Evaluation of Mutagenic Effects of Chronic Renal Disease and Hemodialysis with Micronucleus Test

Evânio Márcio Romanzini¹, Caroline Dani², Jussiene Magnus Justos¹, Kimberly Rosa Martins¹, Marcello Ávila Mascarenhas¹ and Valesca Veiga Cardoso¹*¹

¹Department of Mutagenesis and Toxicology, Centro Universitário Metodista, Brazil
²Department of Biochemistry, Centro Universitário Metodista, Brazil

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Introduction

Chronic renal disease (CRD) causes damage to the kidney tubules and can cause premature cardiovascular death. In the late phase, patients need dialysis or a kidney transplant. The main risk factors in Brazil, the United States (US) and Canada are systemic arterial hypertension (SAH), diabetes mellitus (DM) and family history. CRD in the US is the leading cause of death and affects about 26 million people [1-4].

In Brazil, CRD affects about 1 million people, causing about 15,000 deaths/year. Currently, 100,000 people are estimated to be on HD, and the mortality rate is 17%. The most affected are the elderly, women and ethnic groups such as Asians. The prevalence in different countries is 7.2% in people over 30 years and 28 to 46% in individuals over 64 years [5,6].

The inflammatory process in uremic patients with CRD causes tissue damage, endothelial dysfunction, atherogenesis and cardiovascular disease. Hemodialysis (HD) increases the risk of oxidative stress by the interaction between blood and biocompatible membranes, which may cause DNA damage, genomic instability and inhibition of the repair mechanism [7-11]. In an in vitro study, Ha and colleagues (2000) demonstrated that the solutions used in the process of DP with low pH and or with high glucose levels can promote damage in the peritoneum in addition to the death of the mesothelial cells in the DNA.

Genotoxic agents induce cell damage. The formation of micronuclei (MN) is due to clastogenic agents that promote chromosome breaks in single- and double-stranded DNA. MNs can be used as a biomarker of genotoxic damage and an increase their frequency may represent the action of genotoxic/clastogenic agents. Successive exposure may show cumulative degenerative damage and malignant transformation [10,12-15].

It was estimated that in 2013, there were about 273,518 new cases of renal cancer in the world [16]. Incidence varied by geographic region [17], where it was higher in Europe and North America. [18] The etiology has not yet been identified, but there is evidence of a strong association with genetic and environmental factors such as lifestyle, tobacco use, obesity and occupation [19].

MN analysis can be used as a biomarker for chromosomal DNA damage in a variety of cell types. [13-15,19]. Guven et al. investigated the presence of nuclear damage in peripheral lymphocytes by associating the frequency of micronuclei with hemodialysis (HD) and peritoneal dialysis (DP), concluding that patients in the final stage of renal disease exhibit advanced nuclear damage [20-22].

Oral mucosa is used for the quantitative analysis of nuclear alterations in patients who are smokers of many kinds of tobacco, alcoholics, exposed to chemical agents, pesticides, antineoplastic drugs and other kinds of genotoxic agents [19].

The aim of this study was to evaluate the mutagenic effects of CRD combined with HD, using the micronucleus test, analyzing cells from the oral mucosa of patients with CRD on HD, with CRD not on HD and controls.
Materials and Methods

Samples were randomly obtained from 120 volunteers, and of these 97 participants were analyzed. This was carried out at the hospital care complex, Rio Grande do Sul, Brazil, between January and December 2014. The first group consisted of 32 individuals with CRD undergoing conventional HD for over 3 months with duration of 3 to 4 hr per session, three times a week, with 17 L from Gambro Polyflux dialyzer and peracetic acid sterilant. The second group consisted of 34 individuals with CRD on HD. The control group was formed by 31 individuals without CRD and who never underwent HD. Twenty-three participants was excluded: 12 smokers and 11 over 76 years old or under 21 or those with inflammatory or infectious lesions in the oral mucosa (Figures 1 and 2).

We followed the guidelines and regulatory standards for research involving human beings in Brazil, Resolution number 466 of December 12, 2012, the National Health Council (CNS) under protocols 496,997 and 499,777 of the Research Ethics Committee (CEP) by the Brazil Platform. Informed consent was obtained and confidentiality of subjects’ identity and information was maintained. The participants had no cost or financial advantage.

