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RESEARCH

Haematological and genotoxic profile study of workers exposed to medical waste

Estudo do perfil hematológico e genotóxico de trabalhadores expostos a resíduos dos serviços de saúde

Estudio del perfil genotóxico y hematológico del trabajadores expuestos de los residuos de servicios de salud

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ABSTRACT

Objective: To evaluate the haematological and genotoxic profile of workers exposed to medical waste. **Method:** Descriptive study of an observational nature, performed with two distinct groups: exposed (20 individuals) and unexposed (20 individuals), which had blood samples collected for analysis. **Results:** The results revealed an increased erythrocytes, hematocrit and leukocytes of the exposed group compared to the unexposed group. In the group exposed were identified: eosinophilia (45%), atypical lymphocytes (35%) and neutrophil toxic granulation (25%). It revealed a significant genotoxic effect by the content and frequency of major damage in the exposed group. There was no correlation of these results with the habits and life styles reported. **Conclusion:** It was found that the study group might be undergoing reaction processes caused by some agent, as well as genetic instability. These data highlight the need for greater biomonitoring of these workers in order to prevent neoplastic conditions. **Descriptors:** Medical Waste, Waste Collectors, Occupational Risk.

RESUMO

Objetivo: Avaliar o perfil hematológico e genotóxico dos trabalhadores expostos a resíduos dos serviços de saúde. **Método:** Estudo descritivo, de natureza observacional, realizado com dois grupos distintos: exposto (20 indivíduos) e não exposto (20 indivíduos), onde tiveram seu sangue coletado para análise. **Resultados:** Os resultados encontrados evidenciaram aumento de hemácias, hematócrito e leucócitos do grupo exposto em comparação ao grupo não exposto. Identificou-se no grupo exposto eosinofilia (45%), atipia linfocitária (35%) e granulações tóxicas neutrofílicas (25%). Evidenciou-se significante efeito genotóxico pelo índice e frequência de danos maiores no grupo exposto. Não se obteve correlações destes resultados com hábitos e estilos de vida relatados. **Conclusão:** Avaliou-se que o grupo estudado pode estar passando por processos reacionais ocasionados por algum agente, bem como instabilidade genética. Tais dados salientam a necessidade de maior biomonitoramento destes trabalhadores, a fim de prevenir quadros neoplásicos. **Descritores:** Resíduos dos Serviços de Saúde, Coletores de Resíduos, Risco Ocupacional.

RESUMEN

Objetivo: Evaluar el perfil genotóxico y hematológicos de los trabajadores expuestos a los residuos de los servicios de salud. **Método:** Estudio descriptivo, observacional, realizado con dos grupos: expuestos (20 personas) y no expuestos (20 personas), que tenía su sangre recogida para el análisis. **Resultados:** Los resultados mostraron un aumento, hematocrito y leucocitos grupo expuesto en comparación con el grupo no expuesto. Identificada en el grupo expuesto eosinofilia (45%), linfocitos atípicos (35%) y de neutrófilos granulación tóxica (25%). Se demuestra significativo el índice de efecto genotóxico y la frecuencia de graves daños en el grupo expuesto. No correlaciones obtenidas de estos resultados con los hábitos y estilos de vida reportados. **Conclusión:** Se encontró que el grupo de estudio puede ser sometido a procesos de reacción causada por algún agente, así como la inestabilidad genética. Estos datos ponen de relieve la necesidad de una mayor vigilancia biológica de estos trabajadores con el fin de evitar los condiciones neoplásicas. **Descriptores:** Residuos de Servicios de Salud, los recolectores de residuos, riesgos laborales.

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INTRODUCTION

Currently, the growing increase in the production of solid waste represents a serious and worrying phenomenon by the large amount of trash generated daily, through its high potential in the transmission of pathogens in soil contamination and pollution of air and water,[1] making necessary a correct waste management with proper packaging appropriate containers, for temporary and storage safe from the actions of human beings and animals so that waste collection can be performed.[2]

Occupational garbage collectors are exposed to various types of occupational hazards and, therefore, it is from this panorama of occupational exposure, which is clearly perceived, that the relations between the work process and the health/disease process this professional class deserve attention, study and intervention in public health.[3]

Hospital waste deserves special attention because of a wide spectrum of dangerousness comprising from the potential spread of infectious diseases, to environmental risks derived from the methods employed in its treatment and final disposal. Thus, this study aimed to evaluate and characterize the haematological and genotoxic profile in the peripheral blood medical waste collectors in order to correlate possible changes in their type of occupational exposure.

