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Original Article

Micronuclei in the exfoliated oral epithelial cell: a cross-sectional study in Peruvian artisanal miners

Pizarro-Rojas B[1], Rabanal-Sanchez J[2], Soncco-Llulluy F[3], Rosales-Rimache J[4]

[1]Facultad de Medicina, Universidad Alas Peruanas, Ica 11001, Peru
[2]Escuela de Medicina, Universidad Cesar Vallejo. Trujillo, La Libertad, Peru
[3]Carrera de Medicina Humana, Universidad Científica del Sur. Lima, Peru
[4]Centro de Investigación en Biodiversidad para la Salud, Universidad Privada Norbert Wiener, Lima 15046, Peru

Corresponding author:

Dr. Jaime Rosales-Rimache
Universidad Privada Norbert Wiener, Lima 15046, Peru
Tel.: +51 1 944-457-898,
E-mail:jaime.rosalesr@uwiener.edu.pe
ORCID ID:https://orcid.org/0000-0002-1665-2332

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ABSTRACT

Introduction: Artisanal mining in Peru is an activity that generates a risk of exposure to different compounds, among which mercury and particulate matter stand out. The use of laboratory indicators to assess genotoxicity induced by work activity is a priority need. Our objective was to determine the micronucleus count in buccal cells of artisanal miners in Peru during 2020.

Methods: We designed a cross-sectional study where 80 artisanal miners were evaluated who underwent scraping in the inner area of the cheek to obtain epithelial cells that were stained with Feulgen's staining, and micronuclei and nuclear alterations were identified on a count of 2000 cells.

Results: We found that the mean micronucleus count was 27.5±8.0 (CI95: 25.7 – 29.3, min. 15, max. 48). For the other nuclear alterations such as nucleoplasmic bridges, budding, and binucleation, only up to one alteration was evidenced for each total count. The number of years of work (p=0.004) and age (p<0.001) were the only variables associated with the micronucleus count.

Conclusion: The nuclear alterations in buccal cells of artisanal miners exposed to particulate material were micronuclei, nucleoplasmic bridges, budding, and binucleations, the most frequent being the presence of micronuclei, with a mean value of 27.5 micronuclei per 2000 cells counted.

Keywords: Artisanal mining; Micronuclei; Nuclear alteration

Introduction

Artisanal mining is an activity that is associated with a high rate of occupational mortality and morbidity throughout the world. The most frequent pathological condition associated with it is cancer, with a pathophysiological basis related to DNA damage.[1] According to the reports of the Global Cancer Observatory (GLOBOCAN), it is estimated that 18.1 million new cases occur each year, of which close to 60% are attributed to working conditions, among which mining stands out.[2]

Artisanal mining is an economic activity that involves more than 70,000 miners in Peru; 400,000 people and 40,000 Peruvian families depend on it, who have found an alternative to combat unemployment with little investment, simple technology, and intensive work. For this work, those deposits that, for conventional mining, have ceased to be attractive are used.[3]

Artisanal mining involves using instruments designed to undermine the earth's surface and extract the mineral. This process generates the resuspension of dust in the air, mainly inhalable particulate matter (PM), which generates direct

exposure to the worker. Inhalable PM is the most widely studied pollutant in the world, and it is found in the atmosphere, causing various problems in vegetation and humans.[4,5]

Artisanal miners are a high-risk group due to PM's short- and long-term effects on health. Studies suggest that chronic exposure may be essential to developing diseases, including cancer.[6] The International Agency for Research on Cancer (IARC) includes air pollution as a group 1 agent and bone as a compound or mixture that can cause human cancer with sufficient evidence.[7] A carcinogenic process results from alterations involving continuous generations of cells, which progressively progress toward cancerous growth.[8] It is due to multiple exposures to risk factors that initially produce DNA alterations and damage in target cells or tissues.[9]

