



Biomonitoring through the laboratory evaluation of a population exposed to ores

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ABSTRACT

The mineral exploration in the Amazon, has determined impacts for the environment and for the populations exposed to different agents of the production process. The extraction and the beneficiation developed in the state of Amapá left environmental damages and issues that influence the population's health, such as contamination by metals. Researches have shown that some forms of metals are toxic; causing neurological and genetic complications. The present work considers the toxicological evaluation a fundamental prerequisite in the identification of potential damages to the health. Accordingly, the study's objective is the investigating of the toxic effects through laboratory analysis in a population group of the Elesbao district in Santana-AP municipality exposed to residues of minerals and metals. In this way, the amount of metals in Elesbao water region was evaluated by spectrophotometer, hematological and biochemical tests were accomplished for laboratory evaluation. The paired t-test was applied for statistical evaluation with a significance of 5% ($p < 0.05$). It was concluded that iron and manganese are present in high concentrations in the region water. Hematological and biochemical exams presented statistically relevant alterations. Significant differences in the morphology of the cells were detected by microscopic analysis. This study demonstrates the importance of the investigation of sources of metal exposure and preventing the elimination and minimization of the risks of adverse effects to excessive exposure to multiple metals.

Keywords: Water. Amazon. Laboratory. Metals. Ores. Toxic.

INTRODUCTION

The concern about economic and industrial development and the omission with negative impacts on the environment over several centuries can be presented as two important factors for nowadays high rate of natural resource's degradation, as well as for the gradual reduction levels of health and population quality of life (Facundes, 2011).

In the Amazon region, the last fifty years were marked by the implementation of productive enterprises adopted through Government Development Plans and federal Policies With emphasis on large mining projects. Manganese mineral exploration (Mn) in Serra do Navio – AP and Santana – AP municipality guaranteed the first and most durable large scale and intensive mineral undertaking in the Brazilian Amazon region (Santos et al., 2003).

The mineral exploration in the Amazon, has determined impacts for the environment and for the populations exposed to different agents of the production process. In this context, the Mn extraction and beneficiation project developed by a mining company for approximately 40 years in Santana-AP municipality is addressed, besides environmental damage, other relevant aspects that has influenced over the population health, such as metal contamination (Scarpelli, 2003).

Urbanization is useful as a global agent of the environment. The potential, frequency, and quantity of increased pollutants from a variety of sources is a global trend of concern about water quality by an increasing number of receiving waters that transpire these areas. Among the most frequent forms of human contamination by pollutants, mainly affecting residents, are exposure to the chemical agents: arsenic, cadmium, chromium, lead, dust, iron, manganese, mercury and a few others (Facundes, 2011; de Zwart et al., 2018).

To evaluate the potential impact of exposure on health, it is essential to identify the effects of toxic agents exposure, to recognize exposure conditions that cause damage and to supervise individuals who may be suffering from excessive exposure, aim at to prevent adverse consequences in the population. For the toxicity caused by complex mixtures of substances that can affect the ecosystem and human health occurs in the long run. The ecological risk assessment is

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oriented towards the protection of populations, communities and ecosystems, rather than individual (Holmes et al., 2018; Lima et al., 2015).

The use of laboratory and genetic methodologies for the monitoring of humans exposed to mutagenic has been an effective warning system for pathologies. Thence, the bio monitoring allows, from the obtained data of bio indicators and biomarkers, will be serviceable as guides for future program decisions (Arciszewski et al., 2017).

Biological indicators or biomarkers are reference values considered as guides for assessing the potential health risk of individuals exposed to chemical agents (Costa, 2014). The results demonstrate that there is no risk for the population evaluated until the strong correlation between the exposure to the chemicals and the higher incidence of a particular disease, including genetic alterations and cancer. The fundamental objective of the bio monitoring studies is then the identification of health problems by defining the environmental cause that generate it (Paula, 2010).

This study considers the toxicological evaluation fundamental in the identification of damages to the health. Through the analysis of metals in the water and laboratory tests was fundamental to investigate the suspected influence of metals and mineral residues on possible toxicological and genetic alterations in the residents of the Elesbao district, situated in the mining area in Santana-AP municipality, ensuring, in this way, a reference for genotoxic studies in the area that for many years stores in the open field.

MATERIAL AND METHODS

Compliance with Ethical Standards

- Disclosure of potential conflicts of interest: This study was not funded by any company or organization.
- Research involving human participants and/ or animals: This study involved humans, but all procedures performed in this study were in accordance with the ethical standards of the institutional research committee of the Federal University of Amapá and / or national under CAAE: 62686516.9.0000.0003 and opinion 2.278.622.

Data availability

The data used to support the findings of this study are included within the article and are part of a master degree dissertation. The references data used to support the results and discussions of this study are available on references sections in this article.

This article defined as retrospective case-control study and the present study was approved by the Ethics and Research Committee of the Federal University of Amapa, accredited by the National Health Council under n° CAAE 62686516.9.0000.0003.

The search location corresponds to Elesbao, district that is part of Santana municipality, Amapa State, Brazil.

The study was carried out with volunteer individuals living in Elesbao district, a region close to an active miner located in Santana-AP municipality.