METHODOLOGY

The survey consists of a descriptive study cohort and cross-sectional research, having been held in a private company responsible for the collection, disposal and treatment of Medical Waste (MW) in the city of Teresina, state of Piaui, Brazil. The sample consisted of 40 individuals with age range from 20 to 45 years of age, among these 20 characterize the test group (exposed) and 20 the control group (non-exposed) they were informed of the study and, under their consent, 4 ml of blood was collected by venous puncture, distributed in vacuum tubes (Vacutainer®) with Ethylene Diamine Tetraacetic Acid (EDTA) dipotassium hydrogen phosphate and in heparinized eppendorf and protected from ultraviolet light.

Hemogram

After the collections, the slide smears were made with staining by Fast Panotico. The collection of blood samples were analyzed for hematological tests using the automatic analyzer ABX MICROS 60. Different hematological parameters were used: hemoglobin (Hb), hematocrit (Ht), number of erythrocytes in whole blood, leukogram with leukocyte count and platelet count. Differential counts were performed by the manual method, in which we analyzed 100 leukocytes per slide, as well as its qualitative assessment.

Comet Test in Peripheral Blood

The methodology used for conducting the comet assay was described [4] with some modifications. 2 Slides were prepared for each individual. A sample of 5 µl of blood previously collected in a heparinized syringe, was mixed in 95 µl of low melting agarose, the mixture was placed on the slides pre-coated with normal melting agarose and was covered with a coverslip. Subsequently the slides were maintained in lysis solution, in the refrigerator, for a period of one week. After lysis, the slides were transferred to an electrophoresis tank containing a refrigerated

buffer, with a current of 25V, 300mA. The slides were left in the tank for 20 minutes before running the electrophoresis, which lasted 15 minutes. Following the electrophoresis the slides were neutralized with a buffer for 15 minutes, washed with distilled water and dried at room temperature for 12 hours and fixed with a fixation solution for 15 minutes. The coloring was done with silver nitrate (0.02%).

50 Cells were analyzed per slide, totaling 100 cells per individual. The comets were classified into categories of migration, by the degree of damage to the DNA (0-4), in which the frequency of damage was calculated. The damage can vary from 0 to 4, in accordance with the migration of the DNA, which can be measured by the size of the comet tail.

RESULTS AND DISCUSSION

Analysis of samples with an automatic counter ABX Micros 60 showed statistically significant difference in the values for, hematocrit and leukocytes exposed relative to the unexposed group. There was no significant difference between the other quantitative parameters analyzed, such as hemoglobin, RDW and platelets. Table 1, below, shows the values obtained for mean ± standard deviation of hematological parameters analyzed, both of the exposed and non-exposed groups.

Table 1. Values of hematological parameters analyzed in the exposed and non-exposed groups.

Parameters \ Groups	NON-EXPOSED	EXPOSED	P Value*
Erythrocytes	4.84±0.31	5.34±0.52**	P< 0.0007
Hematocrit	44.65±2.88	48.74±4.51**	P< 0.0023
Hemoglobin	14.87±1.26	14.68±0.91	P< 0.8606
Leukocytes	5980±1111	7636±2681*	P< 0.0335
Platelets	240.1±62.67	246.5±61.85	P< 0.7660
RDW	13.06±0.53	13.29±0.43	P< 0.0083

Source: Direct Research. Mean ± Standard Deviation values, identifying significant **P<0.001 difference between erythrocytes and hematocrit and * P <0.05 for values from the Leukocyte Exposed group compared to the non-exposed group.