Among the biomarkers of early biological effect, the micronucleus count is the most widely used in studies to measure environmental genotoxicity.[10] Micronuclei appear as the most frequent alterations in interphase cells and are formed by eccentric chromosome fragments or complete chromosomes that are not included in the main daughter nuclei during nuclear division and reflect clastogenic and aneugenic events.[11]

Micronucleated cells can be investigated in different types of tissues, such as the oral mucosa, especially exfoliated buccal cells, in which the presence of alterations in the chromosome structure and oxidative stress caused by exposure to air pollutants stand out.[10,12,13]

In the case of Peru, artisanal mining is the oldest and smallest-scale way in which minerals are extracted, and it has become a source of subsistence for people with low resources.[14,15,16,17,18] Artisanal mining is distributed in different regions of Peru, mainly in the mid-south stand out.[3]

Methods

We designed a cross-sectional study to evaluate artisanal miners from Saramarca, in the province of Palpa, Department of Ica in Peru. The intervention area is located approximately 400 km south of the capital of Peru (Lima) (Figure 1). This area is characterized by high gold mining activity. In October 2020, we recruited 80 miners who carried out subsoil mining, "quimbalateo" (use of huge stone-fulling mills where they mix earth, water, and mercury), burning, and gold extraction activities. We included male miners in continuous activity during the last six months and of legal age.

Figure 1: Location of artisanal mining centers in Saramarca, Ica, Peru.

Data collection was obtained through a form; each study participant filled out this format. The sample was taken after the participants signed the informed consent and completed the form. It should be noted that the sheet was prepared based on theoretical aspects that would help determine the presence of a nuclear alteration. Likewise, additional information was collected on variables

that could alter this relationship as potential adjustment variables.

Participants were asked to rinse their mouths with water, and the inner side of the cheek was scraped with a swab; then, the swab was placed in a tube containing isotonic saline solution (NaCl 0.9%), and the swab was struck. The cells broke off at the bottom of the tube and fell into the solution. The collected cells were centrifuged for 5 minutes at 3500 rpm and kept in physiological saline (NaCl 0.9%) at 4°C for transport to the laboratory, where they were fixed in Carnoy (acetic acid and methanol in a 1:3 ratio) and washed. by gentle centrifugation (1500 rpm per minute) until a clean cell pellet is obtained. Subsequently, the smears were prepared on a clean slide in triplicate for each sample and were stained with Feulgen's stain. The reading was in visible light microscopy using 100X magnification in immersion. The average value of the three readings was taken as a measure of internal reliability, evaluating that the readings do not present a coefficient of variation more significant than 15%.

Micronucleus count in buccal epithelium cells. The recommended procedure was used by Holand et al.[19] Cells were stained with the commercial Feulgen kit (Merck, Germany) for subsequent counting of 2000 epithelial cells under a 100X visible light microscope. For the identification of Micronuclei, the following parameters were used: round or oval shape, diameter between 1/16 to 1/3 of the main nucleus, separated from the main nuclei without overlapping; they must not be refractive, they must present the same characteristics in color and condensation of the chromatic than the main nucleus.[20]

Likewise, we identified binucleated cells using the following criteria: they must present two round or oval nuclei and keep their membrane intact and distinguishable from adjacent cells. Nuclei should be separate, similar in size, and have a similar staining pattern. The nuclei can touch but not overlap; the nuclear membrane must be distinguished. A nucleoplasmic bridge can link both nuclei. Finally, we identified nucleoplasmic bridges and budding. For this, we evaluated the microscopic shape, and the homogeneity of the staining and texture of the structures similar to the nuclear ones was evaluated.[20]

The micronuclei count, and other nuclear alterations were presented according to their median and interquartile range. The micronucleus count was dichotomized; we considered 30 micronuclei per 2000 epithelial cells as the cut-off point, according to the criteria used by Shahsavari et al.[21] age and working time were categorized according to their tertiles. We compared micronucleus count ratios using Pearson's Chi-square test. Additionally, we used a Poisson regression model and log linkage from the family of generalized linear models (GLM) and calculated the exponentiated coefficient and its 95% confidence interval. We consider $p<0.05$ as a significant difference. The calculations were made with the statistical program Stata version 17. (StataCorp Collegue Station, TX, USA).