Water Analysis

Water samples were collected at the same time, in October/2017 in 1000 mL plastic containers, duly sterilized, identified, taken from 6 different points, wells, faucets, streams and nearby rivers. The temperature of the samples were stored in icebox during the period and were transported to the laboratory, being stored in a refrigerator, remaining under cooling until the preparation for analysis. The preparations followed the standards established by APHA (American Public Health Association, 2012).

The concentrations of the heavy metals Cd (cadmium), Cr (chromium), Cu (copper), Fe (iron), Pb (lead) and Mn (manganese) in the water samples were measured by Atomic Absorption with Flame Spectrophotometer F-AAS), model AA-6300, from the Bioprospection and Atomic Absorption Laboratory (BAAL) of Federal University of Amapa (UNIFAP). The calibration curves for each metal analyzed were generated from standard AAS solutions provided by the National Institute for Science and Technology of the United States of America (NIST-USA).

Laboratorial analysis

A total of 100 individuals, divided into two groups defined by non-exposed group (50), all volunteers residing in Macapá City, outside the risk region and group exposed to contamination (50) residing in Elesbao district for a minimum period of 10 years. The exposed group was aggregated in a Basic Health Unit in Elesbao district, where the guidelines, filling in questionnaires, consent form and collection of the biological material were carried out, and the control/ non-exposed group was gathered in the clinical analysis laboratory of UNIFAP.

For the hematological study, blood samples were collected in tubes containing anticoagulant (EDTA). Hemogram was performed by a Mindray BC6800 automatic analyzer using the Mindray reagent kits. For biochemical analysis blood samples were collected in gel-free tubes without anticoagulant. After blood coagulation, it was centrifuged for serum withdrawal that were used for biochemical analyzes on a BS800 automatic analyzer and Labtest reagent kits. The morphological analysis was performed using blades (smears) stained by the panoptic kit and analyzed in the 100X optical microscope.

The obtained results in the various analyzes are expressed as mean \pm standard deviation of the mean ($X \pm SEM$) of each experimental group, and the comparison was performed between the exposed female versus the control/ non-exposed group, and another analysis among the male population, exposed versus control/ non-exposed group. To compare the data of the groups evaluated, the paired t-test was applied, with significance of 5% ($p < 0.05$). They were analyzed by the GraphPad Prism® statistical package (version 5.03).

RESULTS AND DISCUSSION

Analysis of metals in water

The amount of metals resulting from Elesbao water analysis are presented in Table 1 and are compared with the maximum permitted values of Ordinance 518/2004 of the Ministry of Health - CONAMA 357/2005 (Brasil, 2005).

The average (Cd), (Cr), (Cu) and (Pb) levels were not significantly altered, but (Fe) and (Mn) above the maximum permitted values.

The influence of environmental factors on the geographical distribution of human health problems have promoted the interaction between the different study areas to evaluate the origins and effects of trace elements such as arsenic, lead, manganese (Rim & Kim, 2015). In this sense, the monitoring of drinking water in Santana-AP municipality, evaluating total metal contents, represents an important action of environmental health surveillance, considering the existence of contaminated sites in nearby areas. For, several studies have correlated high concentrations of metals such as arsenic and manganese in water with high concentrations in biological samples and with neurological damages (Santos et al., 2003; Khan et al., 2012).

The presence of Mn in contaminated water may reflect mineral residues released from mineral practices carried out at the local company. Therefore, our results reinforce the importance of the analysis due to the existence of a potential adverse effect on the central nervous system generated by exposure to high concentrations of Mn, especially through water (Mudgal et al., 2010). In the body, Mn has the ability to cross the blood-brain barrier and may accumulate in the brain. The Mn increase in the brain may cause neuronal injury, which may result in a decrease in the number of dopaminergic neurons. High concentrations of Mn in the hair of children are related to cognitive disorders (Agency for Toxic Substances and Disease Registry, 2012; Mora et al., 2014).

Iron (Fe) present in high concentrations can accumulate in the brain, in addition to being related to the onset of neurodegenerative diseases. The Fe levels in the blood of

the exposed group were also positively correlated with the Fe levels in the water, suggesting this as a probable source of exposure (Schröder et al., 2013).

Besides to neurological damage, the toxicity induced by these metals can cause damage to the DNA, renal damage, disturbances on the biosynthesis of the heme group of hemoglobin, among others. Therefore, the indestructibility added to the bioaccumulation of the metals contributes to the increased concern related to the toxic effects caused by these xenobiotics (Klaassen & Watkins, 2012). Environmental exposure to metals occurs from a variety of sources, with water and food being the most common sources of exposure.

Demographic analysis

The most relevant characteristics of the population included the mean age of the two groups, which were significantly different, the exposed group presented more volunteers with age above 45 years, and the non-exposed group had a younger population with a maximum of 25 years.

In the exposed group, the gender was equally divided between men and women, but more women in the non-exposed group. The non-homogeneity of gender and age present in the non-exposed group is due to the free demand of the volunteers in this research. However, this fact did not influence, since the alterations occurred only in the exposed group.