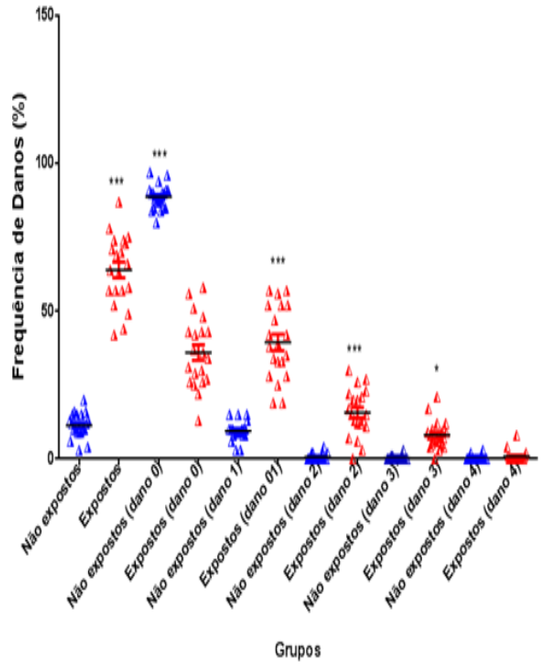
From the microscopic assessments of hematologic slides qualitative and quantitative analysis of white cells from the count of 100 leukocytes per individual were performed. These data are shown in Table 2 below.

Table 2. Frequency and percentage of qualitative and quantitative leukocyte changes.

Variable	Frequency Non-Exposed	Exposed Frequency	Percentage Non-exposed / Exposed
Lymphocytic atypia	0	07	0% / 35%
Neutrophil toxic granulation	0	05	0% / 25%
Eosinophilia	0	09	0% / 45%
Neutropenia	0	03	0% / 15%
Lymphocytosis	0	02	0% / 10%
Lymphopenia	0	02	0% / 10%
Without changes	20	05	100% / 25%

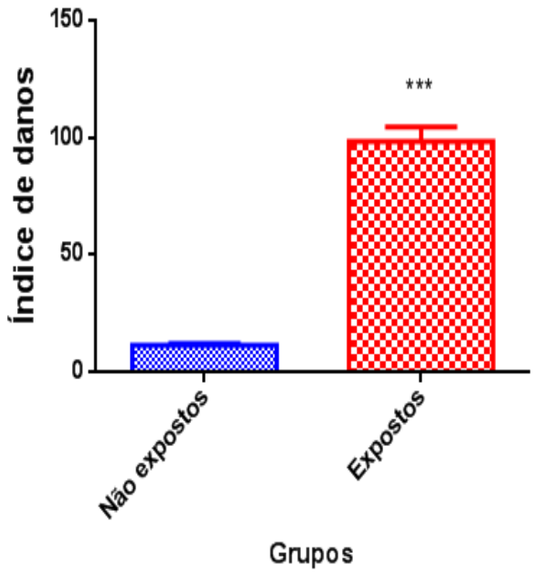
Source: Direct Research.

As far as the genetic damage in blood cells, the results obtained through the Comet Test are illustrated in the latter Figures 1 and 2, regarding the frequency and rate of damage, respectively.



Source: Direct Research.

Figure 1. Frequency of DNA damage in blood cells demonstrating significant (***P<0.0001) increase in the exposed group compared to the non-exposed group. Significant (*** P< 0.0001) number in the frequency of damage 0 in the non-exposed group in comparison to the exposed group. Significant Increase (*** P< 0.0001) of damage 01 in the exposed group when compared to the non-exposed group. Significant (*** P< 0.0001) increase in the frequency of damage 02 of the exposed group compared to the non-exposed group. Frequency of significantly (* P< 0.05) greater damage 03 the exposed group compared to the non-exposed group. Null frequency difference in the occurrence of damage 04 between the groups. One-way multiprocessing Tukey's Multiple Comparison Test.



Source: Direct Research.

Figure 2. Index of damage with significance (*** P< 0.0001) in the exposed group compared to the non-exposed group by the Student's T-test.

With the application of the questionnaire recommended by the International *Commission for Protection against Environmental Mutagens and Carcinogens* Table 3 represents their relationship of significance with the frequency of genetic damage obtained with Chi-square test (X^2).

Table 3. Correlation between Frequency of damage and variables using the Chi-square test. Source: Direct Research. Frequency, Percentage and Value Pearson Chi-Square of correlation

VARIABLE	Frequency Non-Exposed	Exposed Frequency	Percentage Non-exposed / Exposed	Variable X Freq. Damage (P Value)
Smoking	02	03	10% / 15%	P< 0.18
Alcohol	06	07	30% / 35%	P< 0.70
Medicines	04	05	20% / 25%	P< 0.43
Red Meat	20	20	100% / 100%	P< 0.81
White Meat	20	20	100% / 100%	P< 0.06
Genetic Disease	03	02	15% / 10%	P< 0.10
Vitamins	00	04	0% / 20%	P< 0.17

Source: Direct Research. between frequency of damage and variables with null value for all items.