The study was approved on June 18, 2020, by the Universidad Alas Peruanas review committee, with RD N° 237-2020- EPTM-FCS-UAP. The artisanal miners received a talk before they participated in the study. They were informed about the study's objectives, risks, and benefits of their voluntary participation, signing informed consent. The data generated was handled coded and with exclusive access to the research team.

Results

The 80 miners evaluated presented an average age of 46.5 (35.5-62.5) years. Labor seniority in the mining activity averaged 19.5 (10.0-30.0) years (table 1). The average hours worked per day was 8 hours, and the jobs in the mining activity were approximately proportional. About forty-three percent of those evaluated reported consuming cigarettes continuously, and 56.25% alcoholic beverages (table 1). On the other hand, we found a median micronucleus count per 2000 buccal epithelial cells of 27 (IQR:18-32.5). In the case of other nuclear alterations, such as nucleoplasmic bridges, budding, and binucleation, only up to one alteration was evidenced for each total count.

Table 1: Descriptive characteristics of the study population.

Characteristics	N	%	CI 95%
Age (years)	46.5 (35.5-62.5)		
Job seniority (years)	19.5 (10.0-30.0)		
Daily exposure (hours/day)	8.0 (6.0-8.0)		
Job position			
Extraction	17	21.25	13.5-31.7
Transport	21	26.25	17.7-37.1

Pallaqueo	23	28.75	19.8-39.8
Quimbaleteo	19	23.75	15.6-34.4
Smoking			
No	45	56.25	45.1-66.8
Yes	35	43.75	33.2-54.9
Consumption of alcoholic beverages			
No	35	43.75	33.2-54.9
Yes	45	56.25	45.1-66.8

Table 2 shows that micronucleus count and associated factors in bivariate analysis. We found significant differences according to age groups (p=0.004), the count being higher in people over 60. A moderate increase in the micronucleus count is shown concerning the increase in age (R^2=0.504). It can also be seen that the micronucleus count presented significant differences according to job seniority (p<0.001), obtaining the highest count in those workers with more than 25 years of seniority. A moderate increase in the micronucleus count is shown for the increase in job seniority (R^2=0.542). Regarding the number of daily hours worked, this did not generate significant differences (p=0.947) between the micronucleus count. The job position, smoking, and consumption of alcoholic beverages were not significantly associated with the micronucleus count. In the multivariate analysis of the generalized linear model, no independent variables were associated with a micronucleus count more significant than 30 MN/2000 cells.

Table 2.: Micronuclei count and associated factors in bivariate analysis.

Independent variables	**Micronuclei count (MN)**						**p-value***
	MN≤30			**MN>30**			
	n	**%**	**CI95%**	**n**	**%**	**CI95%**	
Age							
<40 years	24	92.3	73.5-98.1	2	7.7	1.9-26.5	0.004
40-60 years	18	54.6	37.4-70.6	15	45.5	29.4-62.6	
>60 years	12	57.1	35.7-76.2	9	42.9	23.8-64.3	
Labor old							
<10 years	19	100.0	--	0	0.0	--	<0.001
10-25 years	23	67.6	50.2-81.3	11	32.4	18.7-49.8	
>25 years	12	44.4	27.0-63.4	15	55.6	36.6-73.0	
Daily working day							
≤8 hours	46	67.7	55.5-77.8	22	32.3	22.2-44.5	0.947
>8 hours	8	66.7	37.2-87.1	4	33.3	12.9-62.8	
Job position							
Extraction	10	58.8	34.9-79.2	7	41.2	20.8-65.1	0.579
Transport	14	6.7	44.3-83.4	7	33.3	16.6-55.7	
Pallaqueo	18	78.3	56.8-90.8	5	21.7	9.2-43.2	
Quimbaleteo	12	63.2	39.9-81.5	7	36.8	18.5-60.1	
Smoking							
No	30	66.7	51.6-79.0	15	33.3	21.0-48.4	0.857
Yes	24	68.6	51.4-81.8	11	31.4	18.2-48.6	
Consumption of alcoholic beverages							
No	25	71.4	54.3-84.0	10	28.6	16.0-45.7	0.508
Yes	29	64.4	49.4-77.1	16	35.6	22.9-50.6	