The number of people who have a job are higher in the exposed group and respectively the exposure to chemical substances was higher in this group. Genetic diseases and cancer are practically the same proportion in the exposed and unexposed ones, however the degree of kinship that presents some disease or mutation is well differentiated.

Health problems such as diabetes, itchy and itchy skin, epilepsy, severe headaches, respiratory and eye problems, as well as hair loss, were present in the narratives of several people residing in Elesbao district. These health problems also coincide with the effects of human exposure to manganese and other metals, notably with regard to respiratory and eye problems as well as neurological disorders (Silva et al., 2014).

Table 1 - Results of the mean trace element contents (mg/ L) in the waters of consumption in 6 different points of Elesbao district and surroundings of the industrial area, in the period of October/ 2017.

ELEMENTS	SAMPLE	CONC. (ppm)	AVERAGE (%)	SD	MAA* (mg/ L)
Fe	A	0.9262	35	0.0133	0.300
	B	11.178	4.03	0.0019	
	C	12.574	15.18	0.0079	
	D	0.4554	114.12	0.0207	
	E	0.3347	86.8	0.0113	
	F	18.748	14.55	0.0114	
Mn	A	-0.0492	102.32	0.0016	0.100
	B	-0.0954	71.39	0.0022	
	C	-0.08	36.88	0.001	
	D	-0.0646	158.51	0.0033	
	E	-0.1046	25.64	0.0009	
	F	0.0523	80.57	0.0013	

Values are expressed as mean \pm SD (n = 6); (Conc./ ppm) in samples A (CAESA water); B (water from the Amazon River); C (water of the Igarape Elesbao); D (well water 1); E (well water 2); F (Igarape water near the company); * Maximum amount allowed for waters for human consumption CONAMA - resolution n^o 357, of March 17, 2005; (Brasil, 2005).

Biochemical evaluation

The biochemical parameters evaluation of the exposed and non-exposed group showed significance ($p = 0.0419$) for alanine aminotransferase (ALT) in the female group. However, no statistical significance was found for aspartate aminotransferase (AST), urea (ERU), creatinine (CREA), iron (RES), and transferrin saturation index (STI). However, 10% of the individuals in the exposed group presented altered values in AST and 25% had alterations in ALT, whereas in the non-exposed group only 6% of individuals had alterations in ALT/AST enzymes.

Biochemical biomarkers are important tools used in epidemiological studies, seeking to establish a relationship between exposure to chemical agents and the health effects of exposed individuals. Exposure to metals is known to produce a variety of biochemical changes, which may be responsible for adverse effects in experimental studies with humans (Costa, 2014; Rodrigues, 2011).

The elevation of ALT is considered as a sensitive and early parameter of liver changes due to exposure to hepatotoxic agents, before irreversible lesions develop (Rodrigues, 2011). When correlating with the metals that are in excess in the waters of the studied environment (Fe and Mn) and the data of the biochemical tests (AST/ALT), it is verified that the exposure to the metals, can cause hepatic pathologies. When Mn is in excess in the body, in high amounts, can cause toxic effects mainly in the nervous system and this one is in higher levels in the liver, conjugated to the bile salts. And the excess of Fe in the blood causes hemochromatosis, a disease characterized by the accumulation of iron in the form of ferritin in muscles, liver, pancreas, joints and heart causing damage to organs and tissues (Agency for Toxic Substances and Disease Registry, 2012; Ruppenthal, 2013).

To evaluate renal function, urea and creatinine were used as biomarkers. The mean and standard deviation data evidenced were considered normal for the reference values, with no significant difference between the groups for either urea ($p = 0.8044$) / ($p = 0.2388$) or for creatinine ($p = 0.8169$) / ($p = 0.0593$). However, even though it did not present statistically significant results, in the renal evaluation differences were evidenced in the results, because in the exposed group 07 volunteers presented alterations in creatinine and 01 with altered urea and in the group not exposed there were no alterations.

The liver and kidneys, the main organs of biotransformation and excretion of toxic substances, are targets most frequently affected by toxic agents. The formation and excretion of urea and creatinine make them useful markers in renal function. Serum levels of these metabolites within the normal range indicate adequate renal function (Rodrigues, 2011; Wallach, 2011).

In serum iron analysis ($p = 0.1447$) / ($p = 0.7451$) and transferrin saturation index ($p = 0.5070$) / ($p = 0.6723$) did not determine statistical significance in the correlations, but both exposed and non exposed groups, presented 10% of individuals with increased iron and changes in STI. Markers

of iron overload include ferritin and transferrin saturation, in addition to iron quantification. If it occurs continuously, iron overload leads to iron accumulation in the liver, pancreas, heart and other organs and, if left untreated, eventually lead to cellular damage and loss of function in some organs (Lozoff et al., 2012; Low et al., 2013).

Hematologic Analysis

The analysis of the samples demonstrated a statistically significant difference in the MCH, platelet and lymphocyte values of the exposed group in relation to the non-exposed group. In the analyzed parameters, the main statistical significance between the exposed and non-exposed groups was in the female group, where MCH determined changes ($p = 0.0458$).

