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Brief Communication

Environmental Biomonitoring Using Cytogenetic Endpoints in a Population Exposed to Mercury in the Brazilian Amazon

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INTRODUCTION

Mercury has been used in the Amazon Basin since the late 1970s in extensive gold-mining operations that employ amalgamation techniques. In addition, increases in the agricultural activities and the population of the region have resulted in deforestation. The mercury released into water systems from slash-and-burn agricultural practices, mining operations, and the leaching of soils after deforestation has been a major contributor to the contamination of the Amazon ecosystem [Roulet et al., 1999, 2000].

There is concern about the health of populations exposed to mercury because this metal and its derivatives, especially methylmercury, are extremely toxic substances [Tchounwou et al., 2003]. A study conducted in 1995 found a clear association between the levels of methylmercury contamination (measured in hair) and cytogenetic damage in lymphocytes for a population living along the banks of the Tapajós River [Amorim et al., 2000]. The results indicated that cells from people with relatively high mercury levels had a decrease in mitotic index (MI) and an increase in the frequency of polyploid cells. The authors of this study proposed that MI could be a useful early indicator for cytogenetic health hazard in people from remote areas where more sophisticated tests were difficult to perform.

In order to follow up on the biomonitoring study started in 1995, new analyses were conducted in 2000 and 2001 using the same cytogenetic endpoints, i.e., MI and polyploidy. It was our intention to develop additional information on the cytogenetic status of the population in order to determine if the endpoints assessed in 1995 were still affected by mercury.

MATERIALS AND METHODS

This study was approved by the ethics committees (Comissão Nacional de Ética em Pesquisa) from the Conselho Nacional de Desenvolvimento Científico e Tecnológico and the Universidade Federal do Pará, Belém,

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Pará, Brazil. The study population lives in a small village, Brasília Legal, situated on the banks of the Tapajós River, a tributary of the Amazon. Brasília Legal is accessible only by water, a 12-hr boat trip from Santarém, a city of several hundred thousand inhabitants. An attempt was made to use exactly the same population from the 1995 study, but after 5 years some people moved or refused to participate in the study. Thus, from the original population (n = 94), a total of only 45 adults, ranging in age from 21 to 65 years, participated in the 2000 study. In 2001, 42 adults, ranging in age from 22 to 65, participated in the study. They were provided with information about the tests that would be performed and the aims of the study, and they gave their written consent. An interview-administered questionnaire was used to obtain information on diet, smoking, alcohol consumption, and other demographic and health-related data.

Venous blood was collected in heparinized vacutainer tubes and used to prepare cell cultures on a boat, using electricity provided by a generator. The use of a mobile laboratory was necessary in order to avoid the loss of cell viability associated with transport and delay. Two independent cultures were initiated per blood sample in RPMI-1640 medium supplemented with 20% fetal calf serum (Cultilab, Campinas, Brazil), antibiotics (100 IU penicillin/ml and 100 μg streptomycin/ml; Gibco, Paisley, U.K.), and 4% phytohemoagglutinin (Cultilab). The cultures were incubated at 37°C for a total of 48 hr, with 0.8 mM colchicine (Sigma, St. Louis, MO) added 2 hr before harvesting.

At harvest, the cells were pelleted by centifugation (1,000 rpm) and treated for 10 min with 0.075 M KCl (Merck, Darmstadt, Germany). They were then treated with Carnoy's fixative (1:3 glacial acetic acid:absolute methanol). The fixed cells were applied to slides, and the slides were air-dried and stained for 10 min with 3% Giemsa stain (Merck) diluted in buffer solution (pH 6.8). The slides were coded and scored blind by light microscopy by a single investigator in the Human Cytogenetics Laboratory at the Federal University of Pará. The MI (frequency of metaphase nuclei in 1,000 nuclei) and the number of polyploid cells in 1,000 cells were determined for each individual.

Analyses for mercury contamination were conducted in the laboratories of the Environmental Research Chair of the University of Québec in Montréal, Canada using cold-vapor atomic fluorescence (CVAF) spectrophotometry. Hair was analyzed for total mercury according to the procedure described by Bloom and Fitzgerald [1988] as adapted for hair. The concentration of inorganic mercury was determined using the methods described by Farant et al. [1981] and adapted for CVAF.

