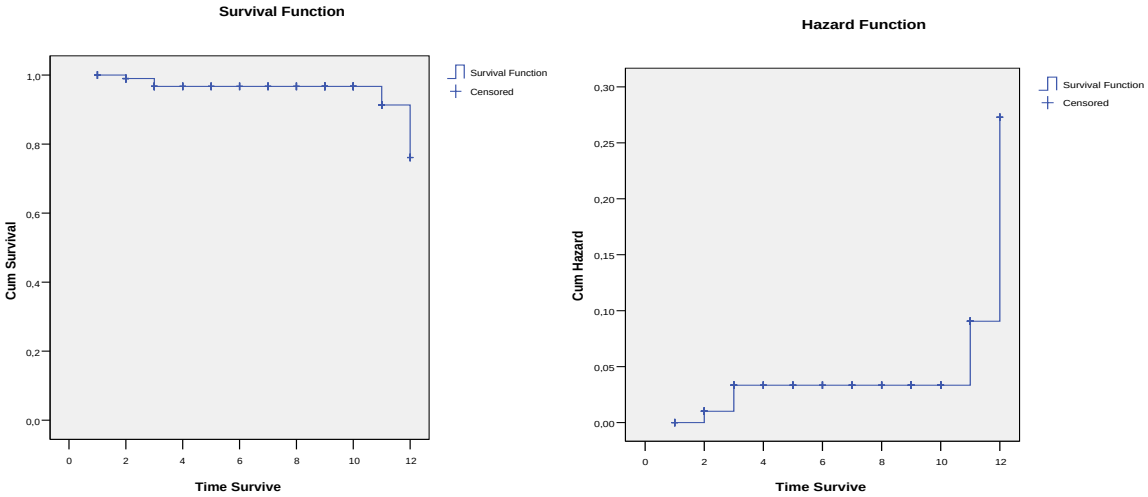

Figure 6. Kaplan-Meier survival function and hazard function

Urinary tract infections are the second most frequent infectious disease in the population [23]. Chronic renal failure is a risk factor for the development of urinary infections due to metabolic disorders resulting in secondary immunodeficiency with a disorder of all components of immunity [23].

In our study from the total of 105 urinary infections, 97 occurred due to the presence of CKD, especially in advance stages. The CKD severity stages associated with UTI are dependent and proportionate to the advancing age. A higher percentage of UTI and CKD was observed in the age group 66-75 years and even over 75 years. Decreasing of glomerular filtration by CKD can be associated also with a progressive decrease in relation to age in elderly. In the group studied UTI and CKD was represented by an insignificantly higher percentage in women. The percentage of 14.75% UTI with CKD in the Nephrology Department in the period studied is close to data from the literature.

It is estimated that almost 10% (< 20 million) adult population in the United States suffers from CKD. The propensity to acquire renal dysfunction increases with age [24]. The physiological decline in nephron number and GFR with advancing age is well known. This makes the elderly more prone to develop chronic kidney disease after various renal insults [25]. After the age of 30 years, glomerular filtration rate (GFR) progressively declines at an average rate of 8 mL/min/1.73 m² per decade [26]. The Australian Diabetes, Obesity and Lifestyle (AusDiab) study suggests that over one-third of people over the age of 65 years have an estimated glomerular filtration rate (eGFR) below 60 mL/min/1.73 m² [26].

Recent evidence suggests that even very elderly patients (> 80 years of age) with modest reductions in eGFR (45–59 mL/min/1.73 m²) have a higher prevalence of CKD-related complications compared to patients with an eGFR ≥ 60 mL/min/1.73 m² [26]. A Taiwanese study revealed that patients with CKD and UTIs were elderly and that females were prone to have more bacteriuria and upper UTIs than males. In addition, patients who had kidney stones were more prone to have upper UTI than other bacteriuria patients [27]. According to the 2016 USRDS (United States Renal Data System) report, CKD is more common than diabetes mellitus in the United States; an estimated 14.8% of adults have CKD, compared to 12.3% with DM [28]. Thirty-three percent of U.S. adults over the age of 40 live with CKD [29]. The risk patients is low - 3-4% as reported by different authors [30]. Despite the high prevalence in older adults and the association with infection, few studies have investigated outcomes following an infectious illness in older people with renal impairment

[31]. In our study the diversity of bacteria increase in final stages of CKD, being more present at advance ages. Thus 8 different types of species are present in stage 5 to 4 species in stage 3. We believe that the phenomenon is due to a marked decrease of immunity, frequencies of hospitalization, hemodialysis and comorbidities.

