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**AVALIAÇÃO DOS EFEITOS CITOGENÉTICOS DA EXPOSIÇÃO
OCUPACIONAL A AGROTÓXICOS EM AGENTES DE SAÚDE
PÚBLICA VINCULADOS À PREFEITURA DE BELO HORIZONTE**

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Belo Horizonte-MG

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Aos meus pais com imenso amor.

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RESUMO

Agentes sanitários responsáveis pela aplicação de pesticidas para o controle de vetores de doenças constituem uma população ocupacionalmente exposta a genotóxicos potenciais. Sendo assim, o objetivo deste estudo foi determinar a relação entre a exposição ocupacional a pesticidas e a presença de danos citogenéticos. Foram selecionados 59 homens (29 agentes sanitários e 30 indivíduos controle) com idade entre 18-57 anos que viviam e trabalhavam na mesma região em Belo Horizonte (Brasil). Através do Teste do Micronúcleo (MN) em linfócitos periféricos, as frequências de micronúcleos (MN), células binucleadas micronucleadas (CBMN), pontes nucleoplasmáticas (PN), células apoptóticas (APOP), células necróticas (NECR) e índice de divisão nuclear (IDN) foram determinados. A análise de covariância (ANCOVA) revelou frequências médias significativamente maiores ($p < 0,05$) de MN ($15,81 \pm 1,31$ vs. $4,71 \pm 0,42$), CBMN ($15,10 \pm 1,22$ vs. $4,62 \pm 0,44$), PN ($4,59 \pm 0,76$ vs. $1,00 \pm 0,34$), NECR ($12,07 \pm 1,45$ vs. $5,17 \pm 0,70$) no grupo exposto, em relação aos indivíduos controle respectivamente. Não houve diferença significativa entre as frequências de APOP entre os grupos exposto e controle, enquanto o IDN foi significativamente menor ($p < 0,05$) nos expostos ($1,49 \pm 0,02$ vs. $1,61 \pm 0,02$). Houve relação direta da idade dos indivíduos e as frequências de MN e CBMN. Não foi observada influência do tempo de exposição ou dos hábitos de fumar e ingerir bebidas alcoólicas sobre os parâmetros citogenéticos analisados. De acordo com estes resultados, a exposição ocupacional à pesticidas mostrou efeito genotóxico e citotóxico nos linfócitos dos agentes sanitários.

INTRODUÇÃO

Atualmente, muitas ocupações levam os trabalhadores à exposição a substâncias químicas que muitas vezes são prejudiciais à saúde dos mesmos. Um exemplo são os agentes sanitários vinculados ao governo, responsáveis pela aplicação de pesticidas para o controle de doenças transmitidas por animais. O Serviço de Controle de Zoonoses da Secretaria Municipal de Belo Horizonte mantém, atualmente, um contingente de 1200 agentes sanitários. Como estes trabalhadores estão expostos a longas jornadas de trabalho com contato direto e/ou indireto com diversos compostos químicos, cujos efeitos crônicos são pouco conhecidos, constituem uma população de risco.

Um dos possíveis efeitos da exposição à pesticidas é a genotoxicidade, que representa um fator de risco primário para a carcinogênese. Tendo em vista que a manifestação clínica do câncer ocorre muito depois de suas causas estarem estabelecidas, é possível o monitoramento e intervenção de indivíduos que estejam com algumas destas causas estabelecidas, mas que ainda não tenham desenvolvido o tumor.

Desta forma, com o intuito de estimar o risco genético da exposição a pesticidas em agentes sanitários vinculados à Prefeitura de Belo Horizonte, este estudo de biomonitoramento genotoxicológico foi realizado. Já que não existem Comissões Internas de Prevenção de Acidentes (CIPA) na Prefeitura de Belo Horizonte, os resultados obtidos com o presente estudo poderão fornecer subsídios para implementação das medidas de proteção e promoção da saúde destes trabalhadores.

EVALUATION OF THE CYTOGENETIC EFFECTS OF OCUPATIONAL EXPOSURE TO PESTICIDES ON SANITARY WORKERS IN BELO HORIZONTE, BRAZIL

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ABSTRACT

Sanitary workers responsible for the application of pesticides in the control disease vectors constitute a population that is exposed to possible genotoxic substances while at work. Thus, the aim of this study was to determine the relation between the occupational exposure to these pesticides and the presence of cytogenetic damages. Fifty-nine men were selected (29 sanitary workers and 30 control individuals) with ages varying between 18-57 years who lived and worked at the same area in Belo Horizonte (Brazil). Through the cytokinesis-block micronucleus assay (CBMN) in peripheral blood lymphocytes the frequencies of micronuclei/1000 binucleated cells (MN/1000 BC), binucleated cells with micronuclei (BCMN)/1000 BC, nucleoplasmic bridges (NB)/1000 BC, apoptotic (APOP) and necrotic (NECR) cells/ 500 cells and nuclear division index (NDI) were determined for all individuals. The analysis of covariance (ANCOVA) showed significantly higher ($p < 0,05$) mean frequencies of MN ($15,81 \pm 1,31$ vs. $4,71 \pm 0,42$), BCMN ($15,10 \pm 1,22$ vs. $4,62 \pm 0,44$), NB ($4,59 \pm 0,76$ vs. $1,00 \pm 0,34$), NECR ($12,07 \pm 1,45$ vs. $5,17 \pm 0,70$) in the exposed group when compared to the control group. There was no significant difference in the APOP frequencies between both groups, while the NDI was significantly lower (1,49

$\pm 0,02$ vs. $1,61 \pm 0,02$) in the control group. Neither the time exposure nor the smoking or alcohol drinking habits influenced the cytogenetic parameters evaluated. According to these results, the occupational exposure to pesticides showed genotoxic and cytotoxic effects on sanitary workers.

Keywords: Pesticide exposure; Biomonitoring; Micronucleus test; Sanitary workers

1. INTRODUCTION

Pesticides compose a group of natural or synthetic chemical substances assigned to combat plagues that generally attack, harm or transmit illness to living organisms including humans [1]. Although they may be selective against specific organisms (as bacteria, fungi, undergrowth and rodent), most of them do not have an absolute selectivity, becoming a potential risk to the human health [2].

Many studies showed an association between the exposure to pesticides and the increase of the incidence of some cancers including the non-Hodgkin's lymphoma [3,4], the multiple myeloma [5], sarcomas [6,7], the pancreatic [8] and the bladder cancer [9]. This is why lots of scientists have evaluated the genetic risk associated with the exposure. The genotoxic effects of a pesticide is a primary factor for carcinogenesis, so the genotoxicologic biomonitoring will become useful in human populations exposed to it [2]. Meanwhile, the results of this kind of biomonitoring obtained until now are ambiguous [10-14], probably due to different conditions of the populations studied, the specific genotoxic effect of the different pesticides employed and due to interindividual variability [2].

In tropical countries illnesses such as dengue, malaria, yellow fever, leishmaniasis and leptospirosis, are health care problems once the ambient conditions favour the development and the proliferation of insects and other vectors [15]. The struggle to eliminate these animals is a task performed by the government at different levels, with the promotion of several control measures, including the application of pesticides in areas with high concentration of cases. The sanitary workers engaged in the application of pesticides are occupationally exposed to possible potential genotoxics, becoming a cancer risk population [2].

This work aimed to evaluate the genotoxic effect of the occupational exposure to pesticides on the population of sanitary workers of Belo Horizonte, Brazil. The micronucleus (MN) lymphocyte culture test was employed. It consists in a cytogenetic method that measures breaks (clastogenic effect) and chromosome losses (aneugenic effect) in binucleated cells [16]. Other cytogenetic parameters such as the nucleoplasmic bridges (NB), apoptotic (APOP) and necrotic (NECR) cells, and the nuclear division index (NDI) were analysed [17]. Factors such as age, smoking and alcohol drinking habits were taken into account because they may influence the expression of the evaluated cytogenetic parameters [18].

