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Article

Diabetes as a risk factor of acute kidney injury in vancomycin users: an observational and prospective study

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Diabetes was investigated as a risk factor for nephrotoxicity induced by vancomycin. In the present study, the drug's nephrotoxic effect was indirectly evaluated by Glomerular Filtration Rate, albuminuria and serum levels of creatinine and urea on the 1st, 7th and 14th days of vancomicyn therapy in a group of diabetic and non-diabetic patients, with and without previous nephropathy. The correlations between investigated variables (including the population's epidemiological profile and hospital care) were measured by the Spearman test. The sample consisted of 132 patients, predominantly male diabetic patients with previous nephropathy, over 40 years, receiving ≥ 10 grams of vancomycin for the treatment of infectious diseases and showing satisfactory clinical outcomes. A risk of vancomycin drug interaction with potential nephrotoxic outcome was observed in 36.4% of patients who used multiple drugs. Furthermore, 80% of patients had an increase of at least 0.5 $mg.dL^{-1}$ in baseline serum levels of creatinine and urea at the end of the study. This was more common among the diabetic patients with previous nephropathy, showing higher albuminuria and a reduction in the Glomerular Filtration Rate. Therefore, it has been recommended that the use of vancomycin in diabetic patients should be in careful dosages and that kidney functioning be monitored.

Keywords: Vancomycin. Nephrotoxicity. Diabetes Mellitus. Kidney damage. Outpatient clinics. Drug synergism.

INTRODUCTION

In recent years, chronic illnesses have caused an increase in the number of deaths around the world. In particular, diabetes mellitus (DM) caused 1.5 million deaths in 2012, 66.6% more than in 2000, taking the eighth place for causes of death (2.6%) around the world (WHO, 2016).

Approximately 90-95% of patients with DM produce insulin but its secretion and functioning is hampered (insulin resistance for Type 2 DM). These patients have a reduction in life expectancy and quality of life, with the onset of cardiovascular disease, strokes, irreversible blindness and the occurrence of premature childbirth. Furthermore, DM is among the main causes of chronic kidney disease worldwide (American Diabetes Association, 2014). In a prospective study conducted in the United Kingdom (2006) (Retnakaran *et al*., 2006), approximately 40% of Type 2 DM patients developed some type of nephropathy and 28% came to have kidney failure in the absence of intensive glycemic

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control by oral hypoglycemic drugs associated with renal protection by anti-hypertensive agents.

Another common complication of DM is the infection of the lower limbs ("diabetic foot") which will eventually occur in over a quarter of all diabetic patients (Singh, Armstrong and Lipsky, 2005). This illness presents high morbimortality mainly caused in infections by aerobic gram-positive microorganisms such as Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus ssp. and Enterococcus ssp (Bowler, Duerden and Armstrong, 2001). According to Niu *et al*. (2008) S. aureus was identified in more than 80% of the isolated cultures of these patients. Murugans, Mani and UmaDevi, (2008) identified strains of methicillin-resistant S. aureus in more than 30% of ulcerations at the lower limbs in diabetic patients, an increasing trend in many countries. In moderate and severe infections, facultative anaerobic gram-negative micro-organisms (such as Proteus mirabilli, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa) and anaerobics (such as Peptostreptococcus, Bacteroides, Prevotella spp. and Clostridium ssp.) have also been isolated in more than 95% of these ulcerations (Bowler, Duerden, Armstrong, 2001). "Diabetic Foot" has been associated with 50-70% of non-traumatic cases of amputation of lower limbs (Singh, Armstrong and Lipsky, 2005).

Vancomycin is recommended worldwide as an antibiotic for the treatment of these opportunistic hospital-spread infections related to the skin, soft tissues, pneumonia, bone, joint and central nervous system - infections produced by Staphylococcus coagulase-negative, Enterococcus sp and, mainly, by methicillin-resistant S. aureus (Rivera and Boucher, 2011). Even though vancomycin is the main choice for severe gram-positive infections, new strains with reduced susceptibility to vancomycin have arisen. This has led renowned clinical guidelines[1] to indicate higher doses of vancomycin than those previously approved (1 gram every 12 hours), aiming to obtain a minimum blood concentration of 15 to 20 μg mL^{-1}. Based on this, the use of 15 to 20 mg kg^{-1} (real body weight) of injectable vancomycin is recommended every 8 to 12 hours, not exceeding 2 grams per dosage, in patients with normal kidney functioning (Rybak *et al*., 2009; Liu *et al*., 2011). However, recent studies have associated longer courses of treatment and higher trough serum vancomycin levels (30-65 $mg.L^{-1}$) to the increase of acute kidney injury (AKI) in these patients (Rybak *et al*., 2009; Beringer *et al*., 2011; Van Hal, Paterson and Lodise, 2013). In 2012, Elyasi *et al*. observed an incidence of dose-dependent nephrotoxicity induced by vancomycin at around 10 to 40%.

In this context, the gradual reduction in kidney functioning, commonly observed in diabetic patients, may contribute to kidney damage induced by vancomycin, as a risk factor for premature and heightened nephrotoxic effects. Our study aims to investigate the renal toxicity of vancomycin in Type 2 DM patients with infectious diseases, contributing to a more effective and safer antimicrobial therapy for these patients.

METHODOLOGY

Case-by-case

A prospective observational quantitative study was conducted on patients treated with vancomycin at the Hospital das Clínicas at the Federal University of Pernambuco (HC/UFPE) in four in-patient sectors (nephrology, internal medicine, infectology, and the intensive care unit) between March and July of 2015.

