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Genotoxicity evaluation in chronic renal patients undergoing hemodialysis and peritoneal dialysis, using the micronucleus test

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ABSTRACT. Patients with chronic renal disease have an increased incidence of cancer. It is well known that long periods of hemodialysis treatment are linked to DNA damage due to oxidative stress. This genotoxic effect may cause the loss of chromosome fragments, or even entire chromosomes, which form micronuclei after cell division, and can be detected by the micronucleus test. In the present case-control study, we evaluated the genotoxic effect of hemodialysis treatment in 20 patients undergoing hemodialysis, and 20 subjected to peritoneal dialysis, matched for gender and age with 40 controls. Genetic damage was assessed by examining the frequency of micronuclei in 2000 exfoliated buccal cells per individual. Our results revealed that patients undergoing hemodialysis treatment have a significantly higher frequency of micronucleated cells (MNC; 5.60 ± 5.31) compared to control subjects (1.50 ± 2.01, $P < 0.01$). Interestingly, the same was not observed for the peritoneal dialysis patients who showed no significant differences in MNC (2.85 ± 2.96) frequency compared to control individuals (3.25

± 3.85). In addition, we evaluated the possible association between creatine levels, smoking, alcohol intake, age, duration of treatment, and incomes of the individuals (separately analyzed according to their gender) and the frequency of micronuclei. The results reported here indicate that the duration of treatment is the only factor associated with increased MNC frequency among hemodialysis patients (Spearman coefficient of 0.414, P = 0.01). The number of MNC found in individuals with six years or less of treatment was significantly lower (2.91 ± 2.74) compared to patients with seven or more years of treatment (8.89 ± 5.96, P < 0.05). Overall, peritoneal dialysis may be a safer choice of treatment, but further studies need to be performed to investigate the risks and benefits of both treatments.

Key words: Micronucleus; Mutagenicity; Peritoneal dialysis patients; Hemodialysis patients; Chronic renal disease

INTRODUCTION

According to the Brazilian Nephrology Society census in 2002, of the total patients undergoing renal substitution therapy in Brazil, 54,523 (89.6%) remained on hemodialysis (HD). Only 7.61% of the total were not kept directly by the Unified Health System. From 1999 to 2002, there was an increase of 27.7% in the number of clients. Our rates of prevalence of terminal chronic renal insufficiency (CRI) treated are about four times lower than in the USA and Japan and half the rates in Italy, France and Germany (Ajzen and Schor, 2002).

Barros et al. (1999) described CRI as a progressive loss of the renal function of depuration, which means glomerular filtration. Independent of the cause, CRI results from the irreversible loss of a large number of functional nephrons (Guyton and Hall, 2002). CRI is associated with the immunoinflammatory multifactorial syndrome which occurs precociously during the disease, getting worse as it progresses (Kan et al., 2002). In 1996/97, the main diseases reported as causes of terminal CRI were arterial hypertension (24%), glomerulonephritis (24%) and diabetes mellitus (17%) (Ajzen and Schor, 2002).

The HD used to compensate for the deficient renal function in uremic patients can be harmful to blood cells, and there is a consensus that this type of patient has a high risk of oxidative stress and, as a consequence, damage to the DNA. Markert et al. (1988) suggest that, in patients kept on HD treatment, the blood interacts with biocompatible membranes where the circulating neutrophils use oxygen and generate reactive oxygen species such as superoxide which leads to a worsening of the inflammatory state. Ross et al. (1997) say that it is probable that the dialysis mechanism acts on the enzymes related to glutathione by removing substrates and essential factors.

In the dialysis process, one of the possible consequences is oxidative stress, which can result in damage to the DNA, including point mutation due to base oxidation, simple and double strand breaks, genomic instability, and inhibition of the repair mechanism (Tarng et al., 2002; Stopper et al., 2004). These breaks can lead to the formation of fragments or losses of whole chromosomes, resulting in a faulty distribution of chromosomes during the mitosis process, and in the subsequent interphase these structures can be detected in the form of mi-

cronuclei (Stopper et al., 1999). The micronucleus frequency, the Comet assay in peripheral lymphocytes, and also 8-hydroxy 2'-deoxyguanosine (8-OH-dG) found in the leukocytes, mitochondrial DNA in skeletal muscle and capillary follicles have been used to establish biomarkers of DNA damage in chronic renal patients. According to Stopper et al. (2004), the alterations in DNA can induce the carcinogenesis mechanism, and may also be involved in the aging process, neurodegenerative diseases, diabetes, and arteriosclerosis.

