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Nutrición Hospitalaria



Trabajo Original

Pediatría

Assessment of micronucleus and oxidative stress in peripheral blood from malnourished children

Evaluación de micronúcleos y estrés oxidante en sangre periférica de niños desnutridos

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Abstract

Introduction: malnutrition is one of the most common health problems among children in underdeveloped countries, including Mexico. Previous studies have indicated increased genetic damage in malnourished humans and animal models, but the essential mechanisms remain unclear. In the present study, we assessed the effects of malnutrition on the frequency of micronucleus (MN) in reticulocytes (RET) from the peripheral blood of well-nourished uninfected (WN), well-nourished infected (WNI), moderately malnourished infected (UNM) and severely malnourished infected (UNS) children. Moreover, lipid peroxidation and the antioxidant status were evaluated to investigate the role of oxidative processes in malnutrition-associated genotoxicity.

Methods: the antioxidant status of the study population was determined by measuring superoxide dismutase (SOD) in the red blood cells and glutathione peroxidase (GPX) in whole blood.

Results: the UNS and UNM groups have increased percentages of MN-RET compared to the WNI group. Moreover, the data showed a significant increase in lipid peroxidation and a decrease in erythrocyte SOD activity and GPX activity in the malnourished group compared to the well-nourished infected children.

Conclusion: the data suggest that the antioxidant system was impaired in the cells of malnourished children and that oxidative stress causes a significant increase in DNA damage, as evaluated by the MN-RET frequency.

Key words:

Oxidative stress.
Malnourished
children. Micronuclei.
Glutathione
peroxidase.
Superoxide
dismutase.

Resumen

Introducción: la desnutrición es uno de los principales problemas de salud entre los niños de los países en desarrollo, incluido México. Estudios previos han mostrado que existe un incremento en el daño genético en humanos y modelos animales desnutridos, pero los mecanismos por los que se desencadena aún son poco claros. En el presente estudio, evaluamos los efectos de la desnutrición en la frecuencia de micronúcleos (MN) en reticulocitos (RET) de sangre periférica de niños bien nutridos no infectados (WN), bien nutridos infectados (WNI), desnutridos moderados infectados (UNM) y con desnutrición severa e infecciones (UNS). Asimismo, se evaluaron la lipoperoxidación y la capacidad antioxidante para investigar el papel del proceso oxidativo en la genotoxicidad asociada a la desnutrición.

Métodos: la capacidad antioxidante de la población de estudio fue determinada midiendo la superóxido-dismutasa (SOD) en los eritrocitos y glutatión peroxidasa (GPX) en sangre completa.

Resultados: los niños UNS y UNM tienen alto porcentaje de MN-RET comparados con el grupo WNI. Además, los datos mostraron un incremento significativo en la lipoperoxidación y disminución en la actividad de SOD y GPX en el grupo de niños desnutridos comparados con el grupo de niños bien nutridos infectados.

Conclusión: los datos sugieren que el sistema antioxidante está deteriorado en las células de los niños desnutridos y que el estrés oxidante causa un incremento significativo en el daño al ADN, el cual se refleja en el incremento en la frecuencia de RET-MN.

Palabras clave:

Estrés oxidante.
Niños desnutridos.
Micronúcleos.
Glutatión peroxidasa.
Superóxido-
dismutasa.

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INTRODUCTION

Malnutrition (also known as undernutrition) is one of the most common health problems among children in developing countries, including Mexico. Malnutrition remains a major cause of morbidity and mortality, with the greatest impact in low-income countries. Epidemiological studies have shown that malnutrition produces a complex cycle of infection, altered nutrition and decreased immune resistance, which in turn promotes a state of malnutrition that favors the occurrence of more severe and frequent infections.

Numerous studies have associated malnourishment with DNA damage, for instance, increased frequencies of both sister chromatid exchange and chromosomal aberrations, as well as an increased frequency of micronuclei, were observed in malnourished rats (1,2). Our group has previously reported the relationship between DNA damage magnitude (assessed by the micronuclei frequency) and malnutrition grade in undernourished children (3). Even though these results provide strong support for malnutrition-induced genotoxicity, the mechanisms underlying DNA damage in malnourished organisms have not been elucidated.

