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Short communication

Evaluation of Pb-210 in urine and frequency of micronuclei in exfoliated cells as indicators of exposure to cigarettes

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ABSTRACT

This study aimed at analyzing the frequency of micronuclei (MN) in exfoliated cells as well as the levels of Pb-210 in urine samples to evaluate the association between the smoking habit and toxic stress of transitional epithelial cells. The frequency of MN was scored from Giemsa-stained slides while exchange resin and beta counting techniques were employed to measure the concentrations of this radioisotope. Urine samples of smokers had levels of Pb-210 up to 158.65 mBq L^{-1}. For nonsmokers, the median was below the detection limit (45 mBq L^{-1}). The analyses of mononucleated cells showed a significant increase of the frequency of MN in smokers when compared to nonsmokers. Statistical tests showed a tight relation between the cigarette consumption and the increase of the frequency of MN, rather than with the levels of Pb-210 present in smoke particles. The results indicate the usefulness of the methodology for the evaluation of human health risks related to chronic contamination with Pb-210.

1. Introduction

The last statistics concerning the number of smoking-related deaths are not optimistic, predicting 8 million deaths in the next decade, especially in emerging countries. Estimates indicate that, in 2015, 5 trillion cigarettes were consumed around the world [1,2].

Concerns about smoking come from the genetic damage caused by toxins found in the cigarettes [3]. Besides chemicals, there are radioactive materials, such as Pb-210 and Po-210 [4]. The incorporation of these radioisotopes into the human body is through food, water, and inhalation. When a cigarette burns, Po-210 and Pb-210 are carried off in the smoke particles, which sublimate into the lungs of smokers, making it available to the bloodstream and to the urine [5]. As a consequence, analyses of Pb-210 in urine have been widely used to assess risks related to smoking habit [6]. Apart from lung cancer, another important risk associated with smoking is the development of cancer of the bladder [7]. In fact, the probability of developing bladder cancer is four times higher for smokers than for nonsmokers [8]. One type of cell found in urine is the transitional epithelial cell, also known as a urothelial cell, and the analysis of these cells is of great importance to monitoring the early stages of individual bladder cancer [7].

The micronuclei (MN) frequency has been proposed and used as bioindicator in the evaluation of cellular toxic stress [7,9,10]. The MN is a separate structure from the main nucleus of a cell, resulting from the non-inclusion of the chromosome or part of the main cell nucleus during mitosis. This structure is a kind of byproduct of unstable chromosomal aberrations [11,12]. The high incidence of MN is related to chromosomal damage caused by exposure to mutations, a defective mitotic process, or inefficiency in DNA repair [12,13]. Moreover, the MN test is a minimally invasive technique and proves to be suitable for monitoring both individual and population exposure [14,15].

This study was designed to evaluate the association between smoking and the toxic stress of transitional epithelial cells from urine samples by combining the analyses of the frequency of MN in exfoliated

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Table 1
General information about the characteristics of the group studied (All from Recife). Distribution of concentration in ^{210}Pb activity in urine, numbers of cells and Micronuclei frequency in exfoliated cells from smokers and nonsmokers.

Subject	Age	Cigarettes (no./day)	Daily seafood Consumption	Smoking Time (in years)	MN Frequency (‰)	Pb-210 (mBq L^{-1})
N1	33	–	Yes	–	1	< 45*
N2	22	–	Yes	–	1	< 45
N3	26	–	Yes	–	5	< 45
N4	36	–	Yes	–	5	< 45
N5	50	–	Yes	–	5	< 45
N6	61	–	No	–	3	< 45
N7	26	–	Yes	–	4	< 45
N8	26	–	Yes	–	3	< 45
N9	20	–	No	–	3	< 45
N10	30	–	Yes	–	4	51.3 ± 3.39
N11	32	–	Yes	–	5	< 45
N12	65	–	Yes	–	5	< 45
N13	21	–	Yes	–	2	< 45
S1	34	< 20	Yes	22	5	158.65 ± 6.06
S2	23	< 20	Yes	7	5	< 45
S3	50	> 20	Yes	30	3	111.26 ± 11.18
S4	50	> 20	Yes	20	9	< 45
S5	52	> 20	Yes	15	6	96.77 ± 5.70
S6	33	< 20	Yes	2	6	< 45
S7	27	> 20	Yes	8	8	49.70 ± 2.90
S8	56	> 20	Yes	36	13	54.19 ± 4.50
S9	44	< 20	Yes	16	7	< 45
S10	62	< 20	Yes	30	6	< 45
S11	25	< 20	Yes	11	7	55.74 ± 5.75
S12	21	> 20	Yes	16	9	75.7 ± 5.10
S13	30	< 20	Yes	5	6	141.16 ± 9.50