Mean corpuscular hemoglobin (MCH) is one of the parameters of the blood count that measures the size and color of hemoglobin within the blood cell. The MCH, as well as the MCV, seek to identify the type of anemia, which may be normochromic or hypochromic. High MCH is due to abnormal increase in red blood cell size, may be characteristic of hyperchromic anemia, thyroid disorders, alcoholism, and may also lead to megaloblastic anemia (Pereira et al., 2013; Melo & Silveira, 2015)

As shown in Table 2, regarding the leukocyte parameters between exposed and non-exposed groups, no significant values were determined for total leukocytes, but, differently, the male group presented lymphocytes with significant statistical evidence ($p = 0.0108$), but they were within the reference range. The other indexes did not present any significant alterations, and the following parameters were analyzed: leukocytes ($p = 0.8812$) / ($p = 0.1506$), neutrophils ($p = 0.6623$) / (0.0879) and lymphocytes $p = 0.3990$.

The number of platelets in the exposed/non-exposed population presented significant changes in relation to the increase. Therefore, in the comparative platelet parameters, the number of platelets in the male group ($p = 0.0189$) and the PDW in the male ($p = 0.0464$) and female ($p = 0.0009$) exhibited statistical significance.

Platelets are the smallest elements formed in the blood, disk-shaped, without a nucleus, with a fragile membrane, produced in the bone marrow by megakaryocyte fragmentation. In order to evaluate platelets, the total number of platelets is analyzed, besides the PDW and MVP index, which is the mean platelet volume, are simple indexes of platelet analysis, since they increase during platelet activation.

High PDW shows that platelets vary considerably in size, which may indicate disorders affecting the bone marrow or platelets, and further testing, are important. It can also be characteristic of certain types of anemia, cancer, inflammatory conditions, infectious diseases and contraceptive use. However, PDW with low values may be indicative of bone marrow disorder or be typical of viral infections such as hepatitis, measles and mononucleosis. Certain types of cancer and certain drugs may lead PDW to low results (Melo & Silveira, 2015; McPherson & Pincus, 2012).

Table 2 - Data from the values obtained in the hemogram - red blood cells/ hematimetric indexes, leukocytes and platelets.

ELEMENTS	SAMPLE	Value Ref. *	EXPOSED (n=45)		NOT EXPOSED (n=45)	
			AVERAGE	SD	AVERAGE	SD
RED BLOOD CELLS (millions / mm ³)	M	4.5 – 6.0	5.1	0.3947	5.18	0.2895
	F	4.1 – 5.5	4.46	0.3095	4.59	0.4432
HB (g/dL)	M	11 – 16	15.06	1.053	15.13	0.6758
	F		13.06	1.088	12.74	1.264
HCT (%)	M	37 – 54	44.39	2.946	44.86	1.795
	F		39.1	3.050	38.38	3.555
RDW (%)	M	11 – 16	13.24	0.7163	13.25	0.5395
	F		13.372	0.792	13.5625	
MCV (fL)	M	80 – 98	87.28	2.907	87.0	3.325
	F		87.57	3.440	84.47	7.938
MCH (pg)	M	28 – 33	29.62	1.132	29.33	1.275
	F		29.24	1.449	27.98	3.026 (S)
MCHC (%)	M	32 – 35	33.92	0.5836	33.70	0.492
	F		33.38	0.838	32.89	1.035
Leukocytes (mm ³)	M	4000 – 10000	6934	1377,7	6999,5	1376,6
	F		6659	2733,4	7628,3	1754,0
Neutrophils (%)	M	50 – 70	54.7	7.92	53.70	6.30
	F		53.8	8.34	58.33	9.55
Lymphocytes (%)	M	20 – 40	29.96	5.96	35.33	6.70 (S)
	F		34.48	8.43	32.48	8.28
Platelets (mm ³)	M	100,000 – 424,000	236,100	61,381	292,900	83,489 (S)
	F		284,916	69,107	310,708	67,145
MPV (fL)	M	7.4 – 10.4	11	1.152	10.47	1.371
	F		10.6	0.931	10.3	0.954
PDW	M	15 – 17	16.28	0.3350	16.07	0.3097 (S)
	F		16.20	0.300	15.80	0.460 (ES)

* Reference value based on the inserts of the reagents used - Mindray and the book - Laboratory of hematology: theories, techniques and practices (2015).
 * Values are expressed as mean ± SD (n = 45). Oneway TEST paired t (p <0.05). HB (Hemoglobin), HCT (Hematocrit), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), RDW (mean corpuscular hemoglobin coefficient), MCHC (hemoglobin concentration) (Platelet Distribution Width).

Cellular morphology

In addition to the investigation of hematological parameters and hematimetric indexes, the blood smears of the patients of the two groups, exposed and not exposed, were submitted to a detailed microscopic analysis in order to find possible morphological differences that could help in the differentiation between them.

100 cells per slide were analyzed, totaling 200 cells per subject. From the microscopic evaluations of the hematological slides, the qualitative and quantitative analyzes of the cells were performed.