A single regression analysis was used for statistical evaluation of the association between mercury levels and the genotoxicity parameters in the population. Differences in hair mercury levels at the sampling dates were assessed by the Kruskal-Wallis test.

RESULTS AND DISCUSSION

Statistical analysis conducted on our data showed no significant relationship between MI and total mercury levels in hair (2000: n = 44, R^2 = 0.000487, P = 0.8869; 2001: n = 26, R^2 = 0.0495, P = 0.2745; Fig. 1). In addition, the frequency of polyploid cells was not related to total hair mercury levels (2000: n = 44, R^2 = 0.00102, P = 0.8373; 2001: n = 26, R^2 = 0.0495, P = 0.2745; Fig. 2). These results indicate that the finding reported by Amorim et al. [2000] in their 1995 study, decreased MI and increased frequency of polyploid cells with increasing hair mercury levels, was no longer true for samples taken in 2000 and 2001. The absence of a significant relationship between hair mercury levels and the cytogenetic endpoints may be related to the decrease in total hair mercury levels since the 1995 study was conducted (median 1995: 13.5 μg/g, n = 94; median 2000: 9.6 μg/g, n = 45; median 2001: 8.3 μg/g, n = 42; Kruskal-Wallis: H = 12.5; P = 0.00191; Fig. 3). Fish is the dietary mainstay of the region and can be a source of human mercury exposure. We believe that improvements in education and the nutritional changes encouraged by our research team may be responsible for this decrease [Lebel et al., 1998; Dolbec et al., 2001; Mergler et al., 2002].

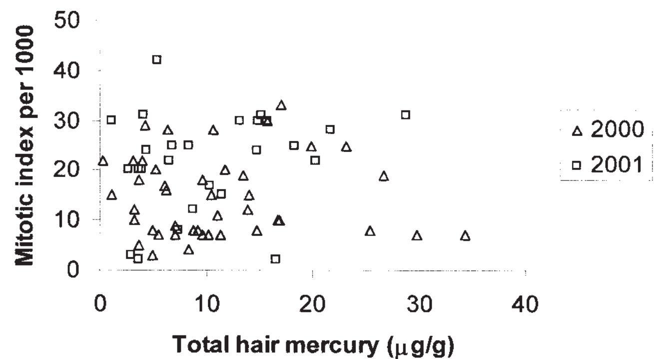

Fig. 1. Relation between mitotic index and total hair mercury (2000: n = 44, R^2 = 0.000487, P = 0.8869; 2001: n = 26, R^2 = 0.0495, P = 0.2745).

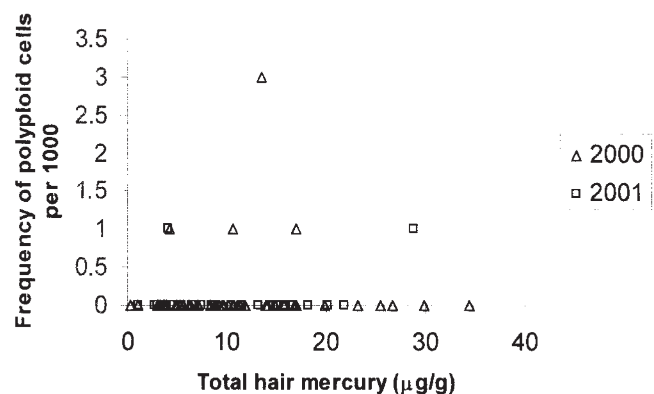

Fig. 2. Relation between polyploid cells and total hair mercury (2000: n = 44, R^2 = 0.00102, P = 0.8373; 2001: n = 26, R^2 = 0.0495, P = 0.2745).

We have also analyzed some confounding factors such as age, sex, smoking habits, alcohol consumption, having worked in gold mining operations, or having suffered from malaria. None of these factors showed any relationship with hair mercury levels, mitotic index, or polyploid cells except for sex in the 2001 study. In that year, women showed significantly lower levels of hair mercury (median: 6.76 μg/g) as compared to men (median: 15.16 μg/g; U = 402.0; P = 0.0381). Lower hair mercury levels in women were also found by Amorim et al. [2000].