The collection and testing of cytogenetic materials followed biosafety guidelines. We used the Cytobrush Plus (Kolplast Commercial Industrial Ltda, São Paulo, SP, Brazil). To obtain samples from the oral mucosa smears the slides were stained by the May-Grünwald/Giemsa (MGG) technique with 10% Giemsa (Merck, Darmstadt, Germany). A total of 2000 cells of each participant were analyzed with a Leica CME® binocular light microscope at 400X, and we counted cells with micronuclei, broken egg cells, binucleated cells, and cells showing apoptosis (karyorrhexis, pyknosis) and karyolysis [20–21].

Statistical analysis was performed with the Statistical Package for Social Sciences (SPSS), version 19.0, and Kolmogorov-Smirnov test for normality of data. Descriptive statistics used frequencies, means and standard deviations (±). Comparisons between groups used the Student t-test and one-way ANOVA followed by the Tukey post-test. p<0.05 was considered statistically significant.

Results

Sample description

We studied 97 subjects aged 21-76 years, for a period of 1 year, including 52 ± 47.17% females and 45 (51.80%) males, with average age of 49.31 ± 19 years. The average with conventional HD treatment time was 4.1500 ± 4.15 years (p=0.0001) (Tables 1-3).

| Variable                        | CRD on HD group | CRD not on HD group | Control group | p    |
|--------------------------------|-----------------|---------------------|---------------|------|
| Number (n)                     | 32              | 34                  | 31            |      |
| Age (years)                    | 53.62 ± 14.20   | 53.26 ± 13.95       | 47.00 ± 15.24 | 0.128|
| Sex (M/F)                      | 22/10           | 15/19               | 8/23          |      |
| Ethnicity (Caucasian/non-Caucasian) | 25/7           | 28/6                | 26/5          |      |
| Years smoking/ex-smoker        | 9.43 ± 15.83    | 9.44 ± 14.05        | 4.58 ± 8.28   | 0.244|
Table 1: Characteristics of the CRD on HD, CRD not on HD and control groups.

Evaluation of nuclear alterations

By morphological analysis, cell quantification and statistical analysis of constant nuclear abnormalities in Table 3, we found a higher prevalence of MN, BNC, BEC, sum of BNC+BEC, karyorrhexis, karyolysis and sum of pyknosis+karyorrhexis in the CRD on HD group, followed by the CRD not on HD and control groups. The prevalence of pyknosis was lowest in the control group. All these differences were statistically significant (p=0.0001).

Table 2: Associated exposure factors in individuals with CRD on HD, with CRD not on HD and control groups (CRD: Chronic renal disease. Note: Values given as N and percentage (%)).

Comparing the number of abnormal cells and the sex of the participants, we found that the CRD not on HD group showed a higher number of BNC in men versus women (p=0.002), and also for the sum of BNC plus BEC in men (p=0.011) versus women, statistically significant differences.

The comparison between the ethnicity of the three groups studied and the number of abnormal cells showed that Caucasians in the CRD on HD group had higher rate of karyorrhexis (p=0.031). In the CRD not on HD group, the number of BNC was greater (p=0.001). However, non-Caucasians showed a significantly greater number of BEC (p=0.007) and BNC plus BEC (p=0.0001), all statistically significant differences.

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Regarding the associated exposure factors (Table 2), in the CRD on HD group, there was a lower prevalence of: beer and wine consumption (28.1%), exposure to chemical (34.4%) and physical (3.1%) agents and chimarrão drinking (31.3%) compared with those in the on HD (p=0.007) and control (p=0.004) groups. Those who drank wine had a greater number of MN (p=0.042). Those who drank distilled alcoholic beverages showed a higher rate of karyolysis (p=0.038).

In the CRD not on HD group, there was greater frequency of exposure to chemical agents (70.6%) and greater rate of karyolysis in those exposed versus not exposed (p=0.041) (Table 2).

Discussion

We observed a higher mean number of MN in the CRD on HD group compared to the CRD not on HD and control groups, indicating that CRD and HD combined have a mutagenic effect on cells causing genetic damage. This increase in MN may also be related to the uremic state caused by CRD, occupational exposures, genetics and lifestyle habits. These results corroborate other studies, where there was a higher frequency of MN in HD patients compared to control groups [22-25].

Long treatments with HD are associated with damage to the genome, due to the inflammatory state and the oxidative "stress." In this study, treatment time with HD averaged 4.15 years (± 4.15). In a previous study, patients on HD for a period ≥ 7 years had a mean MN count of 8.89 (± 5.96), and those on HD for a period ≤ 6 years had a lower mean MN of 2.91 (± 2.74). A similar situation occurred in with BEC with respective values of 3.78 (± 5.09) and 1.00 (± 3.00) (p<0.05). We noted that the greater the time of exposure to HD, the higher the genotoxic damage as evidenced by the increase in the number of MN [7-9,23].