2. MATERIAL AND METHODS

2.1 Subjects

Fifty nine males between 18 and 57 years old agreed to take part in the research, between August and October 2004. All the participants lived and worked in Belo Horizonte (Brazil) and they did not present any serious morbidities at the time of the sample

collection. The exposed group was composed of 29 sanitary workers working for the City Hall of Belo Horizonte who were occupationally exposed to several pesticides (Table 1). The control group was selected from the population which worked next to the exposed individuals and was composed of 30 volunteers who had never been occupationally exposed to pesticides.

All the individuals answered a questionnaire, supplying information related to age, smoking and alcohol drinking habits and, in the case of the exposed group, the duration and the frequency of exposure and the personal protection equipment (PPE) employed. People were considered smokers when they usually smoked 15 or more cigarettes per day through at least one year and people who often drank any alcoholic beverage two or more times per week were considered alcohol drinkers. The main characteristics of both groups are shown in Table 2.

The project was approved by the Committee on Ethics and Research of the Federal University of Minas Gerais (ETIC 373/04) and all the subjects signed the agreement term.

2.2 Blood sample collection and cell culture

Ten millilitres of peripheral blood was obtained from each subject by vein puncture using heparinized vacutainers. Mononuclear cells were fractionated in a density gradient Histopaque[®] (Sigma) and added (10^6 cells/mL) to a RPMI 1640 (Gibco) medium supplemented with 10% (v/v) foetal bovine serum (Gibco), 2mmol/L of ℓ -glutamine (Gibco), antibiotics (penicillin, 100 U/mL, streptomycin, 100 μ g/mL and amphotericine B, 25 μ g/mL; Gibco) and 2% (v/v) of phytohaemagglutinin A (Gibco). The cultures were maintained at 37°C in a 5% CO₂ incubator. After 44 hours cytochalasin B was added to the culture (6 μ g/mL; Sigma) [17].

At the end of the cultures (72 hours) the cells were centrifuged at room temperature and gently dissolved in a cold methanol 70% (v/v): acetic acid (3:1) fixation solution. This procedure was performed twice. The cellular suspension was dropped in three previously identified clean slides. The slides were air dried and stained with Giemsa solution (4% (v/v); Gibco) in Dulbecco's Phosphate Buffered Saline pH 7,1 (Gibco) for 15 minutes.

2.3 Slides analysis

The slides were blindly analysed in an optic microscope with 1000X lens. For each individual, 500 lymphocytes were analysed in order to determine the apoptotic cells (APOP) frequency, the necrotic cells (NECR) frequency and the number of cells with one to four nuclei, employed in the calculus of nuclear division index (NDI). This index is determined by the formula $NDI = MC+2BC+3TC+4QC$ /total viable cells, where MC-QC represents the number of cells with one to four nuclei, respectively. The frequency of the micronuclei (MN), the micronucleated binucleated cells (BCMN) and the nucleoplasmic bridges (NB) were determined by counting 1000 binucleated viable cells (BC) with preserved cytoplasm. BC, MN, NB, APOP and NECR were determined in agreement with the previously described criteria [19].

2.4 Statistical analysis

In order to evaluate the possible differences between the control group and the exposed one in relation to age, an unpaired Student's *t* test between two means was performed. In relation to smoking and alcohol drinking habits, the possible differences between both groups were analysed by a Z test for two independent proportions. The effects of the exposure, smoking and alcohol drinking habits on the cytogenetic variables (MN,

BCMn, NB, APOP and NECR) and on the NDI were evaluated by the analysis of covariance (ANCOVA) including age and time of exposure as covariates. The statistic analysis were performed with the STATISTICA software (5.0 for Windows). Differences were considered statistically significant when p values were under 0.05.

3. RESULTS

The sanitary workers included in this study were exposed to several pesticides. As it is shown in Table 1, some of these pesticides are mutagenic and/or possibly carcinogenic composites and belong to the organophosphate and pyrethroid insecticides and hydroxycumarinic rodenticides groups. The application of the composites was performed by spraying (pyrethroids), powder, pelleting bait, paraffin bait (hydroxycumarinic and indandione) and ultra-low volume nebulization or sand mixed granulated (organophosphate). The composites were applied separately and the PPE used was specific for each composite, except for malathion and temephos whose application was performed without PPE. The majority of the pesticides are used sporadically. All the exposed individuals worked for 40 hours a week.

The main characteristics of the population are described on Table 2. The age and the alcohol drinking habit were similar in both groups. The exposed group had a larger number of smokers ($p < 0.05$, Z test). The average pesticide exposure time of the workers was 5.28 ± 0.60 years.

Table 3 summarizes mean values of cytogenetic variables and nuclear division indexes studied in the groups. The ANCOVA results and the regression coefficients are

presented in Table 4 and 5 respectively. The exposed group showed MN frequencies, BCMN, NB and NECR significantly higher than the control group ($p < 0.01$) (Table 4). The Figure 1 shows the MN frequencies in both groups. Although the APOP frequency difference was not significant, it was higher in the exposed group (Table 4). The exposed group had significantly lower NDI values ($p < 0.01$) (Table 4). The regression coefficients (Table 5) indicated that, from the covariates introduced in the analysis, only the age of the individuals had a significant influence over the MN and BCMN frequencies ($p < 0.01$). The pesticides exposure time did not have an influence over the parameters analysed (Table 5). Neither the smoking habits nor the alcohol drinking ones influenced the analysed cytogenetic variables (Table 4).

4. DISCUSSION

With the increase of the global population, new areas are being rough-hewed and the humans are more exposed to illnesses transmitted by wild vectors [15]. The control of the increase of infected transmitter animals becomes a central issue in the sanitary surveillance kept by governments. The use of pesticides has become routine, mainly in underdeveloped countries, but the genotoxic potential of these substances is yet unknown [2]. Most of the population that lives in the affected areas and the sanitary workers responsible for the application are at cytotoxic and genotoxic risks.

The aim of this study was to evaluate if the occupational exposure to pesticides might cause cytogenetic damage compared to a control group that had never been exposed to these chemicals. Fifty-nine males (29 occupationally exposed and 30 control subjects) were included in the study. The age of the subjects and alcohol drinking habits were similar

in both groups, but there was a larger number of smokers in the exposed group. In spite of that, this difference did not influence the results, since through the analysis of covariance the smoking habit did not have a significant effect on the cytogenetic parameters evaluated.

To evaluate the genetic damages that took place in these subjects the cytokinesis-block micronucleus assay in human lymphocyte cultures (CBMN assay) was used [20,21]. Through this assay the clastogenic and aneugenic effects were detected since the MN are originated from chromosomal fragments or from an entire chromosome non-included in the main nucleus of the descent cell during cellular division [21,22]. Recently, it was proposed the inclusion of other cytogenetic parameters in the CBMN assay [23]. These parameters are the presence of nucleoplasmic bridges (indicators of chromosomic rearrangement), apoptotic and necrotic cells (indicators of cellular viability), and cellular division index [24,25]. Because this test offers simultaneous information on DNA damage and cytotoxic/cytostatic effects caused by possible aggressive agents, nowadays it is a simple and important tool for the monitoring of human population [25].

In this study, the group exposed to pesticides showed a significant higher frequency of chromosome damage (MN, BCMN and NB) compared with the control group. Some studies also showed a positive association between the genotoxicity and the occupational exposure to pesticides [12,26-33], although other studies did not conclude the same [10,13,14,34,39]. Such disagreement may be explained both by different exposure conditions (protection measure used and specific genotoxic potential of the substances used) or by demographic factors and individual habits and genetic features associated [2,18]. By this way each biomonitoring study is unique and, in order to estimate the effects of an occupational exposition, each population should be studied separately and the results should not be generalised.