Oxidative stress, defined as a disturbance in the pro-oxidant/antioxidant balance that favors pro-oxidants (4), has been demonstrated in several diseases. This damage is often called oxidative damage, and has been defined as the biomolecular injury caused by the attack of reactive species upon the constituents of living organisms (5). In particular, reactive oxygen and nitrogen species (ROS/RNS) are the major oxidants that react with DNA and lead to the formation of various lesions, including oxidized bases, abasic sites, and/or DNA strand breaks (6).

There is a large body of experimental evidence suggesting that oxidative processes may substantially contribute to the genotoxicity associated with various pathological conditions, such as diabetes mellitus (7), cancer (8) and other immunological responses (9).

Hence, an interesting working hypothesis might be that the increase in oxidative stress, resulting from elevated levels of free radicals generation and/or a reduced antioxidant response, may contribute to the DNA damage observed in malnourished children.

Increased oxidative damage results not only from increased oxidative stress but also from a failure to repair or replace damaged biomolecules, and mainly oxidative stress can result from decreased antioxidant levels (10). It was previously reported that kwashiorkor-type malnourished children present low reduced glutathione (GSH) levels (11).

Therefore, the correlation between increased oxidative damage and decreased antioxidant defense systems in malnourished children would lead to new insights into the management of this health problem.

The aim of this study was to determine if malnutrition and infection are associated with an increased micronuclei (MN) frequency in reticulocytes, since MN detection is a worldwide-validated assay used to quantify DNA damage (12). Malnutrition and infection were also correlated with erythrocyte hemolysis, as a marker of lipid peroxidation, and the antioxidant status was determined by measuring superoxide dismutase (SOD) in red blood cells and glutathione peroxidase (GPX) in whole blood.

MATERIALS AND METHODS

PATIENTS

Peripheral blood was collected from pediatric patients aged six to 72 months by simple random sampling. This study was approved by the Ethics and Biosafety Committee of the Iztapalapa Pediatric Hospital, Federal District Government, and was conducted in accordance with the Declaration of Helsinki. Signed informed consent was obtained from each participating child's parents.

Children were divided into four experimental groups based upon their nutritional and infectious status as follows: a) well-nourished healthy (uninfected) children (WN); b) well-nourished infected children (WNI); 3) moderately malnourished infected children (UNM); and 4) severely malnourished infected children (UNS).

Malnutrition degree was calculated according to the clinical signs and symptoms presented, as well as the weight-to-height deficit for Mexican children according to the tables previously published by Ramos-Galván (13). The bacterial infections were rigorously diagnosed based upon established medical criteria. Samples from children with viral diseases, birth defects, allergies and traumas were excluded. Similarly, samples with hemolysis, blood clots and/or poor cell quality/number were not included in this study. Uninfected WN children were outpatients at the same hospital and were studied as controls. The samples for the other groups were obtained during hospital admission, before treatment with drugs or supplements.

The age range, gender, weight and height, as well as infection type are described in table I for each group.

SAMPLE PREPARATION

Venipuncture blood samples were collected into heparin-containing tubes as an anticoagulant (250 U/ml, Microlab, Mexico City, Mexico), transported on ice to the laboratory and processed within three hours.

DNA DAMAGE ASSESSMENT: MICRONUCLEUS FREQUENCY

After obtaining blood samples, an aliquot (100 µl) of peripheral blood was diluted (1:2) in BBS (bicarbonate buffered saline solution: 0.9 g NaCl + 0.0444 g NaHCO₃ in 100 ml distilled H₂O) at pH 7.5. A 100 µl aliquot was removed from the diluted sample and fixed by vigorous shaking in cryogenic tubes containing 2 ml of ultra-cold (-70 °C) methanol (Merck Millipore, Darmstadt, Germany). The samples were stored at -70 °C for at least 24 hours prior to cell staining and further analysis.