The number of dacrocytes, echinocytes, hyposegmented neutrophils and macroplatelets were elevated in the group of exposed patients, whereas in the non-exposed group the slides presented normality in their cells and platelets. The information indicate that there were important findings regarding the erythrocyte morphology, characterizing a high poikilocytosis as well as relevant alterations in the white and platelet lineage.

Dacrocytes are erythrocytes in the form of tear drop or pear (Figure 1), it appears when there is fibrosis of the bone marrow or severe dyserythropoese and in some hemolytic anemias. They are characteristic of megaloblastic anemia, thalassemia major and myelofibrosis, both primary and secondary to metastatic carcinoma or other type of bone marrow infiltration (Bain, 2016).

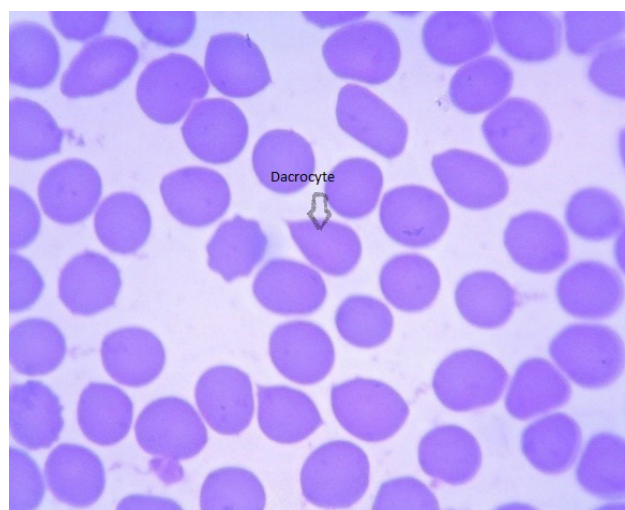


Figure 1 – Dacrocyte – Photo: own authorship

Echinocytes are a type of erythrocytes that have lost the discoid shape and are covered with 10 to 30 blunt spurs, of relatively regular shape and distribution (Figure 2). Echinocytes can be produced in vitro by exposure to fatty acids and certain drugs, or simply by incubation (Melo & Silveira, 2015).

The formation of macroplatelets and giant platelets may occur because the MPV is increased due to accelerated platelet

turnover (Figure 3). Platelets of different sizes characterize platelet anisocytosis, common in myeloproliferative syndromes, myelodysplastic and platelet dysfunction (Melo & Silveira, 2015; McPherson & Pincus, 2012).

Through the smear, it was also possible to identify a nuclear button seems like a micronucleus (Figure 4) in exposed patient slides. However, it is necessary that specific tests, such as the comet assay, be performed for confirmation, since these findings were found only in mononuclear cells. The formation of MN is indicative of genotoxicity of a certain factor capable of causing mutations in the DNA strand, mainly large deletions (Batista & Campos, 2014; Speit, 2013).

In tissues, neutrophils perform phagocytosis of microorganisms and other foreign materials. Alterations in the neutrophil morphology are important in the interpretation of the leukogram as well as to identify the etiology of the disease. Commonly, morphological changes may reflect cell

age, degenerative changes, changes induced by infectious diseases, myeloproliferative or genetic diseases (Kolaczowska & Kubes, 2013).

In the analyzed slides of the exposed patients it was possible to identify an increased number of hyposegmented neutrophils (Figure 5), which are characterized by presenting less than 5 lobes, are young neutrophils but no longer considered rods, are already indicative of left deviation tendency. In the control patients, or those not exposed, all the blades presented neutrophils with structures within normality (Silva, 2015).

The results of the laboratory tests suggest that the population studied did not present alterations in all the statistically significant hematological and biochemical parameters. These results indicate that no metal intoxication occurred, but showed alterations in hematimetric indexes, platelets, red cell morphology and ALT enzyme, which may mean some impairment due to exposure.