The study had the permission of Teaching and Research Management at the Hospital das Clínicas, Federal University of Pernambuco (HC/UFPE), and approval by the Committee of Ethical Research involving Human Beings at the Federal University of Pernambuco (CAAE nº 39569414.6.0000.5208).

Criteria for inclusion

Vancomycin is a restricted antibiotic at the HC/UFPE. Therefore, its administration depended on medical prescription and further clinical evaluation by the hospital infection control commission. In order to be consistent with the period of observed treatment within the medical routine, the sample population consisted of all hospitalized patients over eighteen years old who were under treatment by vancomycin for a period up to 14 days. The sample subjects were divided into four groups: (1) diabetic patients with previous nephropathy, (2) non-diabetic patients with previous nephropathy, (3) diabetic patients without previous nephropathy and (4) non-diabetic patients without previous nephropathy (Control Group), according to the diagnostic criteria for DM and nephropathy adopted by the HC/UFPE. At the HC/UFPE,

1 Examples of protocols have been produced by the National Kidney Foundation [NKF], the American Society of Health System Pharmacists, the Infectious Diseases Society of America and the Society of Infectious Diseases Pharmacists [SIDP].

diabetic and non-diabetic patients with reduced renal function at the time of hospital admission are commonly referred to as nephropathy patients in order to facilitate their clinical evaluation and hospital treatment.

In the present study, DM was diagnosed based on plasma glucose criteria in accordance with recommendations stated by the American Diabetes Association (ADA) (2014). Besides this, the diagnostic criteria for reduced renal function used to categorize the different groups were based on recommendations stated by the ADA (Gross *et al*., 2005) and Kidney Disease: Improving Global Outcomes (KDIGO, 2013). In this case, nephropathy was defined, regardless of its underlying cause, taking into account both early clinical-laboratory evidence (such as decreased Glomerular Filtration Rate (GFR) or increased urinary excretion of albumin) and a series of other specific or non-specific signs and symptoms of reduced renal function. Several risk factors for reduced renal function and its adverse outcomes were also considered by HC-UFPE physicians, such as age, family history of kidney disease, reduction in kidney mass, diabetes, increased frequency of metabolic syndrome components, cardiovascular disease, autoimmune system disease, systemic infections, urinary tract infections, urinary stones and lower urinary tract obstruction.

Criteria for exclusion

Patients who were on dialysis or had prophylactic treatment with vancomycin until the third day of hospitalization were excluded since some studies (Lodise *et al*., 2009; Moh'd *et al*., 2014) showed that the nephrotoxic effects of vancomycin associated with the occurrence of Acute Kidney Injury (AKI) or Chronic Kidney Disease (CKD) and its complications (advanced illnesses and kidney failure) began after 4 to 9 consecutive days of antibiotic administration.

Study design

The incidence of the nephrotoxic effects in patients from different groups was measured through the quantification of biomarkers of kidney function at 3 different moments (before treatment [equivalent to "1st day"] and on the 7th and 14th days after the beginning of the treatment) in accordance with the criteria of NFK and SIDP which foresee the measurement of (1) urinary excretion of albumin, (2) estimated GFR, (3) serum creatinine and urea levels - and their relation to reference values for patients with unstable kidney function, and for those who received antibiotic therapy with vancomycin (between three and five days). The laboratory findings which express kidney functioning were taken from the medical charts of the study population.

The determination of serum creatinine levels was conducted according to the colorimetric method of Beckman, Coulter, JaffeTM (Fillée *et al*., 2011).

The enzymatic method of Beckman, Coulter, Talke & SchubertTM (Talke and Schubert, 1965) was used for the determination of serum urea levels. The reference values for normal adult kidney functioning were 0.5-1.3 $mg.dL^{-1}$ for creatinine and 20-52 $mg.dL^{-1}$ for urea.

AKI diagnosis after vancomicyn therapy was based on criteria of KDIGO and Acute Kidney Injury Network (AKIN). KDIGO defines AKI as the occurrence of (1) an abrupt increase in baseline serum creatinine levels ($\geq$ 0.3 $mg.dL^{-1}$ within 48 hours), (2) an increase of 1.5 times in baseline serum creatinine levels within 7 days and/or (3) oliguria < 0.5 mL kg^{-1} h^{-1} within 6 hours. On the other hand, AKIN correlates AKI with an increase of 0.3 $mg.dL^{-1}$ in baseline serum creatinine levels if the baseline level is $\geq$ 0.6 $mg.dL^{-1}$ or 50% if the baseline level is $\leq$ 0.6 $mg.dL^{-1}$ (Minejima *et al*., 2011; Waikar and Bonventre, 2009).

The GFR was assessed according to the recommendations of the International Society of Nephrology following the Chronic Kidney Disease Epidemiology Collaboration (CDK-EPI) creatinine equation (2009) considering the serum creatinine levels as an endogenous tracer of GFR. In these cases, GFR lower than 60 $mL.min^{-1}$ 1.73 m^{2-1} was considered kidney failure for all ages (Levey *et al*., 2009). Urinary excretion of albumin was determined according to the spectrophotometric benzalkonium chloride particle counting method at 620 nm adapted by Yilmaz, Çelebi and Yücel, (2004). Urinary excretion of albumin $\leq$ 30 mg $24h^{-1}$ was registered as normal, moderately increased between 30-300 mg $24h^{-1}$ [which indicates a risk 20 times greater for the development and progress of kidney diseases] or highly increased (or being able to be taken as evidence of nephropathy) when $\geq$ 300 mg $24h^{-1}$. The urinary excretion of albumin $\geq$ 2200 mg $24h^{-1}$ characterized a proteinuria framework associated with Nephrotic Syndrome. The urinary excretion of protein with a total $\geq$ 3000 mg $24h^{-1}$ was considered an additional risk factor associated to a specific condition, glomerulonephritis (KDIGO, 2013).