*Chi2 Pearson

--Omitted

Discussion

Our results show that the micronucleus count in the evaluated population is high compared to a similar study reported by Rosales-Rimache et al.,[3] where only 15% of the artisanal miners evaluated presented MN. A study carried out in Brazil found that coal miners presented MN in buccal epithelium cells. Therefore the increase in MN was associated with genotoxic damage.[22] This event is related to the evidence found in a systematic review that supports the evaluation of MN in the oral epithelium as a biomarker of genotoxic damage since these MN would be a consequence of chromosomal instability caused by exposure to radiation and toxic chemical agents.[23] In this sense, our results could reflect a certain degree of genotoxicity in buccal epithelial cells related to work activity and open possibilities for the generation of future studies that assess not only the genotoxicity induced by artisanal mining activity but also its evaluation as a rapid, reliable, and valid effect biomarker in the assessment of cancer risk.

Artisanal mining activity in Peru varies, and the vast majority is aimed at extracting gold. For this, it requires chemical inputs such as mercury to achieve amalgamation and subsequent extraction; however, the mercury used is in its liquid form, it tends to evaporate at ambient temperatures above 16°C, and these vapors are inhaled and swallowed by the workers, whose epithelial cells receive direct and continuous damage.[24] In our study, this is reflected by the increase in the micronucleus count, which, in particular, presents higher figures than those reported in other studies.[3]

We found that the micronucleus count per 2000 buccal epithelial cells evaluated was 27.5±8.0, a level that is above that reported in other studies. For example, Rosales-Rimache et al.[3] reported a median of 6 micronuclei per 1000 buccal epithelial cells of Ica artisanal miners exposed to mercury. They also showed that "quimbalateo" was the activity that generated the most significant risk in obtaining high counts. In our study, this was different since labor activity was not associated with the micronucleus count, while the number of years as an artisanal miner was a significantly associated variable. Another study has also reported that mining activity generates dust exposure, which induces micronuclei formation 1.34 times in buccal cells. In addition, micronuclei formation is not the only nuclear alteration; increased rates of condensed chromatin, karyorrhexis, and karyolysis also accompany them. Furthermore, working time is once again the variable determining the highest mining population count.[25] In our study, the formation of other nuclear alterations was not absent; however, its count did not exceed one change per 2,000 cells counted.

In the case of Peru, there have yet to be any national studies that evaluate the nuclear effect on oral cells generated by exposure to chemical agents in mining workers or other work activities. Therefore, our findings constitute a relevant source of information that shows genotoxicity could be related to artisanal mining activity, which implies exposure to multiple chemical agents, among which silica dust in its inhalable fraction and metallic mercury stand out. The main limitation of our study was the declaration of a national health emergency due to COVID-19, a situation that forced many workers to quarantine. As a result, the sample size was small. On the other hand, micronuclei are indicators of cell damage, and therefore, any genotoxic agent can induce the production of these alterations.

Conclusions

We found the presence of micronuclei in mining workers, which is associated with their age and length of work as miners. This finding is similar to other studies in other countries and supports the micronucleus count as a biomarker of genotoxic damage. We recommend carrying out more studies of this type in the mining population to evaluate the effect of this economic activity on Peruvian miners.

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