In peritoneal dialysis (PD), the introduction and maintenance of a foreign body (flexible catheter) in the organism and the distention of the abdominal cavity, periodically or permanently, by the dialysis liquid (sometimes of high osmolarity) and the use of the peritoneum for water and solute transport can generate infectious, mechanical and metabolite complications (Riella, 1996). Ha et al. (2000) have shown *in vitro* that solutions in PD with low pH or high levels of products of glucose metabolism or both promote in the human peritoneum the death of mesothelial cells and DNA damage. Gotloib et al. (2003) exposed mesothelial cells of rats to high glucose concentrations, which are more biocompatible, for 2 h *in vivo*, and found an acceleration of the cell cycle and, after 30 days of culture, fewer cells with nuclear forms and cells with abnormal mitosis due to DNA damage. In agreement with the study of Wieczorowska-Tobis et al. (2001), who used a PD solution with physiological pH and low levels of glucose products in the peritoneum of rats for 6 weeks, there was a decrease in the intraperitoneal inflammatory process and peritoneal fibrosis ($P < 0.005$), when compared to a PD solution with low pH and high levels of glucose products.

The mutagenic and carcinogenic effect of genotoxic agents in human populations, exposed in the work environment accidentally or through medical treatment or because of their lifestyle, has increased considerably. In hospital routines, many mutagenic agents are used for maintenance or diagnosis and treatment of patients. For most of the patients the benefit is obvious, but for some this benefit is only partial (Maluf and Erdtmann, 2000).

A way of studying the genotoxic effects in a population is to conduct monitoring studies, using pertinent biological parameters with short-term manifestation and with micronucleus assays which can identify DNA damage and/or chromosome aberrations resulting from the exposure. The information obtained can be used as an early warning of the potential risk of developing long-term health problems (Au, 1991).

Nowadays, there are many tests, with *in vivo* as well as *in vitro* models, so that the test organisms can be viruses, bacteria, fungi, plants, insects, and mammals including humans (De Flora, 1998). Among the tests that detect chromosomal aberrations in mammals, the micronucleus test stands out, as it is a simple and quick test which detects chromosomal breaks and losses (Hayashi et al., 1994).

A high frequency of cells with micronuclei has been found by some researchers in chronic renal patients who have been undergoing HD for a long time (Stopper et al., 1999). Kan et al. (2002) carried out a study of genotoxicity using the Comet assay in chronic renal patients in HD programs and found a significant increase in DNA damage in comparison with the control group ($P < 0.001$). These results agree with those from other studies, such as the one by Stopper et al. (2001) who also used the Comet assay and found a significant increase in DNA damage in the lymphocytes of 23 patients with chronic renal disease, suggesting that there is a relationship between the duration of HD treatment and the DNA damage induced in these patients.

The aim of the present study was to determine the frequency of micronuclei and other alterations as seen in "broken egg" cells (BEC) and binucleated cells (BNC) in the buccal

mucosa cells of patients with chronic renal disease undergoing HD or PD and of two control groups, to evaluate these treatments for genotoxic effects.

MATERIAL AND METHODS

The current study was carried out in the Genetics Laboratory of the Catholic University of Pelotas. The sample was made up of 80 individuals, including 20 patients with CRI in HD, 20 patients in PD who formed the exposure group, and a control group made up of 40 healthy individuals without known exposure to genotoxic factors. The patients were hospitalized in the Santa Casa de Misericórdia of Pelotas Hospital to undergo elective surgery, and were matched for age and gender. Half of each group was male and the other half female.

The renal patients were in a program of HD for 4 h three times a week, continuous ambulatory peritoneal dialysis or automated peritoneal dialysis and adhered to the treatment involving dialysis and individual prescriptions. None of the patients from the sample had any infectious or inflammatory disease.

The project was approved by the Ethics Committee in Research at the Santa Casa de Misericórdia of Pelotas Hospital and the Federal University of Rio Grande do Sul (UFRGS). The patients signed a written informed consent in order to be interviewed and for the collection of buccal mucosa cells. All of them answered the questionnaire, according to the protocol published by the International Commission for Protection against Environmental Mutagens and Carcinogens (Carrano and Natarajan, 1988). The reliability of the information was guaranteed.