Besides these continuous quantitative variables, dependent variables were also evaluated in the study (clinical outcome and potential risk of drug interaction). Such data was lined up alongside independent variables (vancomycin dosage), intervening (reason for indication of vancomycin), discrete quantitative variable (age) and qualitative (sex, outpatient hospital sector). Data were collected from medical records of the sample population upon consideration of the vancomycin request for these patients in the hospital pharmacy sector.

Statistical analysis

The analysis of data was conducted employing SPSS for Windows® (version 18, SPSS Science, Chicago, IL). The representative sample size calculation was based on observational studies for a finite population. To determine the population's epidemiological profile, their pharmacotherapy and clinical outcome, benchmarks for percentages and respective distributions of frequency were constructed.

The Friedman's test was used to compare serum creatinine and urea levels, GFR values and vancomycin dosage at the 3 assessed moments. Furthermore, to compare the albuminuria values and the dosage of vancomycin between the study's patient groups, the Kruskal-Wallis test was used; and in the pairwise comparison of these groups, the Mann-Whitney test was applied. P value of less than 0.05 was considered significant.

RESULTS

The sample population consisted of 132 patients, predominantly from Group 1 (37.9%), male (55.3%), and aged above 40 years old (73.5%) (Table I).

TABLE I - Distribution of the population (n=132) according to social-demographic profile and groups, HC/UFPE, March to July 2015

	n	%	p-values[1]
Gender			
Males	73	55.3	0.223
Females	59	44.7	
Age group, y			
18-30	10	7.6	
31-40	25	18.9	
41-50	32	24.2	0.005
51-60	35	26.6	
> 60	30	22.7	
Groups			
1. Diabetics with nephropathy	50	37.9	
2. Non-diabetics with nephropathy	29	22.0	0.006
3. Diabetics without nephropathy	23	17.4	
4. Non-diabetics without nephropathy	30	22.7	

[1] p-values from Pearson's chi-squared test, if p-values < 0.05 there is a significant difference in frequency between categorical data sets.

Regarding the clinical treatment of the sample population, 47 patients (35.6%) were hospitalized after attendance at the internal medicine sector, 36 (27.3%) at the nephrology sector and the other patients were distributed through the other sectors. In these cases, the use of vancomycin was preferentially indicated for the treatment of unspecific bacterial infections (61.4%) or other infectious diseases (38.6%), associated or not to DM.

In our study, 57 patients (43.1%) received more than 10 grams of vancomycin in a conventional dosage of 1 gram every 12 hours for 7 days, and 37 (28%) for 14 days. Furthermore, 29 patients (21.9%) received 1 gram every 24 hours for 10 days. The other 9 patients (7%) received vancomycin in dosage schedules with small variations on the above therapeutic schemes reaching up to 10 grams of the antibiotic drug (Table II).

TABLE II - Distribution of the population (n=132) according to outpatient hospital sector, infectious disease related to vancomycin indication and pharmacotherapy, HC/UFPE, March to July 2015

Factor evaluated	n	%	p-values[1]
Outpatient hospital sector			
Infectious Diseases	30	22.7	
Nephrology	36	27.3	0.006
Intensive care unit	19	14.4	
Internal Medicine	47	35.6	
Reason for indication of vancomycin			
Non-specific bacterial infections	81	61.4	
Pneumonia	27	1.5	
Septicemia	16	3.0	<0.001
Ankle and foot injuries	4	12.1	
Osteomyelitis	2	20.5	
Infective endocarditis	2	1.5	
Total dose of vancomycin			
Under 10 g	38	28.8	<0.001
Over 10 g	94	71.2	
DI with nephrotoxic effect			
No potential risk	84	63.6	0.002
Potential risk	48	36.4	

(continuing)

TABLE II - Distribution of the population (n=132) according to outpatient hospital sector, infectious disease related to vancomycin indication and pharmacotherapy, HC/UFPE, March to July 2015

Factor evaluated	n	%	p-values[1]
Drugs with potential risk of DI and nephrotoxic effect			
Piperacillin	14	29.2	
Furosemide	17	35.4	
Polymyxin	5	10.4	
Amikacin	4	8.3	
Ciprofloxacin	3	6.2	<0.001
Tobramycin	2	4.2	
Amphotericin	1	2.1	
Gentamicin	1	2.1	
Moxifloxacin	1	2.1	

[1] p-values from Pearson`s chi-squared test, if p-values < 0.05 there is a significant difference in frequency between categorical data sets; DI, Drug interaction with vancomycin.

For the patients in Groups 1 and 3, there was observed an average administration of 28 grams of vancomycin for 14 days, interspersed with other antibiotics. This therapeutic scheme considered the weight of each patient and the existence of comorbidities with other infectious processes, according to clinical and therapeutic guidelines of the SFDI. In these cases, the use of vancomycin after 10 days was evaluated, every 12 hours, taking into account the clinical outcome of the infected patient after continuous administration of vancomycin.

In the Control Group, a satisfactory clinical outcome with the remission of infections was obtained when lower doses of vancomycin were administered (7 grams for 7 days). In Group 2 the most used dosage schedule was the average dose of 10 grams for 10 days. However, a subtle, or non-satisfactory, clinical outcome was observed and the infections persisted in some cases.