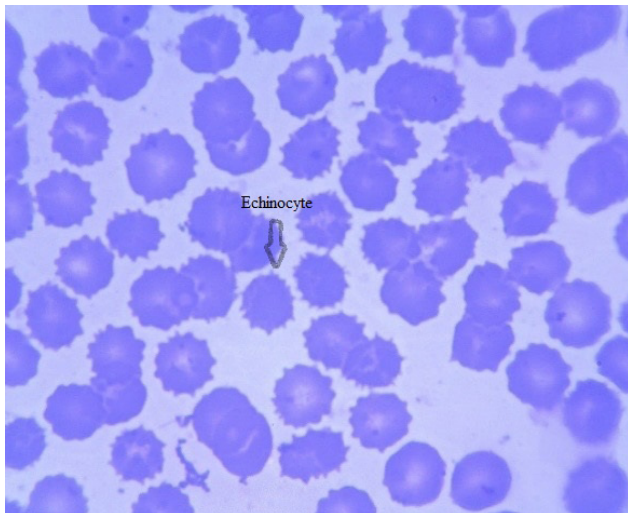


Figure 2 – Echinocyte – Photo: own authorship

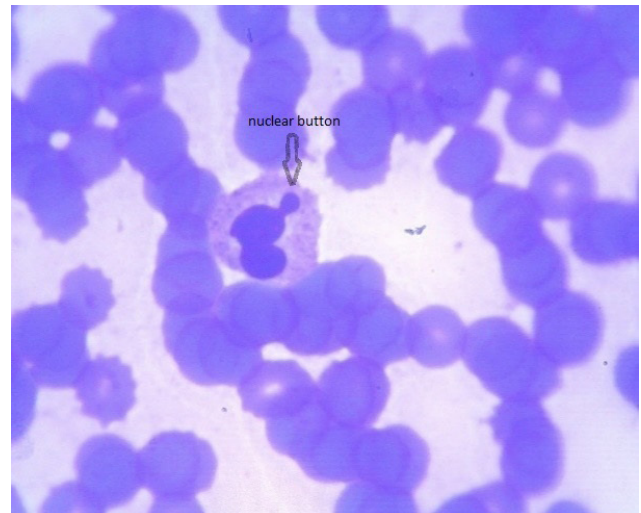


Figure 4 – nuclear button – Photo: own authorship

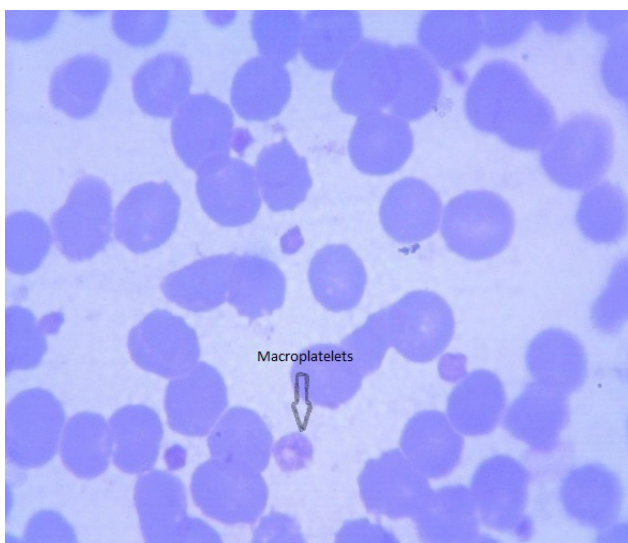


Figure 3 – Macroplatelets – Photo: own authorship

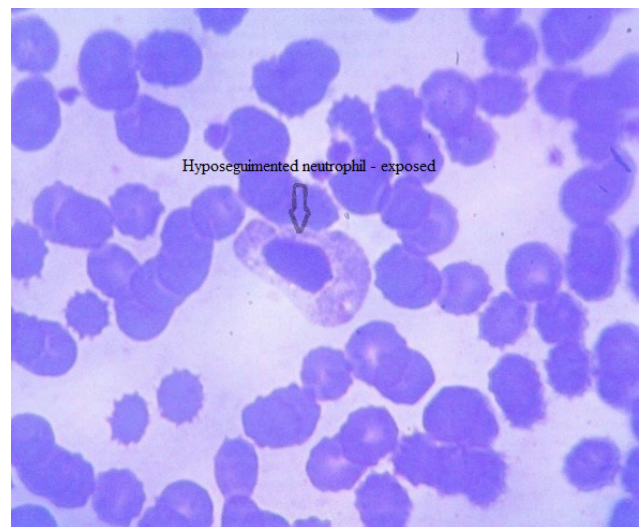


Figure 5 - Hyposegmented neutrophil – exposed – Photo: own authorship

The importance of analyzing serum iron and transferrin to evaluate the iron stores of the organism has been verified, a low number of references is found when these parameters are related to liver disease. Research for this subject is stagnant, owing to the difficulty in finding materials for research from current sources. It is an area that has much to discover and should be explored aiming to help patients who have excess iron before the liver disease develops, impairing the organ function and decreasing the quality of life of the affected patient.

Under these circumstances, morphological changes in red blood cells, neutrophils and platelets in the exposed population suggest further analyzes, as they may be indicative of contamination. The identification of a possible MN indicates a genetic instability in the exposed population. However, it is necessary more specific assays and the biomonitoring in these volunteers.

Although it has a limited diagnostic power, the hemogram under the command of a clinician who knows the cellular functions and pathophysiological bases of the diseases can be an important tool in evaluation of several situations, such as in diagnosis and evolution of hematological diseases, infectious and in therapeutic monitoring.

New laboratory parameters obtained from automated systems may be helpful in blood and bone marrow analysis but should be used with caution. It is important to remember that microscopy is still fundamental in identification of several abnormalities in hematopoiesis. The association of data regarding quantitative aspects, morphological aspects and pathophysiological knowledge of hematopoiesis disorders is important to accurate diagnosis of the changes affecting the blood and bone marrow (Grotto, 2010).

CONCLUSIONS

Water may be indicated as an important source of exposure to these xenobiotics. The results of the water analysis demonstrated that there is contamination of the water by manganese, excess iron with mining tailings, which need to be investigated in the blood of exposed individuals.

This study shows that it is important to investigate sources of metal exposure and to take preventive measures to eliminate or minimize the risks of adverse effects related to excessive exposure to multiple metals.

Accordingly, there is a need for further studies, so that it can investigate different areas of environmental contamination to metals, defining probable sources of exposure, especially studies involving children and the elderly because they are a group especially sensitive to toxicity.