The buccal mucosa of each individual tested was washed with distilled water and then swabbed with a wooden tongue depressor. The scraping of the oral cavity was performed in the molar region to obtain abraded cells in the buccal mucosa. The first exfoliated cells collected were discarded. The wooden tongue depressor, with the scraping, was placed in a centrifuge tube with phosphate buffer, pH 6.8. Two tubes were used, one for the left side and the other for the right side of the oral mucosa. The material was then transported to the laboratory where it was processed.

After removal of the wooden tongue depressors, the tubes were centrifuged for 10 min at 1000 rpm. The supernatants were removed, leaving 0.5 mL of sediment and solution. Twelve milliliters of fixative was added (methanol, acetic acid; 3/1) and the tubes kept for 30 min in the freezer. The tubes were then centrifuged again. The supernatant was discarded, leaving 0.5 mL of suspension. The procedure was repeated adding 8 mL of fixative, leaving at the end 0.3 mL of suspension. With the help of a Pasteur pipette, the pellet was resuspended and 3 drops were placed on a slide pre-heated at 37°C. Afterwards, it was flamed and kept at room temperature overnight to dry. After drying was complete, the following sequence of hydrolysis was carried out: the slide was placed in 1 N HCl for 1 min, and again placed in 1 N fresh HCl at 63°C for 10 min. The slide was then taken out and allowed to drain and cool down for 15 min. Once again, it was placed in 1 N HCl for 5 min. It was then washed three times with distilled water for 5 min each time, kept at room temperature for 15 min and then placed in Schiff dye for 2.5 h in the dark. The slide was then placed in a solution of 80 mL distilled water and 20 mL phosphate buffer for 5 min (also in the dark). Afterwards, it was washed three times with distilled water quickly and left to dry overnight. In the morning, the cytoplasm was stained with fast-green.

The cells were examined with a binocular light microscope, with an objective of 100X and oculars of 10X. The frequency of micronuclei and other nuclear anomalies (BEC

and BNC) were recorded in specific files. A total of 2000 cells per person were evaluated, where only the non-fragmented and non-crowded or overlapping cells were considered. The criteria used for the identification of a micronucleus have been established by Picker and Fox (1986): a) the micronucleus must have a regular contour, round or oval, and must be inside the cytoplasm of a cell; b) it must be Feulgen-positive and of an equal or lower intensity, and must have the same texture and refraction, compared to the main nucleus; c) it must be smaller than the main nucleus, that is, its diameter must be 1/3 of the diameter of the main nucleus; d) it must be in the same focus plane, and e) it must be clearly separated from the main nucleus. Up to three micronuclei were recorded per cell, where questionable micronuclei were excluded. For statistical analysis, a database was created using the program SPSS 10.0 for Windows, and the two-tailed Student *t*-test, the Mann-Whitney test and the Spearman correlation test were used at a significance level of $P < 0.05$.

RESULTS

The purpose of this study was to determine the mutagenic potential of HD and PD treatments, to which chronic renal patients are subjected. Table 1 presents the main characteristics that show the profile of the individuals studied and their controls that were matched for age and gender. The individuals studied were classified by type of treatment, gender, age, duration of treatment, more frequent diseases, creatine level, family income, smoking habit, and alcohol intake.

Table 1. Characteristics observed in patients undergoing hemodialysis, peritoneal dialysis and two control groups.

Variables	Hemodialysis patients	Control group	Peritoneal dialysis patients	Control group
Gender				
Females	10 (50%)	10 (50%)	11 (55%)	11 (55%)
Males	10 (50%)	10 (50%)	9 (45%)	9 (45%)
Age (years)				
Average	50.45 ± 9.87	50.50 ± 10.55	48.65 ± 12.80	49.45 ± 13.08
Variation	30-67	31-67	15-73	15-74
Treatment time (years)				
Average	7.60 ± 5.49		1.80 ± 1.64	
Variation	2-22		0-6	
Creatine level				
Average	10.07 ± 2.51		9.59 ± 2.74	
Variation	5.0-16.40		4.0-15.70	
Diseases				
Hepatitis				
HCV +	17 (85%)	1 (5%)	9 (45%)	0
HBAg +	6 (30%)	0	1 (50%)	0
Herpes	4 (20%)	5 (25%)	5 (25%)	4 (20%)
Family income				
Number of minimum salaries				
Variation	2.35 ± 1.46	6.95 ± 10.91	5.25 ± 9.05	5.05 ± 6.55
	1-6	1-50	1-42	1-30
Smoking habit				
Smokers	3 (15%)	2 (10%)	2 (10%)	2 (10%)
Non-smokers	17 (85%)	18 (90%)	18 (90%)	18 (90%)
Alcohol intake				
Habitual alcoholic intake	6 (30%)	11 (55%)	3 (15%)	12 (60%)
No alcoholic intake	14 (70%)	9 (45%)	17 (85%)	8 (40%)