The presence of chromosome damage in the exposed group can be explained by the genotoxic power of the substance they were exposed to. The pyrethroid group is the most frequently used in solution through spraying. When applying it the workers make use of PPE. Of the pyrethroid used by the workers the U. S. Environmental Protection Agency (EPA) classified only cypermethrin as a possible carcinogen, while the other **pyrethroids** do not have mutagenic and/or carcinogenic activity [40]. This reduces the possibility of the remaining pyrethroids (except cypermethrin) of being responsible for the damage detected. The organophosphate group is applied daily in the solid form without using PPE. In agreement with the EPA classification [40], malathion showed mutagenic activity in the experimental system, but carcinogenic activity was not observed. Other substances (hidroxicumarinic and indandione) are sporadically applied with the use of PPE. So far, most of them haven't been tested for their mutagenic and carcinogenic effects, and so it is not possible to say if the exposure to this substances could be considered safe. This suggests that malathion (organophosphate) and cypermethrin (pyrethroid) should be the main pesticides responsible for the chromosome damage found in the exposed workers. However, it is important to notice that an exposure to a great number of different compounds makes it difficult to know which agent could be responsible for the observed cytogenetic damages.

Another aspect that could contribute to the positive association between the observed cytogenetic damage and pesticide exposure is that most of the biomonitoring studies in occupational exposed populations are done with individuals that used a mix of several chemical compounds [14,38,39,41-43]. In this study, the pesticides are applied separately and probably in higher concentrations than those found in the mixtures and these concentrations may present a higher genotoxic potential.

In spite of the use of the protection equipment in the application by of most products, it was observed an induction of chromosome damage caused by the pesticide exposure. These findings can be explained by the ineffectiveness or inappropriate use of the protection measures. Another explanation could be the fact that malathion organophosphate may be the main responsible substance for the chromosome damage found, because it was applied without PPE.

The cytotoxicity was higher in the sanitary workers than in the control group, since the frequency of necrotic cells was significantly higher in the exposed group. One possibility is the existence of two substance groups: a genotoxic group that causes chromosome damage without killing the cells and a cytotoxic group that causes cell necrosis. The fact is that the surviving cells are perpetuating important mutations proved by the increased frequencies of MN, BCMN and NB.

There was not a significant difference in the APOP frequencies between the exposed and the control groups. This suggests that the damages to DNA caused by the exposure were not sufficient to cause apoptosis, but they caused MN and NB [24].

The reduction in the NDI found in the exposed group, according to other studies [39,41], corroborates the hypothesis that cells with DNA damage delay the cell cycle in order to repair the damage and avoid the fixation of mutations during replication [25]. However, the fact that MN, BCMN and NB frequencies having been higher in the exposed group, show that the repair may not be efficient to correct the induced mutations caused by exposure. Another hypothesis is that the chemicals have cytotoxic properties that affect the cell proliferation kinetics [35,44].

Of the analysed variables that could influence the cytogenetic parameters evaluated, only the age was positively related to MN and BCMN frequencies. These results agree with

many other studies that showed an increase of spontaneous MN frequency with the age [18,45-47]. This effect has been attributed to an increase of aneuploidy mainly of the X and Y chromosomes [48].

It was not observed an association between the cytogenetic parameters frequency and the time of exposure of the workers to the pesticides. This observation differs from the results found by other authors. Bolognesi (2002) [42] found a positive relation between the MN incidence and the pesticide exposure duration when individuals were exposed for more than 10 years, suggesting that chromosome damage is accumulated during continued exposure to pesticides. The lack of association between the cytogenetic parameter evaluated and time of exposure in the present study could be explained by the slight variation in the exposure time. In this study, exposure time was from 1,5 to 18 years and only one individual was exposed for more than 10 years.

The smoking habit did not influence the cytogenetic parameters evaluated. In the biomonitoring studies of populations occupationally exposed to genotoxic agents, the smoking habit influence on the MN frequency is controversial. Few studies showed an association between those variables [49,50], while most of the studies did not find any association at all [14,38,39,41,42,51]. A possible explanation is that the damage caused by tobacco could kill the cells in culture or delay the cell cycle making it impossible the analysis of the MN [52]. However, it was not found an influence of smoking habit on APOP/NECR frequencies and neither on NDI. Another possibility is that the number of smokers in the control group was small (6 individuals) and could constitute a non-representative sample.

Like the smoking habit, the alcohol drinking habit did not influence the parameters evaluated. Data in literature showed positive, preventive or no effects [47,49,53,54] of

alcohol drinking habit on MN frequency. Normally these effects happen as a consequence of the relation between alcohol drinking habit and other variables, such as age [38].

Concluding, the occupational exposure to pesticides by sanitary workers in Belo Horizonte showed a genotoxic and cytotoxic effects, measured by higher frequencies of MN, BCMN, NB and necrotic cells when compared with the control group. The reduced NDI in the exposed group suggests a possible adaptive response to a chronic exposure to pesticides. The age factor presented a direct relation with the MN frequency while the smoking and drinking habits did not influence the cytogenetic parameters evaluated.

These results evidence a genetic hazard related to occupational pesticide exposure and therefore the need for educational programs to stimulate the correct use of the PPE and/or implement new protection measures for the sanitary workers.

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Table 1

Pesticides used by the sanitary workers, with indication of their frequency of use, mutagenicity (M) and carcinogenicity (C) experimental data^a, personal protection equipment (PPE) used and application way.

Type	Product	Group	Freq. use	CAS #	M	C	PPE	Application
I	α -cypermethrin	PYR	Dly	69865-74-0	-	Pos.	Mask, gloves and overalls	Spraying
I	cypermethrin	PYR	Dly	52315-07-8	-	Pos.	Mask, gloves and overalls	Spraying
I	deltamethrin	PYR	Dly	52918-63-5	-	-	Mask, gloves and overalls	Spraying
I	temephos	OP	Dly	3383-96-8	-	-	No protection	Sand mixed granulated
I	malathion	OP	Dly	121-75-5	+	-	No protection	Sand mixed granulated
I	fenithrothion	OP	Ep	122-14-5	-	-	Impermeable overalls, mask and gloves	Ultra- low nebulization
R	brodifacum	HC	Sly	56073-10-0	NA	NA	Rubber gloves and surgical mask	Powder, pelleting bait, paraffine bait
R	coumachlor	HC	Sly	81-82-3	NA	NA	Rubber gloves and surgical mask	Powder, pelleting bait, paraffine bait
R	coumafuryl	HC	Sly	117-52-2	NA	NA	Rubber gloves and surgical mask	Powder, pelleting bait, paraffine bait
R	coumatetralyl	HC	Sly	5836-29-3	NA	NA	Rubber gloves and surgical mask	Powder, pelleting bait, paraffine bait
R	difethialone	HC	Sly	104653-34-1	NA	NA	Rubber gloves and surgical mask	Powder, pelleting bait, paraffine bait
R	flocoumafen	HC	Sly	90035-08-8	-	-	Rubber gloves and surgical mask	Powder, pelleting bait, paraffine bait
R	difenacoum	HC	Sly	56073-07-5	NA	NA	Rubber gloves and surgical mask	Powder, pelleting bait, paraffine bait
R	bromadiolone	HC	Sly	28772-56-7	-	NA	Rubber gloves and surgical mask	Powder, pelleting bait, paraffine bait
R	diphacinone	IND	Sly	82-66-6	NA	NA	Rubber gloves and surgical mask	Powder, pelleting bait, paraffine bait
R	pindone	IND	Sly	83-26-1	NA	NA	Rubber gloves and surgical mask	Powder, pelleting bait, paraffine bait

I, insecticide; R, rodenticide; PYR, pyrethroid; OP, organophosphorate; HC, hidroxicumarinic; IND, indandione. Dly, daily; Ep, during epidemics; Sly, sporadically.

^a Environmental Protection Agency (EPA) classification: -, no observed effect; +, positive effect; Pos., possibly carcinogenic; NA, not available.