RESUMO

Biomonitoramento através da avaliação laboratorial de uma população exposta a minérios

A exploração mineral na Amazônia, determinou impactos para o meio ambiente e para as populações expostas a diferentes agentes no processo de produção. A extração e

beneficiamento de minério desenvolvido no Estado do Amapá deixaram danos ambientais e problemas que influenciam na saúde da população, como a contaminação por metais. Estudos demonstram que algumas formas de metais são tóxicas, causando problemas neurológicos e genéticos. O presente estudo considera a avaliação toxicológica fundamental na identificação de possíveis danos à saúde. Portanto, o objetivo desse estudo é investigar efeitos tóxicos através da análise laboratorial em um grupo populacional do Bairro Elesbão no município de Santana/AP expostos a resíduos de minérios e metais. Desta forma, avaliou-se por espectrofotômetro a quantidade de metais nas águas da região do Elesbão e realizou-se exames hematológicos e bioquímicos para avaliação laboratorial. O Teste t pareado, foi aplicado para avaliação estatística com significância de 5% ($p < 0.05$). Concluiu-se que há ferro e manganês em concentrações elevadas nas águas da região. Os exames hematológicos e bioquímicos apresentaram alterações estatisticamente significantes. A análise microscópica das células detectou diferenças significativas em sua morfologia. Este estudo demonstrou que é importante investigar as fontes de exposição a metais e tomar medidas preventivas para eliminar ou minimizar os riscos dos efeitos adversos relacionados à exposição excessiva a múltiplos metais. Palavras-chave: Água. Amazônia. Laboratorial. Metais. Minérios. Tóxico.

REFERENCES

- Agency for Toxic Substances and Disease Registry – ATSDR. Toxicological profile for manganese. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 2012 [cited 15 de dezembro de 2016]. Available from: <http://www.atsdr.cdc.gov/toxprofiles/tp151.pdf>
- American Public Health Association – APHA. Standard methods for the examination of water and wastewater. 22nd ed. Washington: AWWA; 2012. 1496 p.
- Arciszewski TJ, Munkittrick KR, Scrimgeour GJ, Dubé MG, Wrona FJ, Hazewinkel RR. Using adaptive processes and adverse outcome pathways to develop meaningful, robust, and actionable environmental monitoring programs. *Integr Environ Assess Manag.* 2017;13(5):877-91. <http://dx.doi.org/10.1002/ieam.1938>. PMID:28383771.
- Bain BJ. Células sanguíneas – guia prático. 5. ed. Porto Alegre: Artmed Editora S. A.; 2016. p. 84-85.
- Batista CR, Campos EO. Avaliação da genotoxicidade em células de pacientes fumantes e não fumantes por meio do teste de micronúcleo. *Getec.* 2014;3:49-58.
- Brasil. Conselho Nacional de Meio Ambiente - CONAMA. Resolução nº 357, de 17 de março de 2005. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes, e dá outras providências. *Diário Oficial da União; Brasília; 18 mar. 2005; 58-63. Seção 1.*