In our study, besides vancomycin, another 21 types of drug were concurrently administered to patients: oral hypoglycemic agents [53.3%], diuretics [45%], angiotensin-converting enzyme inhibitors [18.1%], angiotensin II receptor antagonists [3%], calcium channel antagonists [12%], carbapenems [25%], penicillins [42.5%], macrolides [0.75%], lincosamides [1.75%], fluoroquinolones [6.7%], aminoglycosides [12%], lipopeptides [0.75%], gastric shields [50%], anti-inflammatories [25%], anti-depressants [30%], electrolytes [15%], benzodiazepines [30%], anti-retrovirals [10%], immunomodulators [5%], laxatives [50%], parasiticides [5%].

In most cases (63.6%), the prescribed drugs did not show a potential risk for drug interaction with vancomycin. In 48 patients (36.4%) there was observed a potential risk of synergism with nephrotoxic effects being associated (or not) with other clinical effects such as neuropathies and ear diseases. In the pharmacotherapy of these patients, only 9 drugs showed a potential risk of synergic nephrotoxic effects with vancomycin (12% severe, 70.8% moderate and 17.2% mild). Those drugs with greater potential risk of nephrotoxicity and greater frequency of use belonged to the class of beta-lactams antibiotics (41.5%) and loop diuretics (29.3%).

Figures 1 and 2 show the serum creatinine and urea levels of the sample population at 3 different moments (1st, 7th and 14th days, respectively) of hospital treatment.

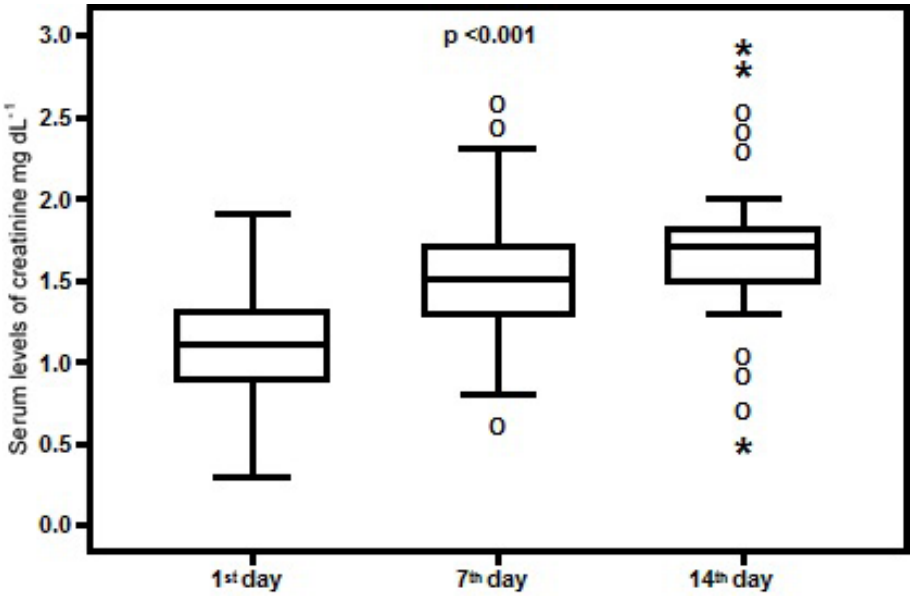

FIGURE 1 - Box-plot of serum creatinine levels on the 1st, 7th and 14th days of vancomycin therapy for the study population.

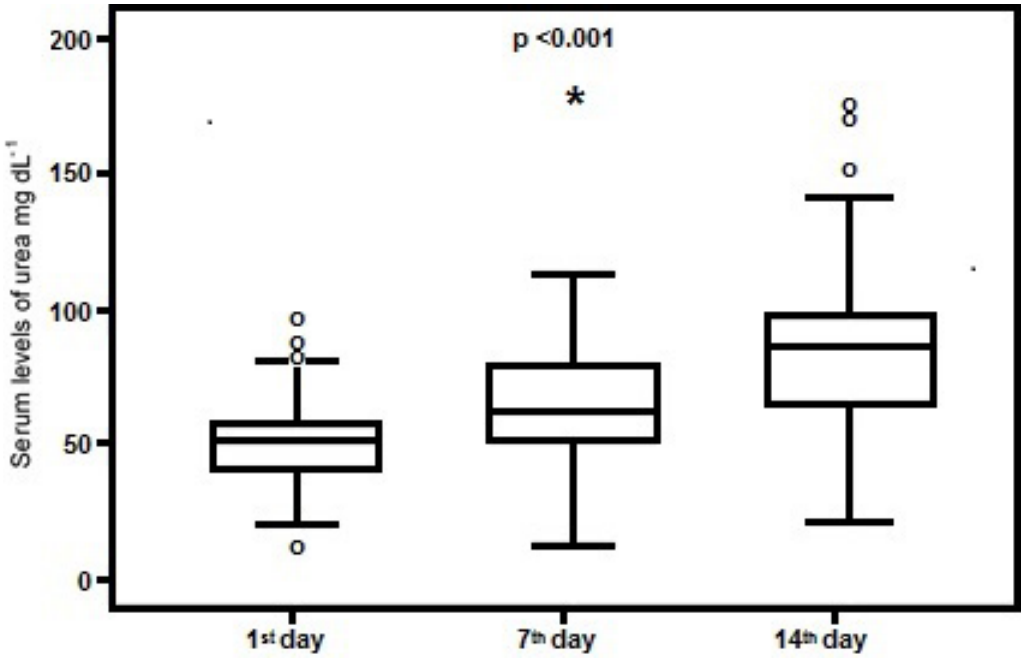

FIGURE 2 - Box-plot of serum urea levels on the 1st, 7th and 14th days of vancomycin therapy for the study population.