CELL STAINING AND FLOW CYTOMETRY ANALYSIS

To process the samples, the cryogenic tubes were removed from the freezer and resuspended. A 1-ml aliquot was

Table I. Clinical characteristics of the study groups

Study group (n)	Mean + SD Age in months (range)	Mean + SD Weight in kg (range)	Mean + SD Height in cm (range)	Mean weight deficit (%)	Type of infection (n)
WN (10)	45.1 ± 16.65 (19-60)	15.47 ± 2.89 (17-22)	98.3 ± 18.49 (90-123)	< 10	No infection (10)
WNI (9)	18.44 ± 1.10 (9-41)	10.04 ± 2.55 (7-10.3)	70.22 ± 28.33 (71-105)	<10	Respiratory (6) Gastrointestinal (3)
UNM (12)	27.6 ± 23.27 (6-60)	14.14 ± 15.35 (5.3-17.5)	79.3 ± 23.11 (65-128)	28 (27-32)	Respiratory (9) Gastrointestinal (3)
UNS (10)	15.85 ± 12.2 (6-44)	5.03 ± 1.02 (4-6.1)	53.7 ± 22.10 (63-60)	51.2 (37-75)	Respiratory (6) Gastrointestinal (3) Mixed* (1)

WN: well-nourished uninfected children; WNI: well-nourished infected children; UNM: moderately malnourished infected children; UNS: severely malnourished infected children. The number of children in each group (n), the averages and ranges for their age, weight, height, and weight deficit, as well as the number of children with specific infections. *The child presented both gastrointestinal and respiratory infections.

removed, washed with BBS at 4 °C and centrifuged at 600 × g for five minutes at 4 °C. The pellet was resuspended, and a 25-μl aliquot was treated with 1 mg of RNase/ml and 5 μl anti-CD71-fluorescein isothiocyanate (anti-CD71-FITC) (to label the RETs and to differentiate them from erythrocytes) (15). All of the samples were incubated in the dark at 4 °C for 40 min followed by room temperature incubation for 90 min. After these incubations, 500 μl of BBS was added, and the sample was analyzed by flow cytometry. Before acquisition of the events, 2 μl (2 μg/ml) of cold IP (for detection of DNA micronuclei-specific fluorescence) was added to detect MN (14).

Flow cytometric analysis was conducted using a FACSCalibur™ flow cytometer (Becton Dickinson, Immunocytometry Systems, San Jose, CA, USA) equipped with an argon laser (488 nm excitation). The data were analyzed with the CellQuest™ Software (version 3.0.1, Becton Dickinson) for data acquisition and analysis (BD Biosciences). We followed the strategy reported by Cervantes et al. (3). Five hundred thousand events were acquired per sample. The percentages of RETs and MN-RETs were calculated according to the method proposed by Litron Laboratories (Rochester, NY, USA).

OXIDATIVE DAMAGE ANALYSIS IN ERYTHROCYTES FROM PERIPHERAL BLOOD

Evaluation of lipid peroxidation

Lipid peroxidation was assessed as an oxidative stress marker using the hemolysis assay reported by Catal F et al. (15) and modified as follows, 250mL of each sample were mixed with 2,2-azo-bis(2-amidinopropane) dihydrochloride (AAPH Cayman Chemicals, USA) for three hours. AAPH is a water-soluble azo compound that has been extensively used as a free radical generator during lipid peroxidation assays and antioxidants characterization (16,17). AAPH decomposition produces molecular nitrogen

and 2 carbon radicals. The carbon radicals may combine to produce stable products or react with molecular oxygen to produce peroxy-radicals. AAPH half-life is approximately 175 h (37 °C, at neutral pH), thus the rate of free radical generation in solution is essentially constant during the first several hours.

One ml of each sample was mixed with 2 ml of phosphate-buffered saline (PBS). The mixture was centrifuged for ten minutes at 1,500 g and the plasma was eliminated. The erythrocyte pellet was washed twice with 2 PBS ml, homogenized by inversion and centrifuged after each wash. A 250 μl sample of the erythrocyte pellet was removed and placed in three polypropylene tubes. PBS (250 μl) was added to the first tube, which was the negative control. AAPH (150 mM) was added to the second tube, and 250 μl of distilled water was added to the third tube in order to hemolyze, and was used as a positive control. All tubes were incubated at 37 °C for three hours. Each hour, 5 μl of the mixture were removed and diluted (1:200) with PBS. The mixture was centrifuged for five minutes at 1,500 g. A 1,000 μl sample from the uppermost part was taken and placed in a cell. The absorbance was read at 410 nm in a Spectra UV-visible Beckman DU® 650 spectrophotometer. The percentage of hemolysis in each sample was calculated and referenced to the positive control.