- Costa TPC. Biomonitoramento citogenético e laboratorial em agentes de endemias expostos a pesticidas organofosforados no município de Valença – PI [dissertação]. Fortaleza: Universidade Federal do Ceará; 2014.
- de Zwart D, Adams W, Galay Burgos M, Hollender J, Junghans M, Merrington G, Muir D, Parkerton T, De Schamphelaere KAC, Whale G, Williams R. Aquatic exposures of chemical mixtures in urban environments: approaches to impact assessment. *Environ Toxicol Chem.* 2018;37(3):703-14. <http://dx.doi.org/10.1002/etc.3975>. PMID:28861906.
- Facundes RS. Danos socioambientais provenientes do manuseio inadequado de rejeitos de manganês e as implicações para a vida e a saúde dos moradores da vila do Elesbão [dissertação]. Macapá: Universidade Federal do Amapá; 2011.
- Grotto HZW. Diagnóstico laboratorial da deficiência de ferro. *Rev Bras Hematol Hemoter.* 2010;32:22-8. <http://dx.doi.org/10.1590/S1516-84842010005000046>.
- Holmes M, Brown CD, Hamer M, Jones R, Maltby L, Posthuma L, Silberhorn E, Teeter JS, Warne MSJ, Weltje L. Prospective aquatic risk assessment for chemical mixtures in agricultural landscapes. *Environ Toxicol Chem.* 2018;37(3):674-89. <http://dx.doi.org/10.1002/etc.4049>. PMID:29193235.
- Khan K, Wasserman KGA, Liu X, Ahmed E, Parvez F, Slavkovich V, Levy D, Mey J, van Geen A, Graziano JH, Factor-Litvak P. Manganese exposure from drinking water and children's academic achievement. *Neurotoxicology.* 2012;33(1):91-7. <http://dx.doi.org/10.1016/j.neuro.2011.12.002>. PMID:22182530.
- Klaassen CD, Watkins JB. Fundamentos em toxicologia. 2. ed. Porto Alegre: Editora AMGH Ltda; 2012.
- Kolaczowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol.* 2013;13(3):159-75. <http://dx.doi.org/10.1038/nri3399>. PMID:23435331.
- Lima DP, Santos C, Silva RS, Yoshioka ETO, Bezerra RM. Contaminação por metais pesados em peixes e água da bacia do rio Cassiporé, Estado do Amapá, Brasil. *Acta Amazon.* 2015;45(4):405-14. <http://dx.doi.org/10.1590/1809-4392201403995>.
- Low M, Farrell A, Biggs B-A, Pasricha S-R. Effects of daily iron supplementation in primary-school-aged children: systematic review and meta-analysis of randomized controlled trials. *CMAJ.* 2013;185(17):E791-802. <http://dx.doi.org/10.1503/cmaj.130628>. PMID:24130243.
- Lozoff B, Castillo M, Clark KM, Smith JB. Iron-fortified vs low-iron infant formula: developmental outcome at 10 years. *Arch Pediatr Adolesc Med.* 2012;166(3):208-15. <http://dx.doi.org/10.1001/archpediatrics.2011.197>. PMID:22064877.
- McPherson RA, Pincus MR. Diagnósticos clínicos e tratamento por métodos laboratoriais. 2. ed. Barueri: Manole; 2012.
- Melo MAW, Silveira CM. Laboratório de hematologia: teorias, técnicas e atlas. Rio de Janeiro: Rubio; 2015.
- Mora AM, van Wendel de Joode B, Mergler D, Córdoba L, Cano C, Quesada R, Smith DR, Menezes-Filho JA, Lundh T, Lindh CH, Bradman A, Eskenazi B. Blood and hair manganese concentrations in pregnant women from the infants' environmental health study (ISA) in Costa Rica. *Environ Sci Technol.* 2014;48(6):3467-76. <http://dx.doi.org/10.1021/es404279r>. PMID:24601641.
- Mudgal V, Madaan N, Mudgal A, Singh RB, Mishra S. Effect of toxic metals on human health. *Open Nutraceuticals J.* 2010;3(1):94-9. <http://dx.doi.org/10.2174/18763960010030100094>.
- Paula SNC. Biomonitoramento como instrumento de detecção de contaminantes ambientais [monografia]. Vitória: Universidade Veiga de Almeida; 2010.
- Pereira AV, Rocha FDL, Oliveira M, Tapety AN, Cavalcante ACM, Chaves TVS. Haematological and genotoxic profile study of workers exposed to medical waste. *Journal of Research Fundamental Care on Line.* 2013;5:160-68.
- Rim KT, Kim SJ. A review on mutagenicity testing for hazard classification of chemicals at work: focusing on in vivo micronucleus test for allyl chloride. *Saf Health Work.* 2015;6(3):184-91. <http://dx.doi.org/10.1016/j.shaw.2015.05.005>. PMID:26929826.
- Rodrigues VRCB. Avaliação das alterações hematológicas, bioquímicas e genotóxicas nos trabalhadores expostos a agrotóxicos em municípios do Estado do Piauí [dissertação]. Fortaleza: Universidade Federal do Ceará; 2011.
- Ruppenthal J. Toxicologia. Santa Maria: Universidade Federal de Santa Maria, Rede e – Tec; 2013.
- Santos ECO, Jesus IM, Brabo ES, Fayal KF, Sá Filho GC, Lima MO, Miranda AMM, Mascarenhas AS, Sá LLC, Silva AP, Câmara VM. Exposição ao mercúrio e ao Arsênio em Estados da Amazônia: síntese dos estudos do Instituto Evandro Chagas/FUNASA. *Rev Bras Epidemiol.* 2003;6(2):171-85. <http://dx.doi.org/10.1590/S1415-790X2003000200010>.
- Scarpelli W. Arsênio do minério de manganês de Serra do Navio. *Novos Cadernos NAEA.* 2003;6:101-33. <http://dx.doi.org/10.5801/ncn.v6i1.85>.
- Schröder N, Figueiredo LS, Lima MNM. Role of brain iron accumulation in cognitive dysfunction: evidence from animal models and human studies. *J Alzheimers Dis.* 2013;34(4):797-812. <http://dx.doi.org/10.3233/JAD-121996>. PMID:23271321.
- Silva IC. Neutrófilos: aspectos clássicos, plasticidade e novas funções imunoregulatorias. *Revista Interdisciplinar de Estudos Experimentais.* 2015;7:35-46.
- Silva JMB, Barrio RJ, Moreira JC. Arsênio - saúde: uma relação que exige vigilância. *Revista Vigilância Sanitária em Debate.* 2014;2(1):57-63. <http://dx.doi.org/10.3395/vd.v2i1.130>.

Speit G. Does the recommended lymphocyte cytokinesis-block micronucleus assay for human biomonitoring actually detect DNA damage induced by occupational and environmental exposure to genotoxic chemicals? *Mutagenesis*. 2013;28(4):375-80. <http://dx.doi.org/10.1093/mutage/get026>. PMID:23644166.

Wallach J. *Interpretação de exames laboratoriais*. 8. ed. Rio de Janeiro: Guanabara Koogan; 2011.

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