Table 2
 Characteristics of the groups studied

Characteristics	Control	Exposed
Number of subjects	30	29
Age (years) (mean \pm SE)	29.17 \pm 1.64	30.31 \pm 1.48 ^a
Range (years)	18-49	21-57
Smoking habit		
Smokers (n, %)	6 (20.00)	14 (48.28) ^b
Non-smokers (n, %)	24 (80.00)	15 (51.72)
Drinking habit		
Yes (n, %)	19 (63.33)	15 (51.72) ^c
No (n, %)	11 (36.67)	14 (48.28)
Years of pesticide exposure (mean \pm SE)	-	5.28 \pm 0.60
Range (years)		1.50-18

^a No statistical difference between both groups (*t*-test).

^b $p < 0,05$ (*Z*-test), compared with control group.

^c No statistical difference between both groups (*Z*-test).

Table 3
 Mean values (\pm SE) of the cytogenetic variables analysed in the groups studied

Variables	Control	Exposed
MN	4.71 \pm 0.42	15.81 \pm 1.31
BCMNI	4.62 \pm 0.44	15.10 \pm 1.22
NB	1.00 \pm 0.34	4.59 \pm 0.76
APOP	11.81 \pm 1.20	18.40 \pm 2.60
NECR	5.17 \pm 0.70	12.07 \pm 1.45
NDI	1.61 \pm 0.02	1.49 \pm 0.02

MN, micronuclei in 1000 binucleated cells; BCMNI, binucleated cells with micronuclei in 1000 binucleated cells; NB, nucleoplasmic bridges in 1000 binucleated cells; APOP, apoptotic cells in 500 viable cells; NECR, necrotic cells in 500 viable cells; NDI, nuclear division index.

Table 4
Summary of the effects for each cytogenetic variable analysed (ANCOVA)

Variables	MS Effect	MS Error	F (<i>df</i> 1.0)	<i>p</i>
MN				
Exposure	615.38	17.19	35.81	<0.01
Smoking habit	58.94	17.19	3.43	0.07
Drinking habit	0.00	17.19	0.00	0.99
BCMNI				
Exposure	513.52	16.30	31.50	<0.01
Smoking habit	47.33	16.30	2.90	0.09
Drinking habit	0.03	16.30	0.00	0.96
NB				
Exposure	30.20	9.82	3.08	0.04
Smoking habit	4.74	9.82	0.48	0.49
Drinking habit	1.96	9.82	0.20	0.66
APOP				
Exposure	187.65	113.31	1.66	0.20
Smoking habit	0.01	113.31	0.00	0.99
Drinking habit	0.55	113.31	0.00	0.94
NECR				
Exposure	348.12	33.33	10.44	<0.01
Smoking habit	109.88	33.33	3.30	0.07
Drinking habit	6.12	33.33	0.18	0.67
NDI				
Exposure	0.11	0.01	8.89	<0.01
Smoking habit	0.00	0.01	0.11	0.74
Drinking habit	0.04	0.01	3.18	0.08

MN, micronuclei; BCMNI, binucleated cells with micronuclei; NB, nucleoplasmic bridges; APOP, apoptotic cells; NECR, necrotic cells; NDI, nuclear division index.

Table 5
Regression coefficients for the covariates introduced in the ANCOVA analysis

Covariates	$\beta \pm SE$	<i>t</i>	<i>p</i>
MN			
Age	0.58 ± 0.08	4.86	<0.01
Years of exposure	-0.19 ± 0.26	-1.58	0.12
BCMNB			
Age	0.57 ± 0.07	4.77	<0.01
Years of exposure	-0.15 ± 0.26	-1.27	0.21
NB			
Age	-0.04 ± 0.06	-0.28	0.78
Years of exposure	0.18 ± 0.20	1.29	0.20
APOP			
Age	-0.23 ± 0.20	-1.61	0.11
Years of exposure	-0.07 ± 0.68	-0.51	0.61
NECR			
Age	-0.06 ± 0.11	-0.40	0.69
Years of exposure	-0.25 ± 0.37	-1.75	0.09
NDI			
Age	0.22 ± 0.00	1.54	0.13
Years of exposure	0.06 ± 0.00	0.45	0.65

MN, micronuclei; BCMNB, binucleated cells with micronuclei; NB, nucleoplasmic bridges; APOP, apoptotic cells; NECR, necrotic cells; NDI, nuclear division index.

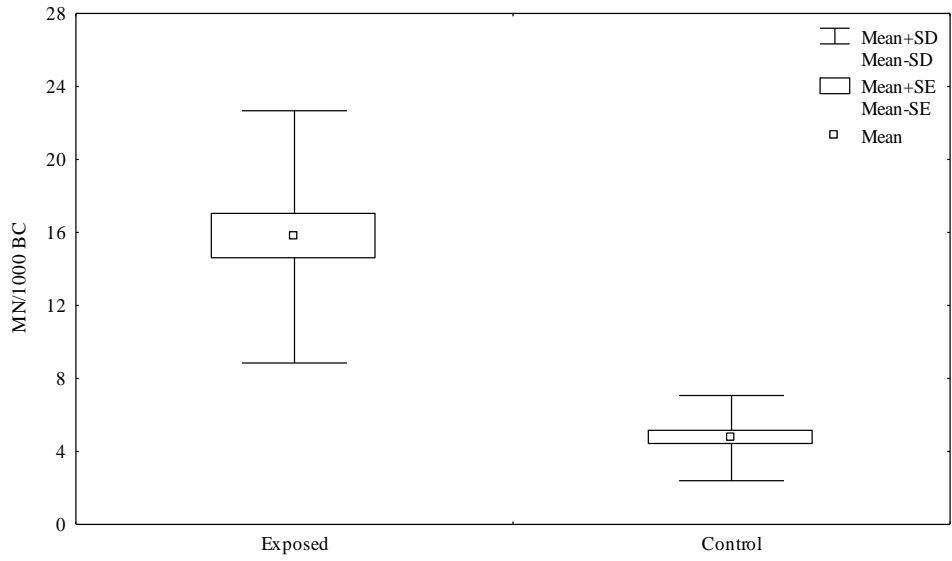


Fig. 1. Micronuclei frequencies in 1000 binucleated cells (MN/1000 BC) in exposed and control groups.

ANEXOS

Figuras

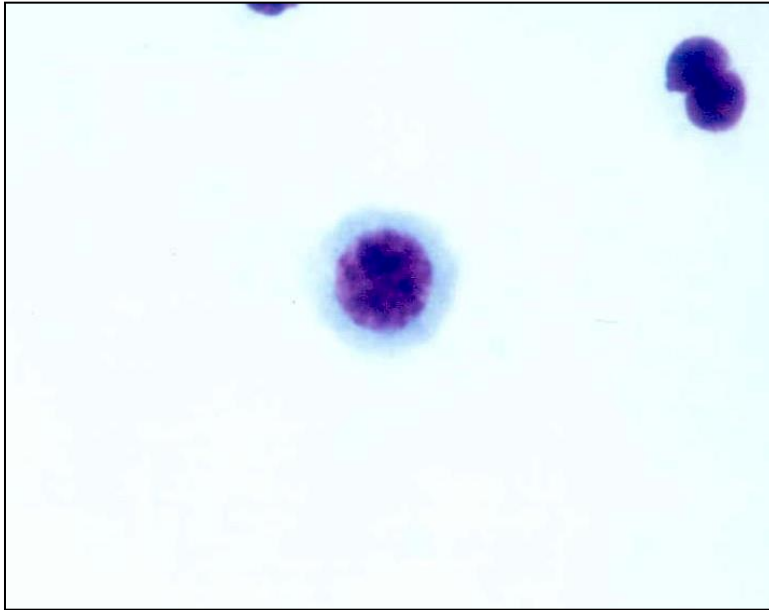


Figura 1 - Fotomicrografia de linfócito mononucleado típico corado por Giemsa (observado sob aumento de 1000 X).