In Brazil, more than 30 000 healthcare facilities producing infectious waste, and in most cities, the issue of handling and disposal are not resolved, and adds that some facilities are unaware of the amount and composition of the waste that they produce.[5]

The solid waste generated in health services account for today an issue that is becoming a focus of concern for health organizations, municipalities, technicians and researchers in the area. This can be seen by the quantity of existing laws and references, which advocate conducts of management of these wastes generated. Such waste constitute a permanent challenge, because in addition to the environmental issues inherent to any type of waste, incorporate a greater concern with regard to control of infections service providers environments, in the aspect of individual/occupational and public/environmental health.[6]

Based on the results obtained in the hematological evaluation, it was observed that individuals belonging to the exposed group showed no significant changes in the data relative to the values of hemoglobin, platelets and RDW, as shown in table 01, when compared to the non-exposed group. However, changes were observed in quantitative parameters: hematocrit (Figure 2), erythrocytes (Figure 1) and leukocytes (Figure 3); and qualitative (Table 2) in relation to the comparative non-exposed group.

The increase in the number of leukocytes does not predict disorders or pathologies, but rather the result of the body's response to eliminate potential pathogens, or when the individual is subjected to situations of physical and / or stressful effort, so the group evidenced with the test result (exposed) from the present study may have been influenced by some of these situations mentioned, since they are related to their work environment.

In this regard, it can be noted that the concomitant increase of hematocrit with leukocytes may have if given by both situations already cited as by care that must be taken during the workday, among them, the hydration and the use of Personal Protective Equipment (PPE's). The measurement of hematocrit allows evaluation practice and rapid of the overall state of individuals, but can present variation in severe cases of dehydration, because there is hemoconcentration, masking cases in which there is no anemia and need for drug interventions.[7]

In a study carried out [8] for the identification of pathogenic microorganisms present in MW the presence of coliforms *Salmonella typhi, Shigella sp., Pseudomonas sp., Streptococcus, Staphylococcus aureus and Candida albicans* was verified. The possibility of survival of viruses in the mass of solid waste has been proven for polio type I, hepatitis A and B, influenza, vaccinia.

The same authors also found the survival time in days of some etiologic agents in the mass of solid waste as follows. *: Entamoeba Histolytica* from 8 to 12 days, Leptospira *interrogans* from 15 to 43, 20 to 170 poliovirus, larvae of worms from 25 to 40, *Salmonella typhi* from 29 to 70, *Mycobacterium tuberculosis* from 150 to 180 and Ascaris *lumbricoides* (eggs) of 2.000 to 2.500. Table 02 shows that 45% of the individuals in the exposed group showed eosinophilia, 35% lymphocytic atypia and 25% neutrophil toxic granulation in their differential count.

The atypical lymphocytes found in 35% of individuals in the exposed group are, in their majority, activated T lymphocytes by infected B cells. At inflammatory sites they act as normal lymphocytes, playing a role in the immune response.[9] The presence and number of atypical lymphocytes are useful information, because they can guide the diagnosis for pathologies and/or infections by viruses, such as the Human Immunodeficiency Virus (HIV), Epstein Barr Virus (EBV), Cytomegalovirus (CMV), Herpes simplex, Rubella, Toxoplasmosis, Adenovirosis and Hepatitis A and B. [10]

Neutrophilic toxic granulation as evidenced in 25% of workers are small formations that appear on granules in the cytoplasm of neutrophils and reflect a disruption of the maturation of these with the azurophilic granules persistence in the mature cell stages, or they may be the result of endocytosis for toxic agents (bacteria, denatured serum proteins) to form new abnormal granules. The term "toxic" is used to indicate the operating status of many cells, which occurs in a variety of diseases, such as systemic infections, cancer, pneumonia, coma diabetic or liver poisoning, chemical and toxic states. [11- 12]

In 45% of the exposed group, it was observed that quantitatively an increase in the number of eosinophils in 100 leukocytes counted

per individual, in this way, such an increase in circulation is usually related to the parasitic diseases, allergic and inflammatory or the situations more rare, clonal or idiopathic, coursing with severe damage to tissues in consequence of eosinophilic infiltration. Thus, the defense cells such as lymphocytes, eosinophils and neutrophils in reactive character may predict a possible contact of workers exposed to pathogens, although not able to confirm the means of contact.