The cytotoxicity of a chemical mutagen can be estimated from several endpoints and one of the most used is MI [Galloway, 2000]. However, MI data alone are considered an insufficient measure of cytotoxicity. The cytotoxic/cytostatic effects measured by MI are indirect, and MI is highly dependent on the time of the measurement [Galloway et al.,

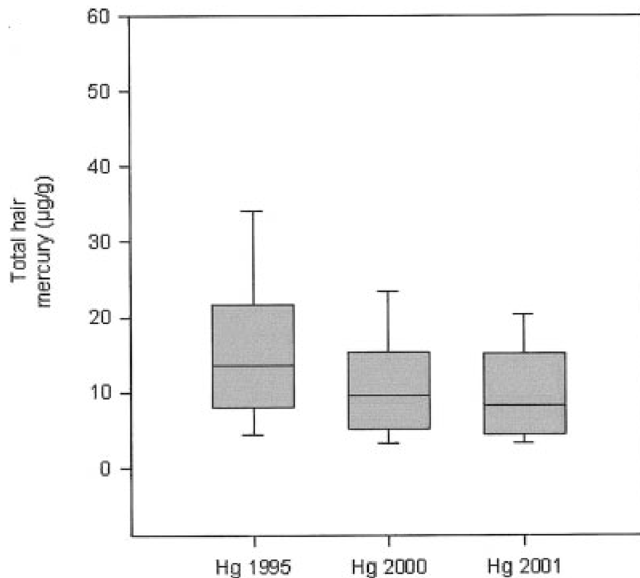

Fig. 3. Median values of total hair mercury in 1995, 2000, and 2001. Median 1995: 13.5 μg/g (n = 94). Median 2000: 9.61 μg/g (n = 45). Median 2001: 8.30 (n = 42). Kruskal-Wallis test: H = 12.5; $P = 0.00191$.

1994; Galloway, 2000]. The limitations of MI as a toxicity parameter are important for cell cultures other than blood lymphocytes. When using lymphocyte cultures, the use of MI is acceptable as a toxicity measure, especially when other toxicity measurements are cumbersome or impractical [Galloway et al., 1994]. In our study, we were able to evaluate only MI as a cytotoxicity endpoint because the laboratory conditions permitted only the use of standard and simple cytogenetic techniques. In addition, the Amorim et al. [2000] study suggested that MI was sensitive to mercury exposure.

Mercury binds to tubulin-SH, leading to the inhibition of microtubule assembly and the disruption of microtubules. This binding results in an impairment of the spindle function, which is believed to be a critical mechanism for cytotoxicity and the inhibition of cell growth [Graf and Reuhl, 1996]. Polyploid cells, which contain an exact multiple of the haploid number of chromosomes (N), e.g. $3N$ and $4N$, can be induced by the impairment of the mitotic spindle with a consequent failure of chromatid migration to the poles. This process yields $4N$ cells [Kirsch-Volders et al., 2002] and is also easily assessed by standard cytogenetic techniques.

The decrease in mercury levels may result in improvements to the health of the population. Exposure to this metal results in a cell intoxication (which presumably is reflected in MI), leading to death via apoptosis. Cell death may result in an immune dysfunction that could be capable of triggering immunologically mediated disease or promoting chronic infection; moreover, immune dysfunction could influence the development and progression of cancer [Shenker et al., 2000; Araragi, 2003]. In addition, the impairment of spindle function and the induction of chromosome segregation errors during cell division induced by mercury poisoning may lead to polyploidy and aneuploidy. Such events could be involved in spontaneous abortion, birth defects, cell transformation, and the tumor progression process [Kirsch-Volders et al., 2002].

MI and polyploidy are endpoints that can be used to assess health hazards in populations living in remote areas because they are measured by standard cytogenetic techniques that are simple, not expensive, and can be carried out virtually anywhere. In the present study, data from these endpoints are consistent with a reduced biological impact on the study population due to a reduction in mercury exposure.

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