BNC indicate delayed cell division and cytotoxicity. We observed a higher mean BNC frequency in the CRD on HD group and CRD not on HD groups, which might have been related to the genotoxic effects of CRD, since the controls did not have CRD. In another study, BNC frequency was also higher in the HD group than in controls, with 46.2% (± 4.3%) versus 24.4% (± 9.5%) [20,25].

The high frequency of BEC can indicate normal adaptation of the epithelium, but we observed a greater incidence in patients with CRD on HD and with CRD not on HD, compared to controls. The sum of BNC plus BEC showed a mean of 37.28 (± 15.78) in the CRD on HD group versus 32.47 (± 22.10) in the CRD not on HD group versus 14.25 (± 6.92) in controls (p=0.0001), reinforcing the notion of this increase being related to the mutagenic effects of CRD and HD [23,26].

The increase in pyknosis in the CRD on HD group can be related to shear stress caused by prolonged HD and by CRD, which results in a state of uremia. The mean frequency of karyorrhexis and karyolysis with the sum of the variables pyknosis plus karyorrhexis between the groups was statistically significant in relation to controls (p=0.0001), demonstrating that CRD and HD determine the increase in these cellular changes [7-9,20,24].

Mean age was 53.62 (± 14.20) years in the CRD on HD group, 53.26 (± 13.95) in the CRD not on HD group, and 47.00 (± 15.24) in the control group, a similar age profile as in other studies [23-25].

There was a greater incidence of BNC and BEC (p=0.002) and greater sum of BNC plus BEC (p=0.011) in males on HD. In another study of males on HD, BNC showed a frequency of 2.20 (± 2.53), BEC 2.50 (± 3.54) and controls 5.40 (± 4.55) e de BEC 4.50 (± 4.20) [23].

There was no statistically significant relation with exposure to chemical agents. In another study, workers exposed to farm pesticides for 16.3 (± 10.0) years had a mean MN of 3.55 (± 2.13), significantly higher than in the non-exposed group with 1.78 (± 1.23) (p=0.001) [27].

We observed in comparing the number of cells with alterations and weekly consumption of chimarrão a statistically significant increase in BEC only in the control group, although it might have been related to the diet restrictions and consumption of chimarrão of those on HD. Another study found that among daily chimarrão drinkers, 28% had BNC, 14% karyorrhexis, 2% karyolysis, 6% BEC, 7% pyknosis and 43% MN [28].

Comparing the cellular changes and the consumption of alcohol, in the CRD on HD group, wine drinkers had a lower mean MN of 5.66 (± 3.60) (p=0.095), while the controls had a lower sum of BNC plus BEC with a mean of 11.50 (± 4.41) (p=0.050). This decrease may further indicate a more protective property of wine related to the reduced

### Table 3: Frequency of abnormalities in exfoliated cells of the oral mucosa of adults examined under a light microscope, at 400 times in 2000 cells

| Effect | Control | CRD on HD | CRD not on HD |
|--------|---------|-----------|---------------|
| MN     | 8.34 ± 5.66* | 1.02 ± 1.05 | 3.54 ± 2.39 | 0.0001 |
| BNC    | 21.03 ± 6.87 | 17.23 ± 11.07 | 9.09 ± 5.74* | 0.0001 |
| BEC    | 16.25 ± 12.49 | 15.23 ± 12.69 | 5.16 ± 2.62* | 0.0001 |
| BNC+BEC| 37.28 ± 15.78 | 32.47 ± 22.10 | 14.25 ± 6.92* | 0.0001 |
| Pyknosis| 20.59 ± 12.06 | 11.47 ± 12.52 | 7.03 ± 5.70* | 0.0001 |
| Karyorrhexis| 13.09 ± 7.92* | 6.97 ± 6.50 | 4.32 ± 4.54 | 0.0001 |
| Pyknosis+karyorrhexis| 33.68 ± 18.19* | 18.44 ± 18.17 | 11.35 ± 8.70 | 0.0001 |
| Karyolysis| 52.03 ± 30.03* | 23.47 ± 18.10 | 24.16 ± 18.05 | 0.0001 |

Note: Values are given as mean ± standard deviation (SD). *p<0.05 statistically significant. Test: one-way ANOVA followed by Turkey's post-test.)
cellular mutagenic effects caused by CRD and HD. Resveratrol, a polyphenol present in red wine, has biochemical, physiological, estrogenic, antiplatelet, anti-inflammatory, cardioprotective and chemopreventive properties [29].