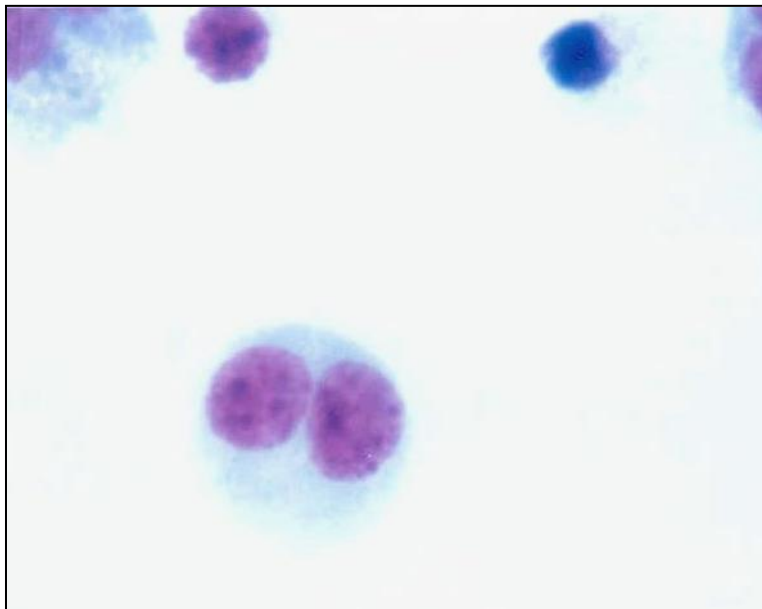


Figura 2 – Fotomicrografia de linfócito binucleado corado por Giemsa (observado sob aumento de 1000 X).

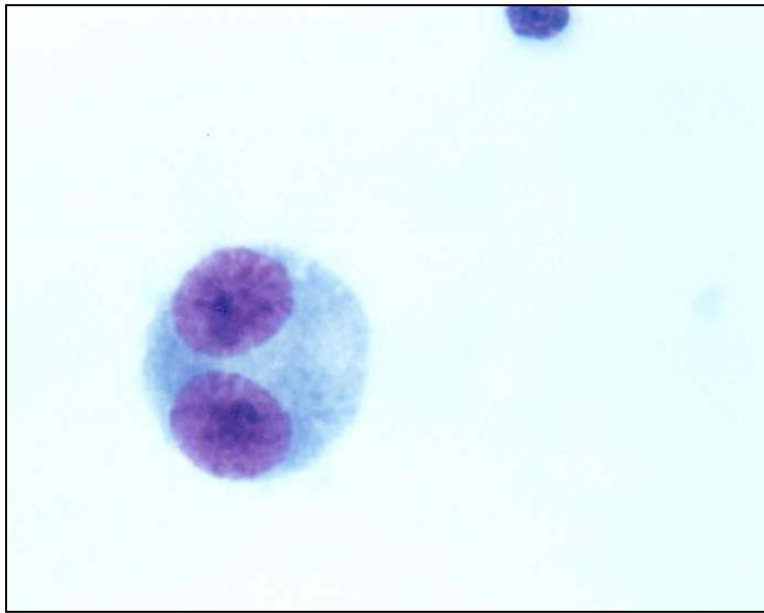


Figura 3 - Fotomicrografia de linfócito binucleado corado por Giemsa (observado sob aumento de 1000 X).

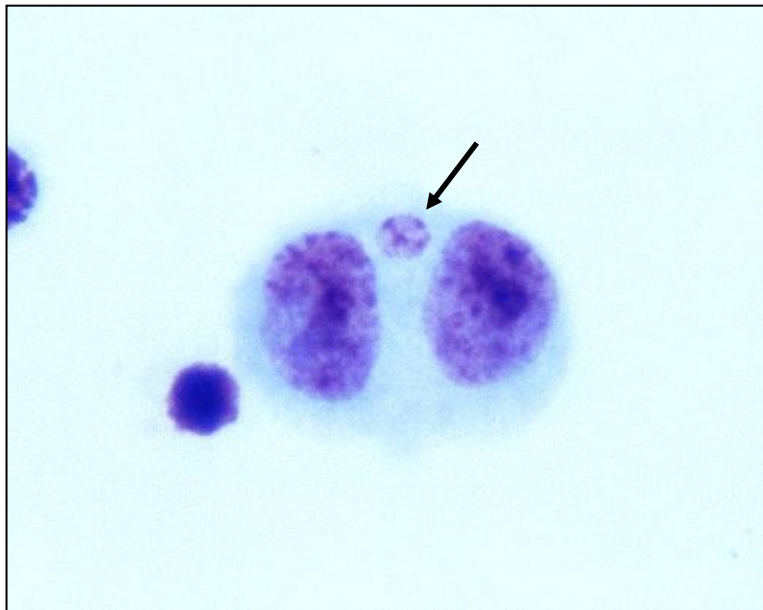


Figura 4 – Fotomicrografia de linfócito binucleado (BC) contendo micronúcleo (MN) corado por Giemsa (observado sob aumento de 1000 X). A seta indica o micronúcleo.

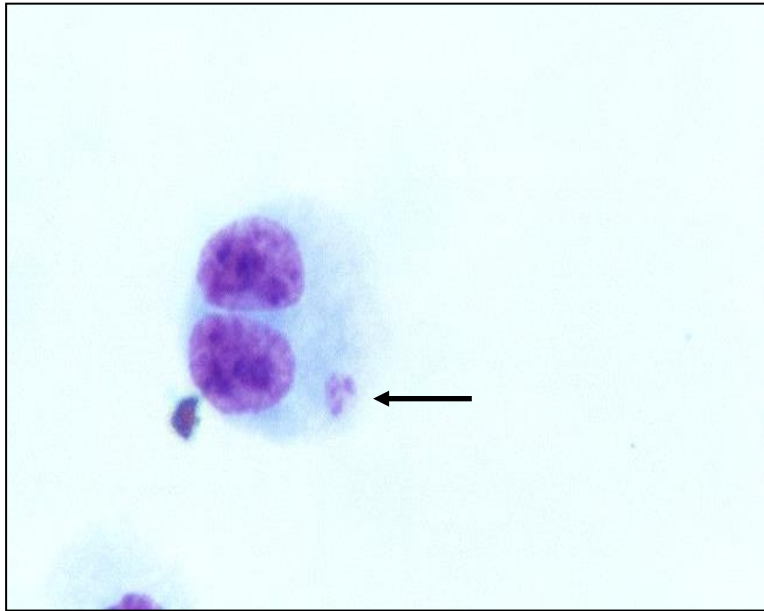


Figura 5 - Fotomicrografia de linfócito binucleado (BC) contendo micronúcleo (MN) corado por Giemsa (observado sob aumento de 1000 X). A seta indica o micronúcleo.

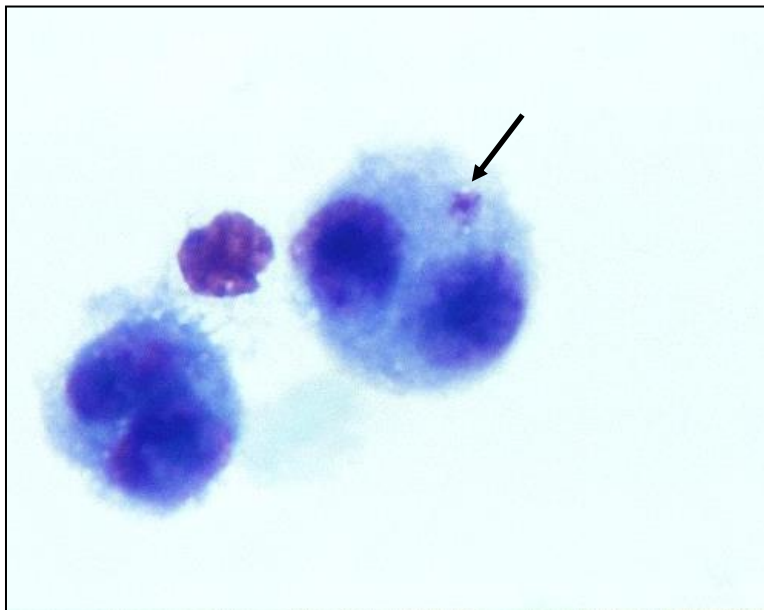


Figura 6 - Fotomicrografia de célula (linfócito) binucleada (BC) contendo micronúcleo (MN) corado por Giemsa (observado sob aumento de 1000 X). A seta indica o micronúcleo.