Genomic comparison of *Staphylococcus spp.* revealed specific urovirulence traits including various transport systems to facilitate adaptation to the varying pH and concentration of inorganic ions, urea and organic compounds [32]. Other Gram-negative uropathogens, like *Proteus* spp. and *Klebsiella* spp., are often associated with the occurrence of bladder stone formation due to urease expression [32]. Additionally, 20–40% of the complicated UTI and approximately 40% of the nosocomial infections in normal wards are UTI due to catheterization. Uropathogenic *Escherichia coli* (UPEC) differ from intestinal pathogenic and many nonpathogenic faecal *Escherichia coli* by the expression of virulence-associated factors [30]. Gram-positive bacteria (*Enterococcus* spp., *Staphylococcus spp.*), also as *Pseudomonas aeruginosa*, Gram-negative bacteria, also play important roles as uropathogens in nosocomial infections [32]. Urinary infections with fungi, especially *Candida* are increasing. In our study, 4 patients with CKD stage 5 had urinary infections with *Candida albicans*, especially males, with diabetes, pre-existing kidney disease (nephropathy, kidney cysts or stones), and with ages between 62 and 76 years. Notice that the occurrence of these infections is after the winter season, when the immunity status may be altered.

UTI caused by fungi is preferentially observed in immune-compromised patients and upon treatment with antibiotics that are excreted via the kidneys [32]. Type 2 diabetes is also a risk factor for fungal UTI, mostly caused by *Candida* [33]. Concerning pre-existing renal impairment (nephropathy without etiology, pyelonephritis, glomerulonephritis, hydro and ureterohydronephrosis, kidney stones, kidney cysts), a significant causal relationship to the CKD installation and evolution and association with UTI was found. The detailed analysis of each individual case did not lead to a significant result due to the reduced number of cases/parameter studied. Incidences of urinary tract infection differed depending on the primary renal disease (12, 13, 41 and 67 percent for chronic glomerulonephritis, diabetic nephropathy, polycystic kidney and chronic pyelonephritis, respectively) [25]. Identification of factors predisposing to chronic kidney disease is important from both individual and community point of view. The reason is to help in planning effective interventions to reduce the risk of this debilitating illness [25]. The nature of the primary renal disease is of paramount importance in predicting the rate of progression. Various risk factors which predispose to kidney damage contribute to the progression of kidney disease [25].

In our study, a causal relationship has also been encountered in the presence of the bladder catheter and kidney stones, both seen as foreign bodies. Most UTIs that develop in hospitalized patients are due to urinary catheters. The longer any urinary catheter is in place, the higher the risk for bacteria growth and infection [34]. All older adults who are immobilized, catheterized, or dehydrated are at increased risk for UTIs [34]. Nearly any kidney disorder, including kidney stones, increases the risk for complicated UTIs [34]. Kidney stones may directly contribute to the development and progression of CKD via urinary tract obstruction and/or infection, nephrocalcinosis and oxalate nephropathy [34]. Patients who had renal stones were more prone to have upper UTI than other bacteriuria patients [27]. The worldwide prevalence of kidney stones among adults is 5% to 9% and is apparently increasing [34]. Major contributors to the CKD burden are the growing frequencies of diabetes, hypertension, and obesity, which are well-established traditional risk factors for CKD [34]. Diabetes mellitus is the leading cause of both CKD and ESRD (end-stage renal disease or also called kidney failure) in developed as well as developing countries. Glycaemic control is of paramount importance in these patients. Pathogenesis of diabetic nephropathy has multiple mechanisms. Rate of decline of GFR is faster in these patients than non-diabetic

CKD [25]. Diabetes is a risk factor for all forms of kidney disease. Diabetes patients are more prone to develop all kinds of clinical renal damage and may suffer more severely from rapid progression [35]. Reduced glomerular filtration rate (GFR) in the absence of albuminuria is a common manifestation of chronic kidney disease (CKD) in diabetes. In people with diabetes, the risk for ESKD (end-stage kidney disease), CKD progression, or rapid decline in eGFR is very low among patients with CKD who do not have baseline moderately increased albuminuria or proteinuria compared with those with albuminuria or proteinuria [36]. Among the infections, urinary tract infections (UTIs) occur more frequently in diabetic patients because of urine glucose excretion and chronic neurologic bladder dysfunction [37].