The relationship between these values shows that the average levels of both biomarkers increased significantly along the treatment for all groups. At the beginning of the treatment (1st day) the sample showed an average of 1.12 ± 0.43 mg.dL^{-1} in baseline serum creatinine levels and 50.9 ± 17.3 mg.dL^{-1} of urea. At the 7th day there was observed an average increase of 0.3 ± 0.13 and 15.2 ± 10.9 mg.dL^{-1} in baseline serum creatinine and urea levels, respectively. In those patients who used vancomycin for 14 days, there was an average increase of 38% over the baseline serum creatinine levels (1.57 ± 0.57 mg.dL^{-1}) and 33.9% for urea (85.1 ± 33.3 mg.dL^{-1}) in relation to the beginning of the

treatment, with $p < 0.001$ in both cases. Compared to the 1st day of treatment, an increase in baseline serum creatinine and urea levels was observed in most cases (120 patients or 91%). 106 (80%) patients developed kidney damage at the end of the treatment.

In Table III the averages and standard deviations of the serum creatinine and urea levels and GFR values of patients in the different groups are described, according to the assessment moment. In these cases, an increase of at least 0.5 $mg.dL^{-1}$ in baseline serum creatinine levels (above the reference value [0.5-1.3 $mg.dL^{-1}$]) was observed in 37.9% of patients in Group 1. On the other hand, in 12 patients of the Control Group the creatinine values remained at baseline levels. In these cases, the laboratory analyses were repeated and the data obtained remained the same.

TABLE III - Mean and standard deviation of kidney function biomarkers values on the 1st, 7th and 14th days of vancomycin therapy, group by group, HC/UFPE, March to July 2015

Measured value	Kidney function biomarkers values			p-values[1]
	1st day (n=132)	7th day (n=132)	14th day (n=37)	
1. Diabetics with nephropathy				
Creatinine ($mg.dL^{-1}$)*	1.13±0.46	1.42±0.50	1.50±0.45	<0.001
Urea ($mg.dL^{-1}$)*	48.57±19.51	61.81±24.46	72.07±20.77	<0.001
GFR ($mL.min^{-1}$ 1.73 $m^{2\,-1}$) *	72.94±27.81	43.90±17.10	35.11±16.88	<0.001
2. Non-diabetics with nephropathy				
Creatinine ($mg.dL^{-1}$)*	1.07±0.46	1.40±0.71	1.93±0.78	0.097
Urea ($mg.dL^{-1}$)*	50.51±14.36	67.73±37.60	119.33±46.61	0.097
GFR ($mL.min^{-1}$ 1.73 $m^{2\,-1}$)*	64.50±24.37	56.12±27.14	44.04±20.92	<0.001
3. Diabetics without nephropathy				
Creatinine ($mg.dL^{-1}$)*	1.14±0.38	1.42±0.48	1.71±0.41	0.002
Urea ($mg.dL^{-1}$)*	54.94±16.05	67.86±22.70	93.57±28.07	0.002
GFR ($mL.min^{-1}$ 1.73 $m^{2\,-1}$) *	61.44±17.43	47.52±14.85	46.00±21.32	0.097
4. Non-diabetics without nephropathy				
Creatinine ($mg.dL^{-1}$)*	1.13±0.40	1.45±0.57	1.48±0.73	0.121
Urea ($mg.dL^{-1}$)*	52.07±16.97	70.20±27.78	88.00±40.64	<0.001
GFR ($mL.min^{-1}$ 1.73 $m^{2\,-1}$) *	75.97±26.90	46.60±16.77	47.60±22.84	0.011

[1]p-values from Friedman test, if p-values < 0.05 there is a significant difference in kidney function biomarkers values between categorical data sets.

In Groups 1 and 3, a significant increase in baseline serum creatinine and urea levels was observed ($p < 0.001$ and $p \leq 0.002$, respectively) along the 1st, 7th and 14th days of treatment. The GFR showed a significant reduction among the different assessment moments in Group 1 ($p < 0.001$) and non-significant for those in Group 3 ($p = 0.097$). In Group 2 a significant reduction in the GFR ($p < 0.001$) was also observed, but with a non-significant increase in baseline serum creatinine and urea levels among the different assessment moments ($p = 0.097$ for both biomarkers). On the other hand, in the Control Group a significant increase in baseline serum urea levels and a significant reduction in the GFR were observed ($p < 0.001$ and $p = 0.011$ for each of the measures, respectively) with a low increase in the creatinine values between different days of treatment ($p = 0.121$).

In Table IV the average and standard deviations for the albuminuria values are described, as well as for the doses of vancomycin alongside the assessed groups. Although non-statistically significant differences in doses of vancomycin were observed among the assessed groups, it was observed that the comparison, two by two, of albuminuria data distribution was significant for all groups ($p < 0.001$), with greater values for diabetic patients in Groups 1 and 3, followed by Groups 2 and Control Group.

Regarding the age group and the total dose of vancomycin administered to the study population, vancomycin dosage increased proportionally according to age group. An administration of higher doses was observed among individuals aged 51 years or older (≥ 18.14±7.37 grams per patient) compared to younger subjects (ranging from 14.07±8.07 to 16.14±7.26 grams).

DISCUSSION

DM is a significant risk factor for nephropathies with unfavorable prognosis in advanced stages. Diabetics commonly develop chronic wound infections. Problems in healing associated with repetitive antibiotic treatment could lead to resistant bacterial infections in these patients. In this context, vancomycin has been the first-choice antibiotic in clinical health care of gram-positive bacterial infections recommended by WHO guidelines, in distinct clinical protocols that use different vancomycin dosing schedules to an efficient pharmacological management of infected patients. However, some of these pharmacological protocols could lead to a very high drug concentration. Unfortunately, longer pharmacological treatment courses and higher trough vancomycin doses have a toxic effect on kidneys, particularly in kidneys already damaged with impaired kidney function, such as those of adult diabetics. Based on this, in order to evaluate the effect of DM on kidney dysfunction associated with vancomycin therapy, hospitalized adult patients with several infection indicators were assessed by us along the course of treatment with vancomycin.