In the CRD on HD group who drank distilled spirits had a higher mean rate of karyolysis at 93.00 ± 52.32 (p=0.038). The effects of alcohol together with a HD may result in potentiated cell damage, since ethanol acetaldehyde may promote carcinogenesis and since alcohol consumption is a risk factor for cancers of the head and neck, intestine, liver and breast. In another study, workers exposed to pesticides with a habit of alcohol consumption had higher mean MN levels compared to controls. In this study, we found that CRD, by affecting renal filtration, and HD, by causing oxidative stress, together influence this process [27,30,31].

This study differs from similar ones by analyzing CRD and HD separately in three groups which made it clear that patients undergoing HD had a significant increase in genetic changes, evidenced by a higher frequency of MN and BNC, compared to patients with CRD not subjected to HD [22-25].

New studies are warranted to evaluate genetic polymorphisms and susceptibility to the development of renal cancer by genotyping patients with CRD undergoing HD.

Conclusion

We demonstrated using the micronucleus test that there were statistically significant differences in mean number of cells with MN, BNC, BEC, pyknosis, karyorrhexis and karyolysis between groups, with higher numbers in the CRD on HD group in relation to the CRD not on HD and control groups.

The increase in cellular changes might have been related to CRD, the HD and also other occupational exposure factors, eating habits, underlying diseases, genetic and ethnic factors denoting greater susceptibility to mutagenic effects.

We noted that those on HD, Caucasian males had higher rates of cellular changes that may be associated with the occurrence of mutagenic damage. We showed that CRD and HD contribute to the emergence and increase of metanuclear changes such as pyknosis, karyorrhexis and karyolysis.

Finally, we believe that CRD and HD are mutagenic factors alone, together or in combination with other occupational exposure factors, genetics and lifestyle habits, and that they significantly contribute to the emergence and increase of micronuclei and other nuclear changes. Therefore, CRD and HD may be factors that are related to the emergence of renal cancer. In addition, our results show that the MN test and the frequency test of other nuclear alterations using the oral mucosa can be an extremely sensitive and non-invasive biomarker to evaluate the deleterious effects of various xenobiotic substances, including procedures such as HD.

Conflict of Interest

The authors of the manuscript declare not have a conflict of interest.