In the presented research, we had a close correlation between CKD stage 5 and hypertension (HT). Because HTN may cause or result from CKD, HTN prevalence is higher and control more difficult with worse kidney function [38]. Hypertension and type 2 diabetes (DM2) are important causes of chronic kidney disease (CKD) [37]. In DM2, the main characteristics of CKD, that is, reduction of glomerular filtration rate (GFR) and albuminuria, are important predictors of cardiovascular complications both in general population and in patients with DM2 [39]. A high percentage of hypertensive patients with and without diabetes move each year toward more severe stages of CKD [39]. Based on a national survey of a representative sample of noninstitutionalized adults in the USA, it is estimated that hypertension occurs in 23.3% of individuals without CKD, and 35.8% of stage 1, 48.1% of stage 2, 59.9% of stage 3, and 84.1% of stage 4-5 CKD patients [17]. Prevalence of hypertension also varies with the cause of CKD; strong association with hypertension was reported in patients with renal artery stenosis (93%), diabetic nephropathy (87%), and polycystic kidney disease (74%) [17]. Patients with CKD should maintain a BP (blood pressure) that is consistently below 140/90 mmHg, although, if a patient has diabetes or microalbuminuria, a consistent BP below 130/80 mmHg is recommended [26].

CKD is a major risk multiplier in patients with diabetes, hypertension, heart disease and stroke, all of which are key causes of death and disability among older people [16]. The prevalence of chronic kidney disease is higher in the elderly, in whom it contributes to the effects of ageing. In our study, hemodialysis was strongly correlated with CKD stages and UTI. The current understanding on many aspects of UTI in patients with renal insufficiency and/or on dialysis remains incomplete. The populations are not homogeneous and many factors influence the deterioration of kidney function. Recent reports indicate that dialysis may not provide a clear benefit over conservative care regarding survival and quality of life, especially in those patients with extensive comorbidities. In a number of small comparative studies, dialysis for elderly patients with extensive comorbidities did not confer a significant survival advantage over conservative management [40]. Due to prolonged hemodialysis that may compromise their immune system, they are vulnerable to infection, including UTI [25]. A recent larger study found that elderly patients with extensive comorbidities treated by dialysis had a median survival that was only 5 months longer from entry into CKD stage 5 than patients who had undergone conservative kidney management [40]. This suggests that an individualized approach is necessary.

Anemia is one of the side effects of CKD. Overall, in our study we do not have a significant causal relationship between anemia and CKD with UTI: Chi-test 7.64; $p = 0.1$. However, isolate in CKD stage 5, a significantly higher incidence of anemia was observed: 83% of patients in stage 5 also had anemia ($p = 0.001$). The prevalence of anemia increases as GFR declines due to reduced erythropoietin production by the kidney and increasing resistance to its action due to hyperparathyroidism [26].

The true incidence and prevalence of hyperkalemia is not known, but it has been estimated to be 2-3% in the general population [41, 42] and 1% to 10% among hospitalized patients [43, 44]. People with CKD heart failure, DM, and those taking blood pressure

medicines called renin-angiotensin-aldosterone system inhibitors (RAASi) have an estimated 2 to 3 times higher risk for hyperkalemia [43, 45]. More than half of predialysis CKD patients develop hyperkalemia. In our study, the frequency of hyperkalemia was correlated with CKD stages. Proteinuria is not correlated with CKD (X^2 0.36, p = 0.54), but the proteinuria frequency is higher in patients with CKD than in patients without CKD, of the total of 43 patients with proteinuria, 38 patients had CKD (88% of cases with proteinuria) and only 5 patients with proteinuria were without CKD (12% of cases with proteinuria), $p <$ 0.001.

In the analyzed period 6 patients each has two hospital admissions with recurrent UTI or with urinary reinfections with other bacteria and CKD. These patients were mostly female, with an average age of 55.16 years (with a minimum and maximum of 49 years and 77 years), predominantly from rural areas. Of the 6 patients, 5 were in CKD stage 5 and one in stage 3. All 5 patients with CKD stage 5 were on hemodialysis at the second hospitalization or both.

Relapses were with *Escherichia coli* (3 cases), *Klebsiella spp.* (1 case), and reinfections were with different bacteria, one case was with *Klebsiella spp*. and others with *Enterococcus spp*., *Enterobacter spp.* and *Pseudomonas aeruginosa*. The majority of bacteria were multi-drug resistant, and at second admission in the hospital have increased the number of antibiotics to which bacteria are resistant. Although in the literature it is shown that reinfection is more common than relapse [46], in our study there were more frequent relapses, especially with *E. coli*.