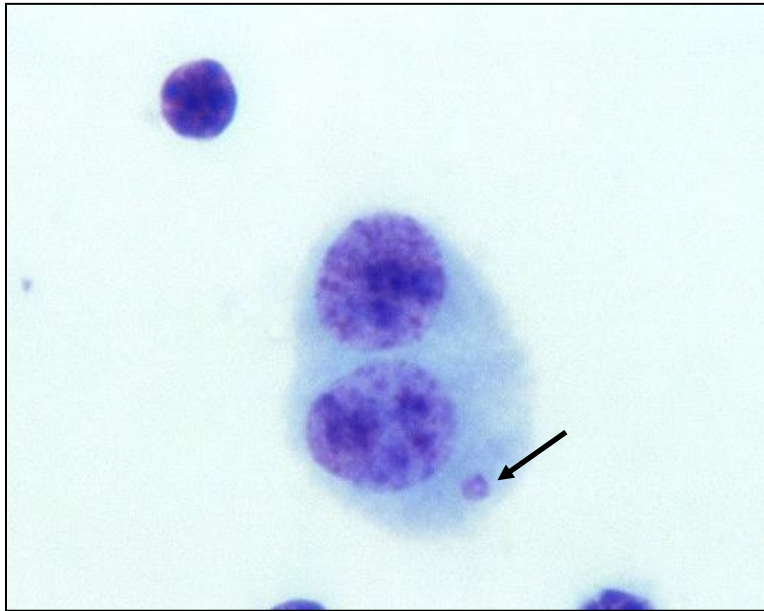


Figura 7 - Fotomicrografia de linfócito binucleado (BC) contendo micronúcleo (MN) corado por Giemsa (observado sob aumento de 1000 X). A seta indica o micronúcleo.

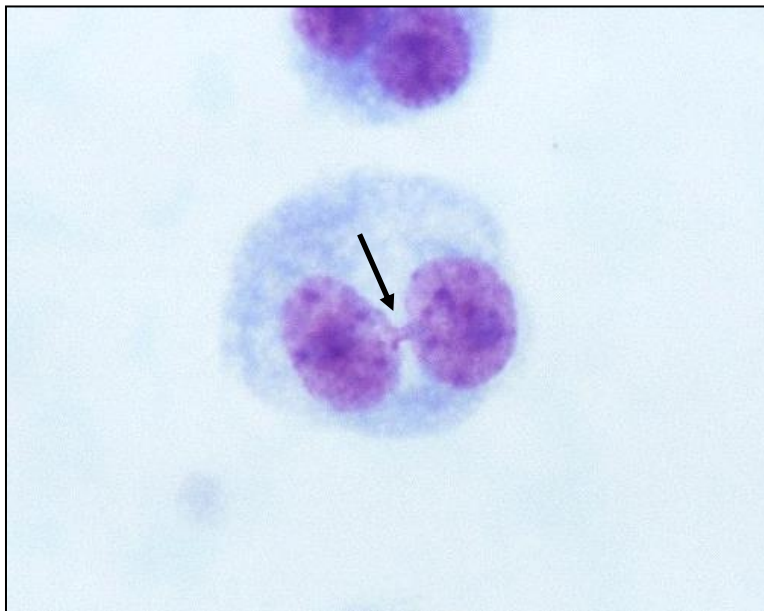


Figura 8 - Fotomicrografia de linfócito binucleado (BC) contendo ponte nucleoplasmática (NB) corado por Giemsa (observado sob aumento de 1000 X). A seta indica a ponte nucleoplasmática.

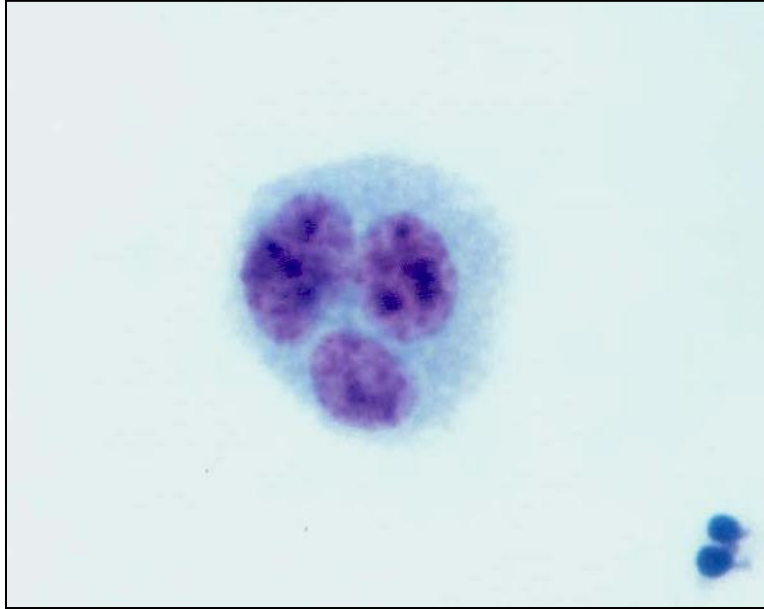


Figura 9 - Fotomicrografia de linfócito trinucleado (TC) típico corado por Giemsa (observado sob aumento de 1000 X).

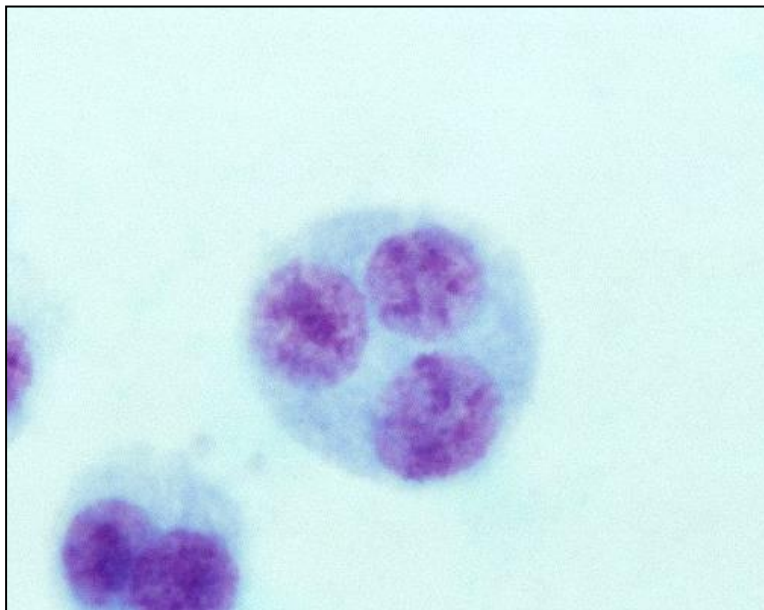


Figura 10 - Fotomicrografia de linfócito trinucleado (TC) corado por Giemsa (observado sob aumento de 1000 X).