References

1. http://www.cdc.gov/diabetes/projects/pdfs/CKD_Factsheet.pdf
2. Lordsleem AG, José ER, Filho BM, Victor EG (2012) Cardiac evaluation of patients with chronic kidney disease: what lessons? J Bras Nefrol 34: 8-15.
3. Zamponato TK, Thomé FS, Gonçalves LFS (2008) Socioeconomic profile of patients with chronic kidney disease on dialysis in the northwestern region of Rio Grande do Sul. J Bras Nefrol 30: 192-199.
4. Inker LA, Astor BC, Fox CH, Iakova T, Lash JP, et al. (2014) KDOQI US Commentary on the 2012 KDIGO Clinical Practice Guideline for the Evaluation and Management of CRD. Am J Kidney Dis 63: 713-735.
5. http://www.sbn.br/censo
6. http://www.sbn.org.br
7. Dummer CD, Thomé FS, Veronese FV (2007) Doença renal crônica, inflamação e aterosclerose: novos conceitos de um velho problema. Rev Assoc Med Bras 53: 446-450.
8. Bahia L, AGUIAR LGK, Villela NR, Bottino D, Bouskela E (2006) The endothelium in the metabolic syndrome. Arq Bras Endocrinol Metab: 50.
9. Moreesco RN, Silva SH (2011) Biomarcadores cardíacos na avaliação da síndrome coronariana aguda. Scientia Medica Porto Alegre 1: 132-142.
10. Stopper H, Schupp N, Bahner U, Sebekova K, Klassen A, et al. (2004) Genomic damage in end-stage renal failure: potential involvement of advanced glycation end products and carbonyl stress. Semin Nephrol 24: 474-478.
11. Tarng DC, Chen TW, Huang TP, Chen CL, Liu TY, et al. (2002) Increased oxidative damage to peripheral blood leukocyte DNA in chronic peritoneal dialysis patients. J Am Soc Nephrol 13: 1321-1330.
12. Stich HF, Stich W, Parida BB (1982) Elevated frequency of micronucleated cells in the buccal mucosa of individuals at high risk for oral cancer: betel quid chewers. Cancer Lett 17: 125-134.
13. Silva J, Erdtmann B, Henriques JAP (2003) Genética Toxicológica. Porto Alegre, Alcance. pp: 424.
14. Stopper H, Meyen T, Röckenförd A, Bahner U, Heidland A, et al. (1999) Increased genomic damage in lymphocytes of patients before and after long-term maintenance hemodialysis therapy. Am J Kidney Dis 34: 334-337.
15. Carrard VC, Costa CH, Ferreira LA, Lauxen IS, Rados PV (2007) Teste dos micrônucleos – um biomarcador de dano genotóxico em células descamadas da mucosa bucal. Rev Fac Odontol Porto Alegre 48: 77-81.
16. http://globocan.iarc.fr
17. Ferlay J, Autier P, Boniol M, Heanue M, Colombet M, et al. (2007) Estimates of the cancer incidence and mortality in Europe in 2006. Ann Oncol 18: 581-592.
18. Mathew A, Devesa SS, Fraumeni JF, Chow WH (2002) Global increases in kidney cancer incidence 1973-1992. Eur J Cancer Prev 11: 171-178.
19. Majer BL, Laky B, Knasmüller S, Kassie F (2001) Use of the micronucleus assay with exfoliated epithelial cells as a biomarker for monitoring individuals at elevated risk of genetic damage and in chemoprevention trials. Mutat Res 489: 147-172.
20. Tolbert PE, Shy CM, Allen JW (1992) Micronuclei and other nuclear anomalies in buccal smears. Methods Development Mutat Res 271: 69-77.
21. Sarto F, Finotto S, Giacomelli L, Mazzotti D, Tomanin R, et al. (1987) The Micronucleus Assay in Exfoliated Cells of the Human Buccal Mucosa. Mutagenesis 2: 11-17.
22. Guven GS, Altıparmak MR, Trabulus S, Yalin AS, Batar B, et al. (2013) Relationship between genomic damage and clinical features in dialysis patients. Genet Test Mol Markers 17: 202-206.
23. Roth JM, Restani RG, Gonçalves TTS, Sphor SLS, Ness AB, et al. (2008) Genotoxicity evaluation in chronic renal patients undergoing hemodialysis and peritoneal dialysis, using the micronucleus test. Genet Mol Res 7: 433-443.
24. Sandoval SB, Stoyanova E, Colli E, Pastor S, Reyes J, et al. (2010) Genetic damage in chronic renal failure patients is associated with the glomerular filtration rate index. Mutagenesis 25: 603-608.
25. López AR, Medina MEP, Reyes UM, Arredondo MC, Aguilar CA, et al. (2013) Genetic damage in patients with chronic kidney disease,
peritoneal dialysis and haemodialysis: a comparative study: Mutagenesis 28: 219-225.

26. Cerqueira EMM, Filho ISG, Trindade S, Lopes MA, Passos JS, et al. (2004) Genetic Damage in Exfoliated Cells from Oral Mucosa of individuals Exposed to X-rays During Panoramic Dental Radiographies. Mutat Res 562: 111-117.

27. Bortoli GM, Azevedo MB, Silva LB (2009) Cytogenetic biomonitoring of Brazilian workers exposed to pesticides: Micronucleus analysis in buccal epithelial cells of soybean growers: Mutat Res 1: 675.

28. Ferigolo PC, Sagrillo MR (2013) Genotoxicidade relacionada ao consumo de chimarrão: Disciplinarum Scientia. Série. Ciências da Saúde 14: 1-13.

29. Das DK, Mukherjee S, Ray D (2010) Resveratrol and red wine, healthy heart and longevity. Heart Fail Rev 5: 467-477.

30. Balbo S, Meng L, Bliss RL, Jensen JA, Hatsuksi DK, et al. (2012) Time course of DNA adduct formation in peripheral blood granulocytes and lymphocytes after drinking alcohol. Mutagenesis 27: 485-490.

31. Ha H, Yu MR, Choi HN, Cha MK, Kang HS, et al. (2000) Effects of conventional and new peritoneal dialysis solutions on human peritoneal mesothelial cell viability and proliferation. Perit Dial Int 20: S10-S18.