In a study in a primary care setting 53% of women above the age of 55 years and 36% of younger women report a recurrence within 1 year [47]. Recurrent UTI can either be a relapse or reinfection. A relapse UTI is caused by the same bacterial strain implicated in a previous UTI within 2 weeks of the completion of treatment for the original infection [47]. By contrast, a recurrent UTI arising more than 2 weeks after treatment or after sterile culture is considered to be a reinfection, even if the infecting pathogen is the same as the original [47]. For postmenopausal women, the risk factors are markedly different and include oestrogen deficiency, cystocele, urogenital surgery, high post-void residual volume and a previous UTI [47]. These women also have a relative depletion of vaginal lactobacilli and an increase in vaginal *E. coli* compared with premenopausal women. This age-related alteration of the normal vaginal flora, especially loss of hydrogen peroxide-producing lactobacilli, may predispose women to introital colonization with *Escherichia coli* and also to UTI [47]. Estrogen loss at menopause results in thinning of the vaginal epithelium and decreased amounts of glycogen [46]. The resulting environment is hostile to lactobacilli, and the numbers decrease. The vaginal *pH* increases, and there is an increased propensity for colonization with uropathogens [46]. Women who are non-secretors of histocompatibility blood-group antigens are at increased risk of recurrent UTI [46]. Uremia, present in all patients with relapses in our study, is associated with a state of immune dysfunction characterized by immunodepression that likely contributes to the high prevalence of infections among these patients as well as by immune responses resulting in inflammation that may contribute to CVD [48].

Of the 6 patients with relapses of urinary tract infections, 2 died at second hospitalization. Deaths occurred in females in the postmenopausal period with age over 70 years, possibly due to more complex pathophysiology of recurrent UTI in this population [47]. Uremia is associated with a state of immune dysfunction characterized by immunodepression that likely contributes to the high prevalence of infections among these patients [48]. Other significant factors for recurrent UTI in postmenopausal woman are diabetes mellitus and a previous history of UTI, and in our study of the 6 patients with relapses, 4 (66.66%) had diabetes. All five deaths in the period analyzed were in patients diagnosed with UTI and CKD stage 5. Same to relapses, deaths occurred mainly in females

from rural areas and with an average age of 69.6 ± 10.45 years. Death occurred in patients having a number of risk factors, pre-existing CKD, co-morbidities such as diabetes, hypertension, hemodialysis, hyperkalemia, modification of kidney function parameters (urea, creatinine, uric acid, and proteinuria), recurrences of urinary infection.

In the literature is shown that the infection in advanced chronic kidney disease leads to increased risk of cardiovascular events, end-stage kidney disease and mortality [49]. End-stage renal disease (ESRD) is associated with significantly increased morbidity and mortality resulting from cardiovascular disease (CVD) and infections, accounting for 50% and 20%, respectively, of the total mortality in ESRD patients [48]. We found in our study that UTI germs in deceased patients are those types that cause multidrug-resistant nosocomial infections and infectious relapses. Other authors considered previously also that multidrug-resistant uropathogens are common in patients of chronic kidney disease [50]. All causal factors, including UTI, have altered cardiovascular function, the immediate cause of death being cardiopulmonary arrest due to cardiovascular disorder.

4. CONCLUSIONS

The incidence of patients with UTI in the Nephrology Department during the study period is 14.75%. The ratio of female/male in patients with UTI is insignificantly higher for females. Female gender predominates in CKD stages 2 - 3, but a statistically significant difference is in stage 5. The mean age of women in relation to the mean age of men in patients with UTI and CKD was no different (p = 0.15). In women, UTI appears at an older age with CKD compared with the age of women without CKD.

In addition to the decrease of glomerular filtration value with age, for women occur hormonal changes related to menopause and comorbidities such as hypertension, diabetes, which can determine the decrease of immunity responses and function of urogenital epithelial as defense factors. Statistical analyses reveal that the severity of CDK in relation to UTI is dependent and proportionate to ageing.

Bacterial species have predominated in the urine of patients with UTI and UTI - CKD compared to *Candida albicans,* which was present only in patients with CKD in stage 5. In the urine of patients with CKD, *Escherichia coli, Klebsiella spp*. and *Proteus spp*. were isolated predominatly.

The statistical correlation of hemodialysis with CKD stages with a predominance in stage 5 emphasizes that hemodialysis is especially common in stage 5 when the kidney no longer responds to its purification function. Especially in this stage, biological factors are modified which show that kidney function is altered (high levels of urea, creatinine, uric acid and the presence of proteinuria).

Comorbidities, such as diabetes and hypertension, increase the risk of CKD aggravation to the final stage.

Diversity of germs in urine increases to the final stages of CKD, possibly through a more frequent contact of the patient with hospital environment by multiply hospitalization or conducting hemodialysis under precarious conditions.

Investigating the risk of recurrent (6.18%) or death (5.15%) during the study period, we noticed a risk shift towards to the final stages of CKD associated with UTI with the involvement of a large number o multiresistant microbial strains. The use of statistical relationships allowed to make some correlations between CKD evolution with or without UTI. However, for a small group of patients, over a period of one year, with few subgroups of demographics, causality, comorbidity and biological factors, risk factors for reinfection or

death, the proper complex assessement of the data obtained from a retrospective observational study may be difficult. We consider necessary to find predictive correlations by involving these factors in CKD evolution associated with UTI.

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