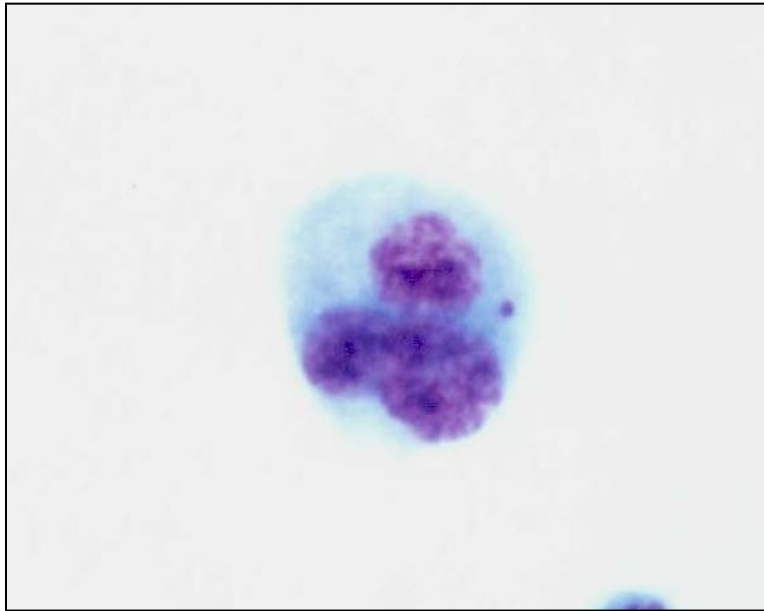


Figura 11 Fotomicrografia de linfócito trinucleado (TC) contendo micronúcleo (MN) corado por Giemsa (observado sob aumento de 1000 X).



Figura 12 - Fotomicrografia de linfócito tetranucleado (QC) típico corado por Giemsa (observado sob aumento de 1000 X).



Figura 13 - Fotomicrografia de linfócito tetranucleado (QC) contendo micronúcleo (MN) corado por Giemsa (observado sob aumento de 1000 X).



Figura 14 - Fotomicrografia de linfócito em apoptose corado por Giemsa (observado sob aumento de 1000 X).

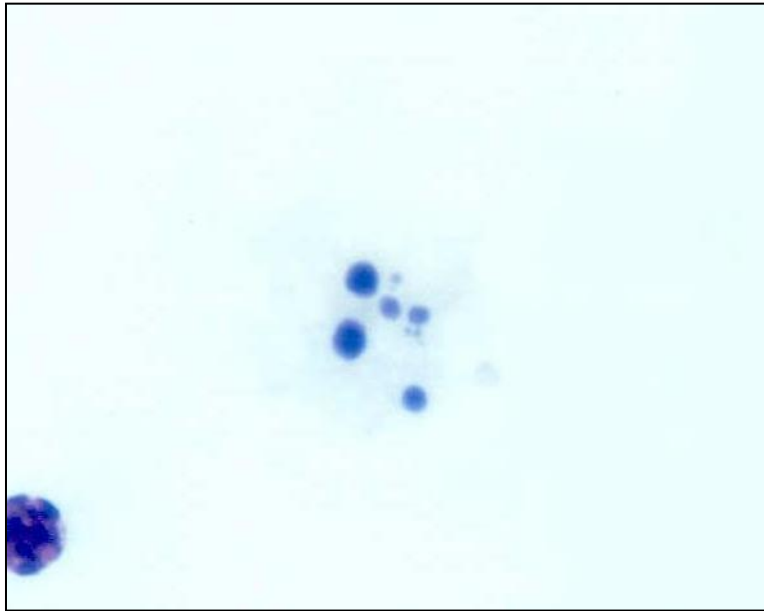


Figura 15 - Fotomicrografia de linfócito em apoptose corado por Giemsa (observado sob aumento de 1000 X).

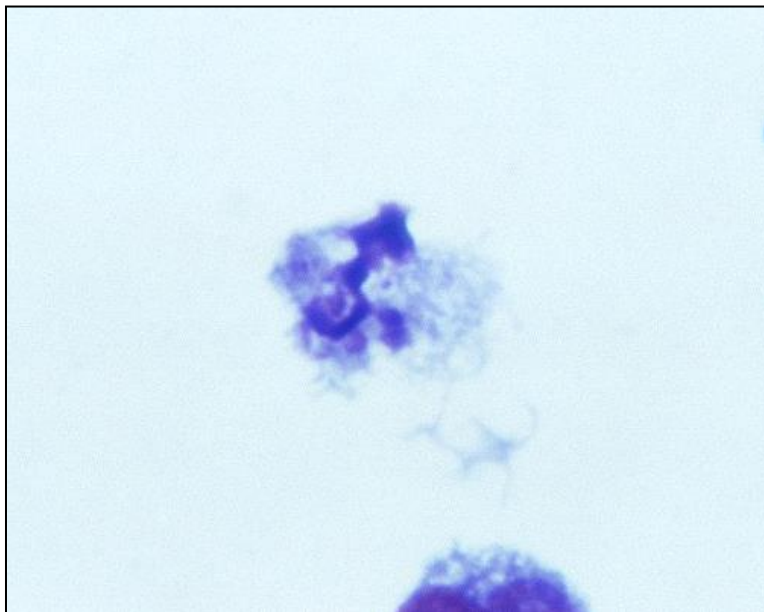


Figura 16 - Fotomicrografia de linfócito em necrose corado por Giemsa (observado sob aumento de 1000 X).

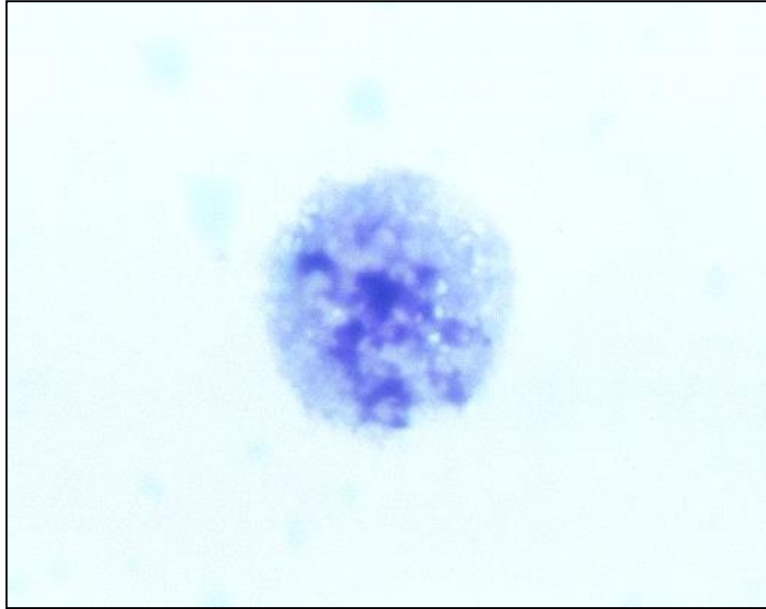


Figura 17 - Fotomicrografia de linfócito em necrose corado por Giemsa (observado sob aumento de 1000 X).

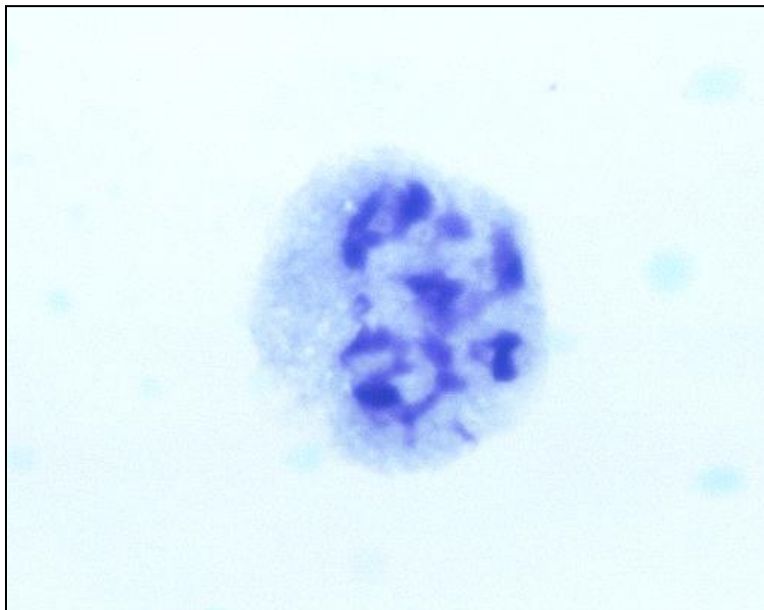


Figura 18 - Fotomicrografia de linfócito em necrose corado por Giemsa (observado sob aumento de 1000 X).