In our study, the number of patients increased significantly (11.4%) between the 1st (18-30 years old) and 2nd age group (31-40 years old), showing not so

TABLE IV - Mean and standard deviation of albuminuria and dose of vancomycin, group by group, HC/UFPE, March to July 2015

Group	Albuminuria (mg $24h^{-1}$)	Vancomycin (g)
1. Diabetics with nephropathy	1904±457	17.56±7.50
2. Non-diabetics with nephropathy	707±268	14.35±8.25
3. Diabetics without nephropathy	1609±474	17.85±8.17
4. Non-diabetics without nephropathy	258±209	18.03±8.53
p-values[1]	<0.001	0.108

[1]p-values from Kruska-Wallis test, if p-values < 0.05 there is a significant difference in albuminuria and dose of vancomycin between categorical data sets.

significant differences between groups for patients above 40 years old. This is probably related to the profile of patients assisted at the HC/UFPE, a public health unit without an emergency sector, which mostly receives patients with chronic diseases, such as diabetics - a predominant group to be observed by our study and whose morbidity frequently involves individuals above the age of 40 years. According to WHO, diabetics are often adult individuals with a history of insulin resistance associated with obesity and sedentarism (WHO, 2016). Based on this, the diabetics observed in our study were assumed to be only composed by patients with Type 2 DM, considering that one of the inclusion criteria adopted by our study was that patients should be 18 years or older.

Some authors suggest that differences in lifestyle related to sex category may lead to differences in the risks of developing Type 2 DM and, in consequence, to differences in the prevalence of this condition in women and men (Malhão *et al*., 2016). However, different studies have shown a higher, or non-significant, Type 2 DM prevalence for both genders (Hilawe *et al*., 2013). In our study, non-significant gender differences were observed for all groups.

Besides the nephropathy commonly observed among diabetics, the progressive kidney anatomical and functional changes in the adult population reported by Esposito and Dal Canton (2010) may have contributed to the development and progression of kidney failure widely observed in our study.

HC/UFPE is renowned as a public health entity committed to nephropathy assistance. Therefore, our sample consisted predominantly of patients with nephropathy and a clinical profile involving infections, assisted at the hospital's internal medical sector, probably due to the direct or indirect effects of the chronic complications of kidney failure, such as azotemia and hyperkalemia (Needham, 2005).

Furthermore, the hyperglycemia symptoms among diabetics contribute to the development of immunological system dysfunctions (such as alteration in the neutrophils, humoral immunity and depression of the antioxidant system) which may have contributed to the bacterial infection cases observed by our study (Casqueiro, Casqueiro and Alves, 2012). Besides these circumstances, micro and macro angiopathies, neuropathies, decreases in urinary antibiotic activity, gastrointestinal and urinary motility reduction - besides prolonged hospital stay, a large number of medical interventions and the indiscriminate use of antibiotics - could also have made patients prone to nosocomial infection (Rivera and Boucher, 2011).

In our study, 97 patients (73.5%) received empiric therapy with vancomycin after clinical assessment suggested an infectious disease. This was reinforced by laboratorial outcomes that showed leukocytosis and increased levels of reactive protein C, according to the protocol of the hospital control commission of the HC/UFPE. For the other patients (n=35), bacterial culture and antibiogram were used to decide upon the administration of vancomycin. However, this microbiological diagnosis was carried out only after the failure of the previous empirical treatment with other antibiotics.

It is worth noting that vancomycin is the first choice antibiotic in the clinical treatment of serious infections caused by methicillin-resistant S. aureus, including skin and soft tissues infections, pneumonia and other infections of the blood stream (Liu *et al*., 2011). Considering that the use of vancomycin is one of the criteria for inclusion in our study, different infectious processes motivated the indication for its use in all groups, such as non-specific bacterial infections and osteomyelitis due to severe aggravation of wounds in the lower limbs. Some factors that also contributed to the use of vancomycin inside hospital sectors were: acute pneumonia acquired within the environment of a community; septicemia with loss of consciousness; as well as infections of the blood stream associated with severe infectious endocarditis in those patients who were submitted to invasive medical procedures such as the peripherally inserted venous catheter. In some cases, the confinement of infected patients to the hospital infectious diseases sector was necessary to minimize the spread of infectious processes.

Wounds in the ankle and feet ("diabetic foot") - the 4th motive for the indication of vancomycin in our study - are common, severe and difficult to treat morbidities related to DM (Niu *et al*., 2008; Rivera and Boucher, 2011). Therefore, in the treatment of osteomyelitis and diabetic foot a gradual increase in the prevalence and dose of vancomycin was observed in our study. It could be related to the greater predisposition towards nephrotoxicity observed among diabetics.

In our study, a satisfactory clinical outcome and the remission of infection was observed for most of our sample, mainly for those patients diagnosed with unspecific bacterial infection and treated with a conventional dose of vancomycin of 1 gram every 12

hours for 7 days, in accordance with recommendations stated by the FDA guidelines. Only in a small group of patients (26%) was there observed an unsatisfactory response to antibiotic treatment. The persistence of the infectious disease may be due to an over infection of gram-negative or anaerobic bacterial strains, or bacterial strains' resistance to antibiotic agents due to a mutation in the DNA of the bacteria, or by the acquisition of a new genomic material in these patients.