Data are reported as number with the percent in parentheses or as means ± SD and variation, for N = 20 in each group.

Table 2 shows the mean numbers (in 2000 cells observed) of micronucleus cells (MNC), BNC, and BEC, and the number of total micronuclei (TMN) in HD and PD patients, as well as in the control groups. A significant difference ($P < 0.01$) was found in HD patients (5.60 ± 5.31 MNC; 5.60 ± 5.30 TMN) and the control group (1.50 ± 2.01 MNC; 1.76 ± 2.24 TMN) in relation to the number of MNC and of TMN, but differences between the PD patients and their control group were not found.

Table 2. Average and standard deviation of the number of cells with micronuclei, bionucleated, broken egg, and the total of micronuclei (in 2000 cells) of patients in hemodialysis, peritoneal dialysis and the two control groups.

Variables	Homodialysis patients	Control group	Peritoneal dialysis patients	Control group
MNC				
Average	5.60 ± 5.31	1.0 ± 2.01	2.85 ± 2.96	3.25 ± 3.85
Variation	0-16	0-6	0-11	0-11
	P = 0.004			
TMN				
Average	5.60 ± 5.30	1.76 ± 2.24	2.95 ± 3.13	4.05 ± 5.14
Variation	0-16	0-6	0-11	0-16
	P = 0.007			
BNC				
Average	2.20 ± 2.71	4.20 ± 3.85	5.40 ± 3.72	4.60 ± 4.57
Variation	0-8	0-16	0-13	0-15
	P = 0.061			
BEC				
Average	2.25 ± 4.20	2.85 ± 3.58	4.15 ± 3.59	3.90 ± 3.88
Variation	0-16	0-2	0-14	0-14
	P = 0.507			

Data are reported as means ± SD and variation, for N = 20 in each group.
MNC = micronucleus cells; TMN = total micronucleus cells; BNC = binucleated cells; BEC = broken egg cells. Data analyzed by the Mann-Whitney U-test.

Comparisons were made among the variables of gender, age (in the intervals: ≤50 and ≥51 years old), family income (≤1 salary, from 2 to 4 salaries and ≥5 salaries), creatine level (≤10 and ≥10.01 mg/dL), smoking habit, and alcohol intake among the HD and PD patients and the control groups. The results suggest that there is no association between these variables and the number of nuclear anomalies (Tables 3 and 4).

A comparison was also made between two different treatment duration times, between ≤6 and ≥7 years of treatment. The results showed that there is an association between the length of treatment and the number of chromosomal alterations detected. The number of MNC (2.91 ± 2.74) for the interval of ≤6 years of treatment was significantly ($P < 0.05$) lower than the value found (8.89 ± 5.96 MNC) for the interval of ≥7 years of treatment. Also significant were the differences for the number of TMN (≤6 years of treatment: 3.09 ± 3.08; ≥7 years of treatment: 10.11 ± 7.04; $P < 0.05$) and for BEC (≤6 years of treatment: 1.00 ± 3.00; ≥7 years of treatment: 3.78 ± 5.09; $P < 0.05$) for HD patients. The Spearman correlation coefficient (0.414) with $P = 0.01$, showed a positive correlation between length of treatment and the number of MNC. The same was not found for the PD patients.

Table 3. Average of micronucleated cells (MNC), total micronucleus cells (TMN), binucleated cells (BNC), and broken egg cells (BEC) in relation to the socio-demographic and habits analyzed from the hemodialysis patients and control group.