With regard to workers from the test group, 100% mentioned the use of PPE during their working day. It is recommended [13] that every device or product, for individual use used by the worker, intended for risk protection susceptible to threaten the safety and health at work is an Individual Protection Equipment.

The use of PPE is provided for in the labor legislation. The Consolidation of Labor Laws (CLL) provides for the obligation of the company to provide to employees, without charge, proper PPE to risks and in perfect state of conservation and operation, as well as the obligations of the employer to provide the PPE, and it is up to employees the responsibility for its use, storage and conservation.[13]

In accordance with Art. 4 (2), the Resolution no. 005/1993 the treatment method for hospital waste incineration, is the method by which occurs the burning these wastes at a temperature above 100ºC with excess oxygen. Studies have reported that heavy metals such as lead, cadmium, arsenic, mercury and chromium, are not destroyed during incineration, and are often released to the environment in ways even more concentrated and dangerous than the original trash.[14] In addition to being related to cause adverse effects on human health, the heavy metals can develop severe clinical conditions of the gastrointestinal system, peripheral circulatory and nervous, as well as neoplastic conditions in these organs. [15]

Figures 01 and 02 display the results that are related to the Frequency and the Index of damage, respectively, assessed by Comet Assay and as demonstrated, the group of workers exposed showed a significant increase of DNA damage when compared to the non-exposed group. In this respect, the literature with near incinerators populations demonstrate a potential exposure to certain compounds through inhalation of air and other means such as skin contact and eye mucous. Although few reports with workers occupationally exposed to incineration of hospital waste, were found in other countries (United Kingdom, Spain and Japan) significant increases in the levels of dioxins in tissues of individuals who live close to incinerators, probably as a result of exposure.

It is important to stress the importance of studies in populations exposed to physical, chemical agents or environmental, now that these can undergo mutations, cancer and birth defects. [16] Such causative agents are produced by man himself, although they are natural occurring toxic substances

from bacteria, fungi, plants and animals. The damage caused can be chemical, physical or biological and, in some cases, genetic. The toxic substances that act on genetic material, especially in DNA, causing changes in your code are called genotoxic or mutagenic, which may cause gene and/or chromosome mutations.[17]

The comet assay (SCGE assay - Single Cell Gel Electrophoresis assay) has been a tool increasingly used in studies of exposed populations by biomonitors by being an easy test, fast and highly sensitive in the detection of damage to genetic material. [18] Thus this test shows it is quite reliable in the genotoxic assessment of workers exposed to medical waste in this survey.

The damage observed in this study can be probably related to single and double breaks, DNA adduct formation and bridges intra and inter chain

able to be detected in the alkaline version of the comet assay. Despite some DNA damage being repaired, it is necessary the permanent biomonitoring for genotoxicity in MW, with the use of biomarkers for prevention of future DNA lesions, which may induce neoplastic growths in damaged somatic cells. In recent years the monitoring of genotoxic effects of chemicals in humans with the objective of assessing the risks have increased and as a result has been identified markers of human exposure to mutagens and carcinogens. [19]

The cells after the completion of the Comet Assay methodology have classification based on five classes, called class 0 to class 4 displayed in figure 06 the results and shows all the types of damage that the test makes it possible to visualize them. The class 0 corresponds to cells that do not have suffered any kind of damage; class 1 the comets with minimal damage; the class 2 with average damage; the class 3 the comets with intense damage and, finally, the class 4 the comets with maximum damage.[20]

It was not possible to divide the sample into subgroups that would deliver to the authors, a correlation between the frequency of damage with variables such as age, smoking, alcohol, drug use, red meat consumption, consumption of white meat, hereditary diseases and consumption of vitamins (table 03). Therefore, the data obtained are possibly related to the exposure of these individuals to medical waste.

CONCLUSION

The data obtained in this work with the realization of hematological and genotoxic tests, have provided evidence that the individual focus of research present reaction processes towards exposure to some agent. It is added that they present risks of genetic instability; probably it is likely as a result of their medical waste duties, because there was no significance with the habits and lifestyles reported. It emphasizes the need for continuous assessment and the use of other biomarkers of the study population in order to prevent the emergence of possible future neoplasms arising from their occupational exposure.

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