In these cases, even with higher doses of vancomycin, and/or shorter intervals of drug administration, that were also used in our study, as recommended by several clinical protocols and therapeutic guidelines (Liu *et al*., 2011; Rybak *et al*., 2009), these other vancomycin dosing schedules were not capable of eradicating the infectious process completely, which reinforced the possibility of bacterial resistance (Tacconelli and Cataldo, 2008). On the other hand, Hamada, Kuti and Nicola, (2015) posted that tissue penetration of vancomycin can be affected by the degree of inflammation and infected tissue. Furthermore, these authors found that epithelial drug penetration is significantly low in diabetics (average of 0.1 $mg.L^{-1}$) compared with non-diabetic patients (average of 0.3 $mg.L^{-1}$). Therefore, in these cases - mainly for diabetics - the level of effectiveness for microbicidal concentration of vancomycin in situ could be difficult to achieve even when blood-plasma concentrations levels of vancomycin followed theoretically therapeutic effective doses. These data corroborate with our study, in which the patients who did not show satisfactory clinical outcomes and/or remission of infection were diabetics with acute inflammation.

As reported by Moore *et al*. (2011) and as observed in our study, although the use of vancomycin in the healthcare of infected patients is a standard therapy for the hospital context, reported cases of failure to combat recurring infection are becoming increasingly common. Casqueiro, Casqueiro and Alves, 2012 suggest that this therapeutic ineffectiveness has led to increase the use of vancomycin in combination with other antibiotics, seeking the eradication of bacterial infections. Unfortunately, the concomitant administration of some of these drugs is considered a high risk factor for nephrotoxicity induced by vancomycin (Rybak *et al*., 2009). In this context, in spite of the greater potential risk of drug interaction with vancomycin and nephrotoxic effects, the piperacillin β-lactam antibiotic was one of the most prescribed drugs in our study (29.3%), mainly for the therapy of diabetic patients with nephropathy at the beginning of treatment (Group 1). Similarly, furosemide (35.4%), a loop diuretic with greater potential risk of drug interaction with vancomycin and nephrotoxic effects, is widely used for the remission of edemas caused by kidney failure among patients with nephropathy.

Although the interaction of vancomycin with aminoglycosides (gentamicin, amikacin and tobramycin) and fluoroquinolones (ciprofloxacin, moxifloxacin) was less frequently observed in our study, it has a high potential risk of nephrotoxicity, ototoxicity and neurotoxicity (Paquette, *et al*., 2015; Lomaestro, 2000). In 2009, Rybak *et al*. (2009) reported a rate of nephrotoxicity induced by vancomycin varying from 7 to 35% when this drug was used concomitantly with aminoglycosides. Therefore, these side effects from drug interaction with vancomycin could be hampering clinical results and increasing the morbidity observed in some patients within our sample. According to Droege, Van Fleet and Mueller (2016), the incidence of kidney damage has been increasing along with the maintenance of a combined therapy using different nephrotoxic antibiotics, such as the aminoglycosides, amphotericin B and piperacillin/tazobactam. For this reason, the concomitant use of vancomycin with antibiotics from these different classes should be avoided in order to prevent debilitating of renal function, a risk factor for serious infections in older patients with CKD - an important group in our study. Since they are contraindicated, drug interactions with toxic effects are usually avoided in hospital environments. Nevertheless, in our study potential risks of drug interaction with vancomycin and nephrotoxic effects were observed for 36.4% of prescribed drugs.

In vancomycin dosing schemes for patients of different age groups it was observed that older patients received higher doses of vancomycin, with small differences for patients over 51 years old. In this case, it is worth noting that most of our patients above 50 years old were diagnosed as DM, a condition that contributes to bacterial infection development and the higher doses of vancomycin in this group (51-60 years). On the other hand, patients above 60 years old had a greater disposition towards the development and progression of kidney diseases compared to the other groups of our study. In these cases, the medical staff did not increase the dose of vancomycin administered to these patients as a preventative measure in order to delay or reduce the risk of kidney damage. Although, in this group, a

statistically significant reduced kidney function was also observed 14 days after the beginning of treatment.

A significant increase in baseline serum creatinine and urea levels was observed in our study for all groups. These laboratorial outcomes corroborate with the studies of Davies *et al*. (2013) and Moh'd *et al*. (2014) where the similarity in the growth pattern of baseline serum levels for biomarkers was observed in more than 30% of patients in intensive care with vancomycin therapy for 14 days.

However, unlike these authors, our study also showed a reduction in GFR suggesting deteriorated kidney function in our patients.

In our study, high serum creatinine levels were observed in diabetic patients with previous nephropathy (Group 1) seven days after the beginning of the treatment with vancomycin. The increase of 0.3 $mg.dL^{-1}$ in baseline serum creatinine levels observed in the 2sd assessed moment (7th day) for all groups, including the Control Group, could also be a signal of AKI related to the administration of vancomycin. Cano *et al*. (2012) observed signs of kidney damage in patients of intensive care units 7 days after the beginning of the treatment with vancomycin, even with intermittent intravenous administration of conventional doses of 2 grams per day. Similarly, Elyasi *et al*. (2012) observed an incidence of vancomycin renal toxicity at around 10-20% in patients using conventional doses. Although vancomycin-induced nephrotoxicity is uncommon and usually reversible, several conditions present in our study have been proposed as predisposing factors. High-risk group (including those with obesity or older), those receiving a longer duration of therapy (even more than 7 days) and/or therapy with concomitant nephrotoxic agents and those who are critically ill (including those with severe infections such as endocarditis, severe sepsis and osteomyelitis) or who already have a compromised renal function, are particularly at risk for vancomycin-induced nephrotoxicity (Elyasi *et al*., 2012). Furthermore, some researchers have suggested that patients with preexisting kidney dysfunction would be less likely to eliminate vancomycin and thus could achieve high trough levels of vancomycin with subsequent development of renal dysfunction even with lower-dose regimes (Moffett, Kim and Edwards, 2010).