Variables	MNC		TMN		BNC		BEC	
	Patients	Control	Patients	Control	Patients	Control	Patients	Control
Gender								
Females	6.10 ± 5.24	1.60 ± 2.41	6.90 ± 6.43	2.20 ± 3.29	2.20 ± 3.01	3.00 ± 2.71	2.00 ± 4.97	1.20 ± 1.87 (P = 0.034)
Males	5.10 ± 5.61	1.40 ± 1.65	5.60 ± 6.27	1.40 ± 1.64	2.20 ± 2.53	5.40 ± 4.55	2.50 ± 3.54	4.50 ± 4.20
Age groups								
≤50 years	5.70 ± 4.79	1.56 ± 2.13	6.40 ± 5.56	2.22 ± 3.19	2.00 ± 2.54	4.11 ± 4.88	1.30 ± 2.26	2.56 ± 4.00
≥51 years	5.50 ± 6.04	1.45 ± 2.02	6.10 ± 7.12	1.45 ± 2.01	2.40 ± 2.99	4.27 ± 3.00	3.20 ± 5.49	3.09 ± 3.39
Treatment length								
≤6 years	2.91 ± 2.74 (P = 0.038)		3.09 ± 3.08 (P = 0.030)		1.64 ± 2.50		1.00 ± 3.00 (P = 0.014)	
≥7 years	8.89 ± 5.96		10.11 ± 7.04		2.89 ± 2.93		3.78 ± 5.09	
Family income (minimum salary)								
≤1 minimum salary	5.25 ± 4.62	1.00 ± 1.73	5.00 ± 4.78	1.60 ± 2.60	2.13 ± 2.75	4.00 ± 2.55	2.63 ± 3.96	2.60 ± 2.41
from 2 to 4 minimum salaries	6.27 ± 5.98	2.86 ± 2.54	7.27 ± 7.29	3.28 ± 3.30	2.36 ± 2.91	6.86 ± 4.63 (P(2-3) = 0.012	2.09 ± 4.72	4.43 ± 5.13
≥5 minimum salaries	1.00 ± 0	0.63 ± 0.92	1.00 ± 0	0.62 ± 0.91	1.00 ± 0	2.00 ± 2.33	1.00 ± 0	1.63 ± 2.20
Creatine levels								
≤10.00 mg/dL	6.36 ± 5.43		7.27 ± 6.48		2.45 ± 2.84		3.09 ± 5.22	
≥10.01 mg/dL	4.67 ± 5.32		5.00 ± 6.02		1.89 ± 2.67		1.22 ± 2.39	
Alcohol intake								
Yes	4.00 ± 4.69	1.27 ± 1.74	4.00 ± 5.00	1.54 ± 2.11	1.17 ± 0.98	4.09 ± 4.59	1.17 ± 2.86	3.64 ± 4.37
No	6.29 ± 5.87	1.78 ± 2.39	7.07 ± 6.67	2.11 ± 3.14	2.64 ± 3.10	4.33 ± 2.96	2.71 ± 4.68	1.89 ± 2.20
Smoking habit								
Yes	3.67 ± 3.51	2.00 ± 2.83	4.33 ± 4.50	3.00 ± 4.24	0.67 ± 1.05	4.00 ± 0	0	2.50 ± 3.54
No	5.94 ± 5.57	1.44 ± 2.01	6.58 ± 6.52	1.66 ± 2.47	2.47 ± 2.83	4.22 ± 4.07	2.65 ± 4.46	2.89 ± 3.69

Data are reported as means ± SD.

Table 4. Average of micronucleated cells (MNC), total micronucleus cells (TMN), binucleated cells (BNC), and broken egg cells (BEC) in relation to socio-demographic variables and habits analyzed from the peritoneal patients and control group.