Legend: (N − Nonsmoker); (S − Smoker); * DL (detection limit).

cells with the concentration of Pb-210.

2. Material and methods

2.1. Subjects

This study was approved by the Ethics in Research Committee of the Federal University of Pernambuco (number 1 472.005). The research subjects were 26 adult men (13 smokers and 13 nonsmokers). A questionnaire collected information about lifestyle and factors such as age, diet, cigarette smoking habits, and sanitary hygienic conditions were evaluated. The volunteers were included in the study since they did not make use of prescription drugs or undergo any radio or chemotherapy procedures and had no history of urinary cytological abnormalities, bladder cancer, or any other type of cancer. Table 1 presents the profiles of such subjects.

All cigarettes consumed by the subjects are in accordance with the rules of the Brazilian National Health Surveillance Agency – ANVISA.

2.2. Collection of urine samples

For the MN analyses in exfoliative cells from urine samples, the methodology employed was based on Fortin et al. [7]. All volunteers received the following instructions: (a) wash your hands, then wash the penis, pulling back all the foreskin, rinse and dry it well with sterile gauze or clean towel; (b) After that, starts urination, discarding the first jet of urine without interrupting urination. For each volunteer, the sample final volume was 250 mL of urine in the specific container given to each subject. Some volunteers performed two or three urine collections until reaching 250 mL. Each sample was immediately transported to the laboratory to be processed. Generally, the period between the urine collection and delivery to the laboratory was of about 2 h.

To evaluate the concentrations of Pb-210 in the urine samples, this study employed the same methodology used by Costa-Júnior et al. [16]. For this, the volunteers received polyethylene containers with a capacity of 2 L. The same hygienic procedures of urine collection for MN analyses were used. The subjects have collected urine several times in 24 h until obtaining at least 1.5 L. In this study, the amount of material collected ranged from 1.5 to 2 L. At the end of the collection, each sample was stored in a refrigerator until analyzed according to the protocol described in Section 2.5.

2.3. Isolation, fixation and staining of exfoliated cells in urine

Since all volunteers were male, squamous cell contamination was unlikely. The presence of squamous cells is more common among women and hinders differentiation with urothelial cells, which is a limiting factor for biomonitoring when compared to the male population [17]. For cell isolation, we used the methodology of Lehucher-Michel et al. [18] and adopted by Fortin et al. [7]. It begins with the centrifugation of the samples at 400 x g for 10 min. The supernatant was carefully discarded, leaving 10–15 mL of urine in the bottle. The liquid was shaken and transferred to 15-mL conical tubes. A second centrifugation was performed at 400 x g for 10 min. The supernatant was discarded, leaving only 0.5 mL to ensure minimal loss of cells. The cells were washed with 10 mL of 0.9% NaCl and centrifuged again at 400 x g for 10 min. A second washing with 0.9% NaCl was done. After the second wash, the final pellet was resuspended in 1.0 mL of 0.9% NaCl, and it was necessary to transfer the sample to a 1.5 mL micro-tube and perform centrifugation at 10,000*g* for 1 min. Most of the supernatant should be removed and the pellet resuspended in 0.2 mL of sterile 0.9% NaCl. The pallet obtained in the previous step were resuspended in fixative and fixed twice in 1.5 mL for 10 min using Carnoy I fixative (methanol to glacial acetic acid, 3:1). Cells were spread on the previously clean microscope slide and air dried overnight, and staining was performed using 5% Giemsa [7].

2.4. Micronuclei scoring

Slides were screened with 400× magnification on Leica Laborlux microscope, following the criteria described by Fenech et al. [14] and adopted by Fortin et al. [7]. Thus, for the scoring of MN, the following