Regarding the duration of antimicrobial therapy in vancomycin-induced nephrotoxicity, individuals that used vancomycin for 14 days in Groups 1 and 2 showed an increase in baseline serum levels of both biomarkers, suggesting a progression in the kidney damage of these patients. Even those diabetic patients without previous nephropathy (Group 3) showed an increase in baseline serum creatinine and urea levels above their reference values and GFR decreased. However, this reduction was less than those with nephropathy at the beginning of our study (Groups 1 and 2).

On the other hand, for some patients in the Control Group, the creatinine values remained at baseline levels. According to Denic, Glassock and Rule, (2016), in some cases the GFR in the injured kidney region can be compensated by another healthy and hypertrophied kidney region.

Laboratorial outcomes suggestive of kidney damage were observed in more than 80% of patients at the end of our study, being more prevalent in Group 1. This is in accordance with Wong-Beringer *et al*., (2011) where an increase $\geq$ 0.3 $mg.dL^{-1}$ in baseline serum creatinine levels (with baseline level 0.6 $\geq$ $mg.dL^{-1}$) or 50% (with baseline level 0.6 $\leq$ $mg.dL^{-1}$) was observed in 11.6 to 42.6% of hospitalized patients after prolonged use of vancomycin. Furthermore, creatinine is usually a more accurate marker of kidney function than urea, which may have contributed to the higher correlation between the dose of vancomycin and creatinine values (compared to urea) observed in our study.

In vancomycin therapy, a significant increase in baseline serum biomarkers levels are usually observed 2 to 5 days after discontinuing antibiotics. In the cases of mild nephrotoxicity, a return to baseline serum biomarkers levels is expected 10 to 14 days after antibiotics are stopped and 14 to 21 days in the most severe cases (Minejima *et al*., 2011). However, as observed in our study, chronic non-transmissible illnesses and their complications (such as DM and diabetic nephropathy) can aggravate the bacterial infection to impair therapy with antibiotics, since they limit the renal metabolic reestablishment of these patients. Furthermore, diabetics are a high-risk group for cardiovascular disease associated to the significant reduction of GFR and consequent destruction of renal glomeruli by vascular micro-angiopathy (Ninomiya *et al*., 2009).

Ninomiya *et al*. (2009) suggested an association between inefficient glomerular selectivity - promoted by tubular lysosomal dysfunction and progressing to kidney damage - and urinary excretion of albumin in diabetic patients. Based on this, the potential risk of kidney damage associated with the administration

of vancomycin (dose-dependent effect) was also investigated in our study for the determination of albuminuria in different groups receiving different vancomycin dosage schemes. In agreement with Ninomiya *et al*., (2009), the highest albuminuria values were also observed among diabetic patients with and without previous nephropathy (Groups 1 and 3) receiving high doses of vancomycin - at the end of our study. According to the Kidney Disease Outcomes Quality Initiative (KDOQI) of the National Kidney Foundation (NKF) (Levey, Coresh, National Kidney Foundation, 2002) and KDIGO (KDIGO, 2013), high albuminuria and the GFR reduction observed in all groups of our study - mainly in Group 1 - are also important signals of AKI with risk of death, mainly when kidney damage is being masked by the asymptomatic increase of baseline serum creatinine and urea levels. Furthermore, GFR values between 30-44 $mL.min^{-1}$ $1.73m^{2-1}$ suggested moderate-serious CKD. Therefore, in our study, diabetic patients with previous nephropathy (Group 1) were considered a high-risk group, according to KDOQI and KDIGO.

On the other hand, some prospective studies described a high-risk of early Diabetic Nephropathy and death in patients showing normoalbuminuria and reduced GFR (< 60 $mL.min^{-1}$ $1.73m^{2-1}$) (Kramer *et al*., 2007). Based on this, the American Diabetes Association suggests the simultaneous determination of both biochemical parameters for evaluation of early Diabetic Nephropathy (Kramer *et al*., 2007).

The limitations of our study involved the coexistence of other diseases in the sample population and concurrent use of nephrotoxic drugs. Serum creatinine and urea levels were taken as belated biomarkers of kidney damage, since they were available in hospital routine. The authors also point out the possibility of employing other, earlier biomarkers with greater sensibility and specificity, such as cystatin C and low molecular weight proteinuria in the characterization of kidney damage. Furthermore, in our study, the precise effects of DM and underlying diseases on the nephrotoxicity induced by vancomycin, or those related to the use of each nephrotoxic agent with vancomycin, could not be assessed. This was not possible due to the interference of some factors such as the stage of diabetes, body mass index, trough serum vancomycin levels and severity of the disease, which, although directly influencing the severity of kidney damage, were absent in the medical records of the study population.

CONCLUSION

Our study strongly suggests that the antimicrobial therapy with vancomycin could lead to AKI in diabetics (with nephropathy and without nephropathy). Based on this, vancomycin must be carefully dosed and monitored, with a close look at the kidney functioning of those hospitalized patients with severe nephropathy risk factors such as DM.

Although a prospective double blind, randomized study is required to investigate the possible relationship between vancomycin administration and kidney damage in diabetics, our data suggest that renoprotective strategies must be adopted in the antibiotic therapy of these patients, such as shorter treatment courses and lower trough serum vancomycin levels or its changing to another drug with similar broad-spectrum antibiotics.

CONFLICTS OF INTEREST

The authors of the manuscript report no conflicts of interest. This work was financially supported by the Ministry of Education and Culture of Brazil.

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