Variables	MNC		TMN		BNC		BEC	
	Patients	Control	Patients	Control	Patients	Control	Patients	Control
Gender								
Females	1.82 ± 2.18	3.55 ± 4.46	1.90 ± 2.42	4.27 ± 5.74	4.91 ± 3.21	4.27 ± 4.41	3.64 ± 3.98	4.91 ± 4.28
Males	4.11 ± 3.41	2.89 ± 3.18	4.22 ± 3.56	3.77 ± 4.63	6.00 ± 4.39	5.00 ± 5.00	4.78 ± 3.15	2.67 ± 3.12
Age groups								
≤50 years	3.09 ± 3.42	3.42 ± 4.01	3.18 ± 3.57	4.50 ± 5.66	5.64 ± 3.44	5.50 ± 5.02	4.00 ± 4.05	4.33 ± 4.12
≥51 years	2.56 ± 2.46	3.00 ± 3.85	2.66 ± 2.69	3.37 ± 4.53	5.11 ± 4.23	3.25 ± 3.69	4.33 ± 3.16	3.25 ± 3.65
Treatment length								
≤2 years	2.69 ± 2.36		2.81 ± 2.63		4.88 ± 3.46		3.88 ± 2.92	
≥3 years	3.50 ± 5.20		3.50 ± 5.19		7.50 ± 4.51		5.25 ± 6.08	
Family income (minimum salaries)								
≤1 minimum salary	2.25 ± 1.71	4.67± 3.79	2.25 ± 1.70	6.00 ± 6.08	5.75 ± 3.30	1.33 ± 0.58	3.00 ± 2.94	3.33 ± 3.51
from 2 to 4 minimum salaries	3.89 ± 3.89	4.09 ± 4.35	4.11 ± 4.13	5.09 ± 5.71	4.89 ± 4.54	5.09 ± 5.03	2.56 ± 1.67	5.27 ± 4.47
≥5 minimum salaries	1.86 ± 1.77	1.00 ± 2.00	1.85 ± 1.77	1.16 ± 2.40	5.86 ± 3.18	5.33 ± 4.59	6.86 ± 4.41	1.67 ± 1.37
Creatine levels								
≤10.00 mg/dL	2.83 ± 3.27		2.91 ± 3.39		5.75 ± 4.16		3.33 ± 2.53	
≥10.01 mg/dL	2.88 ± 2.64		3.00 ± 2.92		4.88 ± 3.14		5.38 ± 4.69	
Alcohol intake								
Yes	1.00 ± 1.00	3.58 ± 3.92	1.00 ± 1.00	4.25 ± 4.90	2.67 ± 3.06	6.08 ± 5.12	2.00 ± 2.65	4.50 ± 3.97
No	3.18 ± 3.09	2.75 ± 3.96	3.29 ± 3.27	5.82 ± 2.05	5.88 ± 3.69	2.38 ± 2.45	4.53 ± 3.66	3.00 ± 3.82
Smoking habit								
Yes	5.50 ± 3.54	2.50 ± 3.54	6.00 ± 4.24	3.00 ± 4.24	5.00 ± 4.24	6.50 ± 6.36	1.50 ± 2.12	2.50 ± 0.71
No	2.56 ± 2.85	3.33 ± 3.97	2.61 ± 2.95	4.16 ± 5.32	5.44 ± 3.79	4.39 ± 4.53	4.44 ± 3.63	4.06 ± 4.07

Data are reported as means ± SD.

The possible influence of the level of creatine, smoking habit, alcohol intake, age, and family income was considered in the determination of the frequency of nuclear alterations in relation to gender in both types of treatment and in the controls. No association was detected.

DISCUSSION

According to Stopper et al. (2001), one of the consequences of chronic renal disease is the high risk of cancer. This happens in patients with CRI without dialysis treatment as well as in those who are kept on HD (Goodkin et al., 2004). This fact can be related to failures in the DNA repair mechanism and, according to the latest studies, to the high frequency of MNC found in these patients. The results of this study are in agreement with the findings of Stopper et al. (2001) mentioned above, since a significantly higher number of MNC was found among the HD patients than in the control individuals, as well as the number of TMN.

In a previous investigation, Stopper et al. (1999) evaluated 19 patients in the final stages of renal insufficiency as well as 20 control individuals, and observed a slight increase in the MNC frequency, albeit not significant, in these patients. Nevertheless, in this study, the controls were not matched for age. In a second study, 16 patients with CRI in HD treatment, 19 patients without dialysis treatment and 23 control individuals matched for age were selected. In this evaluation, the frequency of MNC in BNC was determined in lymphocyte cultures and a significantly higher number of MNC was seen among the patients kept on HD in comparison to the control group.

In another study, Stopper et al. (2001) evaluated 26 chronic renal patients involved in HD treatment, using the Comet assay, and also obtained significant differences between patients and controls.

Liu et al. (2001) carried out a study that examined the deletion of 4977 bp of mitochondrial DNA in 162 patients with chronic renal disease, 125 patients being in HD and 37 in PD. They classified the individuals studied in age groups of 20-30, 31-40, 41-50, 51-60, and 61-70 years and found an incidence of deletion of 4977 bp, of 30.0, 31.9, 40.0, 43.9, and 44.8%, respectively. In the controls, the incidence was 8.6, 14.0, 14.3, 20.4, and 31.6%, respectively. The study showed that there was a significant increase in deletions of 4977 bp in mitochondrial DNA of the chronic renal patients in relation to the controls, due to the existence of factors that promoted genomic instability, leading to the occurrence of mutation.