Termo de Consentimento
UNIVERSIDADE FEDERAL DE MINAS GERAIS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS

TERMO DE CONSENTIMENTO

**Aos funcionários da FUNASA e do Centro de Controle de Zoonoses da Regional
Venda Nova da Prefeitura Municipal de Belo Horizonte**

O projeto “Avaliação dos efeitos genotóxicos da exposição ocupacional a pesticidas sobre agentes sanitários da Fundação Nacional de Saúde do Estado de Minas Gerais e do Centro de Controle de Zoonoses da Regional Venda Nova da Prefeitura Municipal de Belo Horizonte” tem como objetivo avaliar os efeitos prejudiciais da exposição ocupacional a pesticidas em agentes sanitários vinculados a FUNASA e ao Centro de Controle de Zoonoses da Regional Venda Nova da Prefeitura de Belo Horizonte. Esta avaliação será feita através da análise da frequência de danos genéticos em células de sangue destes trabalhadores. Nossos esforços se concentram no sentido de fornecer aos serviços de vigilância a saúde subsídios para implementação das medidas de proteção a estes trabalhadores.

Para a realização deste estudo contamos com a colaboração de funcionários da FUNASA e do Centro de Controle de Zoonoses que têm ou tiveram exposição ocupacional a pesticidas. Para as análises a serem realizadas, serão coletados 10 ml de sangue dos colaboradores com seringa descartável e estéril, **NÃO ACARRETANDO RISCOS À SAÚDE DO COLABORADOR.**

O material coletado será transportado pelos responsáveis pela pesquisa para o Laboratório de Genética de Neoplasias e Mutagênese do Departamento de Biologia Geral do Instituto de Ciências Biológicas da UFMG, onde será efetuada a manipulação do mesmo, sob responsabilidade da Prof^a Maria Cristina Lima de Castro.

Lembramos ainda que os colaboradores não terão suas identidades reveladas e que todas as informações fornecidas e os resultados obtidos serão mantidos em absoluto sigilo, sendo utilizados apenas para divulgação em reuniões e revistas científicas.

As pessoas que concordarem em colaborar com a pesquisa deverão assinar este termo de consentimento e fornecer, através de um questionário a ser aplicado, algumas informações a respeito de seu trabalho e de alguns hábitos.

Maria Cristina Lima de Castro (orientadora)

Comitê de Ética em Pesquisa da UFMG

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TERMO DE CONSENTIMENTO
Aos colaboradores voluntários do projeto

O projeto “Avaliação dos efeitos genotóxicos da exposição ocupacional a pesticidas sobre agentes sanitários da Fundação Nacional de Saúde do Estado de Minas Gerais e do Centro de Controle de Zoonoses da Regional Venda Nova da Prefeitura Municipal de Belo Horizonte” tem como objetivo avaliar os efeitos prejudiciais da exposição ocupacional a pesticidas em agentes sanitários vinculados a FUNASA e ao Centro de Controle de Zoonoses da Regional Venda Nova da Prefeitura de Belo Horizonte. Esta avaliação será feita através da análise da frequência de danos genéticos em células de sangue destes trabalhadores. Nossos esforços se concentram no sentido de fornecer aos serviços de vigilância a saúde subsídios para implementação das medidas de proteção a estes trabalhadores.

No entanto, para a realização deste estudo precisamos, além da colaboração dos agentes sanitários, da colaboração de doadores voluntários sem história de exposição a pesticidas para constituírem o grupo controle. Os danos genéticos encontrados no grupo controle servirão como referência para serem comparados com os encontrados nos agentes sanitários ocupacionalmente expostos a pesticidas.

Para as análises a serem realizadas, serão coletados 10 ml de sangue dos colaboradores com seringa descartável e estéril, **NÃO ACARRETANDO RISCOS À SAÚDE DO COLABORADOR.**

O material coletado será transportado pelos responsáveis pela pesquisa para o Laboratório de Genética de Neoplasias e Mutagênese do Departamento de Biologia Geral do Instituto de Ciências Biológicas da UFMG, onde será efetuada a manipulação do mesmo, sob responsabilidade da Prof^a Maria Cristina Lima de Castro.

Lembramos ainda que os colaboradores não terão suas identidades reveladas e que todas as informações fornecidas e os resultados obtidos serão mantidos em absoluto sigilo, sendo utilizados apenas para divulgação em reuniões e revistas científicas.

As pessoas que concordarem em colaborar com a pesquisa deverão assinar este termo de consentimento e fornecer, através de um questionário a ser aplicado, algumas informações a respeito de seu trabalho e de alguns hábitos.

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DECLARAÇÃO DE CONCORDÂNCIA

Eu, _____,
nascido em _____ de _____ de _____
documento (tipo e número) _____
endereço _____

concordo em participar da pesquisa de “Avaliação dos efeitos genotóxicos da exposição ocupacional a pesticidas sobre agentes sanitários da Fundação Nacional de Saúde do Estado de Minas Gerais e do Centro de Controle de Zoonoses da Regional Venda Nova da Prefeitura Municipal de Belo Horizonte” desenvolvida na Universidade Federal de Minas Gerais. Para isto concordo, sem nenhum pagamento, em doar uma amostra de 10 ml de sangue periférico e autorizo o uso deste material para trabalhos científicos.

Belo Horizonte _____ de _____ de _____.

Assinatura do participante

Maria Cristina Lima de Castro (pesquisador responsável)

Testemunha

Questionário



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QUESTIONÁRIO

Data: ____/____/____

Caso n° ____

Idade ____ anos

1) Exposição ocupacional a pesticidas?

Não

Sim

A quais pesticidas está ocupacionalmente exposto?

Há quanto tempo exerce esta função?

_____ anos

Com que frequência está ocupacionalmente exposto?

mais de 1 vez por semana (_____ dias por semana)

1 vez por semana

de 15 em 15 dias

1 vez por mês

Equipamentos de proteção individual utilizados:

2) Hábito de fumar cigarro industrializado?

Não

Fumou mas parou

Parou há quanto tempo? _____ meses _____ anos



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Fumou durante quanto tempo? _____ meses _____ anos

Número de cigarros consumidos por dia:

- de 1 a 5 por dia
- de 6 a 10 por dia
- de 11 a 15 por dia
- de 16 a 20 por dia
- de 21 a 30 por dia
- mais de 30 por dia

Sim

Há quanto tempo? _____ meses _____ anos

Número de cigarros consumidos por dia?

- de 1 a 5 por dia
- de 6 a 10 por dia
- de 11 a 15 por dia
- de 16 a 20 por dia
- de 21 a 30 por dia
- mais de 30 por dia

3) Hábito de ingerir bebidas alcoólicas?

Não

Parou de beber

Há quanto tempo? _____ meses _____ anos

Com que frequência bebia?

- diariamente
- 4 a 6 vezes por semana
- 3 a 5 vezes por semana
- 1 a 2 vezes por semana
- 2 a 3 vezes por mês
- 1 vez por mês
- menos de 1 vez por mês



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Sim

Há quanto tempo? _____ meses _____ anos

Com que frequência bebe?

- diariamente
- 4 a 6 vezes por semana
- 3 a 5 vezes por semana
- 1 a 2 vezes por semana
- 2 a 3 vezes por mês
- 1 vez por mês
- menos de 1 vez por mês