Kan et al. (2002), using the Comet assay, carried out a study on DNA damage in chronic renal patients kept on HD and found a significant increase in the number of DNA breaks. However, they did not find a positive correlation between the duration (3.5 years) of HD treatment and the DNA damage that was found in the study of Stopper et al. (2001), using the Comet assay in lymphocytes.

In the present study, significant differences were found in the frequency of MNC, as well as in the number of TMN in relation to the length of HD treatment. There was a significant increase ($P = 0.038$) with treatment of 7 years and longer, confirmed by Spearman's correlation coefficient ($P = 0.01$). Kan et al. (2002) conclude that the length of treatment of the patients evaluated by them was very small, approximately 3.5 years, while Stopper et al. (2001) detected in their patients this increase with treatment of 10 years or more.

This correlation between length of treatment and nuclear alterations can be explained by the fact that the blood of these patients, maintained on HD treatment cycles, interacts with

biocompatible membranes and in this process many reactive oxygen species can be produced. It is also suggested that the dialysis process acts on the enzymes related to glutathione, by removing substrates and essential factors (Kan et al., 2002).

Among the factors that were evaluated for their influence on the formation of MNC, BNC, BEC, and TMN, one of the most relevant was the creatine level, as it is related to the severity of chronic renal disease (Combe et al., 2004). The patients were classified into two levels of ≤10.00 and ≥10.01 mg/dL. The results analyzed did not show differences in the number of nuclear anomalies. However, some studies report that the increase in creatine level is related to the number of MNC. Stopper et al. (2001) found a correlation between creatine level (severity of the disease) and DNA damage, determined using the Comet assay. Nevertheless, Stopper et al. (1999) did not find this correlation. In this second study, similar to the present one, the creatine level of the patients was very high, indicating the gravity of the disease in most of the patients.

Contrary to the results found in the patients on HD, significant differences were not found in PD patients. This finding may be due to the fact that all patients were under treatment for a short time, at most for 6 years, which limited this study. However, differences were not detected between the patients on HD and PD in relation to the cell alterations observed. It could be that the patients on PD were in a better general state of health than the ones on HD and that possibly PD has a smaller genotoxic effect.

Tarng et al. (2002) evaluated the oxidative DNA damage of leukocytes of peripheral blood, quantifying 8-OH-dG, by the electrochemical method in 24 control individuals, 22 patients with chronic renal disease without dialysis treatment and 42 patients on PD. The highest average level of 8-OH-dG was found in the PD patients, followed by the chronic renal patients and the controls. The authors concluded that a significant increase in the level of 8-OH-dG occurs with chronic renal disease and that this level tends to increase with the progression of the disease, meaning that peritoneal dialysis treatment exacerbates this DNA damage. Ishibashi et al. (2002) observed 8-OH-dG in patients that were starting PD (3-5 months). However, 8-OH-dG was observed in higher numbers of mesothelial cells of patients on long-term PD.

In conclusion, the patients under HD treatment showed more MNC than did their controls, indicating that there are factors related to their condition that increase the incidence of nuclear anomalies, which are a consequence of alterations in the genetic material as well as failures in repair mechanisms. According to Stopper et al. (2004), the formation of reactive oxygen species, due to chronic renal disease and the reactions with biocompatible dialysis membranes, as well as a reduced antioxidant defense in these patients, can also contribute to the increase in chromosomal and/or genomic damage, taking into account that the formation of the micronucleus can result in DNA strand breaks, as well as the loss of whole chromosomes, resulting in abnormal distribution of chromosomes among the daughter cells during the mitosis process.

This fact was confirmed by the correlation between the length of treatment and the number of MNC, showing that the longer the treatment, the higher the genotoxic effect.

Factors, such as creatine level, gender, age, family income, smoking habit, and alcohol intake do not influence the probable genotoxic action of hemodialysis treatment.

The patients on PD did not show differences in the number of MNC in comparison with the control individuals, which suggests that this treatment is possibly less harmful for the patient. However, due to the short duration of treatment of these patients, it is necessary to continue this study.

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