ANTIOXIDANT RESPONSE IN PERIPHERAL BLOOD

Analysis of superoxide dismutase activity

SOD enzymatic activity in erythrocytes was evaluated using the quantitative *in vitro* determination of superoxide dismutase (SOD) RANSOD kit (Randox Laboratories Ltd., County Antrim, UK), according to the manufacturer's instructions.

Heparinized whole blood (500 μl) was centrifuged for ten minutes at 1,500 g and the plasma was then aspirated. The erythrocyte pellet was washed four times with 4 ml of a

0.9% NaCl solution, homogenized by inversion and centrifuged at 1,500 g for ten minutes after each wash.

The washed erythrocytes were then brought to 2.0 ml with cold redistilled water, mixed and left to stand at 4 °C for 15 minutes. One hundred μ l of the diluted erythrocytes were mixed with 0.01 mM phosphate buffer. Fifteen μ l of these diluted erythrocytes were removed and mixed with 1.7 ml of the Mixed Substrate (reconstituted according to the manufacturer's instructions) and 250 μ l of xanthine oxidase. The absorbance was determined after 30 seconds (A_1) and three minutes (A_2) using a Beckman DU® 650 spectrophotometer at a wavelength of 505 nm and temperature of 37 °C. The activity was expressed as SOD units/ml of blood.

Analysis of glutathione peroxidase activity

GPx enzymatic activity was determined using the Paglia and Valentine method (18). GPx catalyzes the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP⁺. The absorbance decrease at 340 nm was measured.

The GPx activity in whole blood was evaluated using the quantitative *in vitro* GPx determination RANSEL kit (Randox Laboratories Ltd., County Antrim, UK), according to the manufacturer's instructions. Fifty μ l of whole blood were diluted with Ransod Sample Diluent, mixed and incubated at 37 °C for one minute. Twenty μ l of diluted blood, 1 ml of mixed substrate (glutathione 4 mmol/l, glutathione reductase > 0.5 U/l, NADPH 0.34 mmol/l) and 40 μ l of cumene (cumene hydroperoxide 0.18 mmol/l) were mixed. The absorbance of the mixture was determined after one minute and two minutes using a Beckman DU® 650 spectrophotometer at 650 nm. The GPx activity was expressed as U/l of blood.

STATISTICAL ANALYSIS

For each group, we calculated the mean and the standard error of the mean for the RET+ and MN-RET+ frequency, hemolysis percentages, USOD/ml in red blood cells and U/l GPx in whole blood. The frequency of MN between the study groups was compared using a Kruskal-Wallis test, followed by Mann-Whitney U test and Bonferroni multiple comparison tests. Statistical analyses were performed using NCSS version 07.1.9 and NOPANDEV software packages. Differences with a p value of less than 0.05 were accepted as statistically significant.

RESULTS

PATIENTS

Forty-one children, 17 girls and 24 boys, 6-60 months old, participated in this study and were classified in four groups as follows:

a) well-nourished healthy (uninfected) children (WN); b) well-nourished infected children (WNI); c) moderately malnourished infected children (UNM); and d) severely malnourished infected children (UNS). Their age range, weight and height, as well as infection type, are described in table I.

RETICULOCYTE (RET) FREQUENCY

The RET frequencies for the individual children in the various study groups are shown in figure 1. The RET average percentage for the WN group was $1.77 \pm 0.42\%$, which was significantly lower than the value found for the WNI group, $2.73 \pm 0.43\%$ ($p < 0.05$). The UNM group had a RET average percentage of $2.45 \pm 0.41\%$. The UNS group showed RETs increased frequency compared with the WN and WNI groups, and had an average of $3.93 \pm 0.97\%$ ($p < 0.05$).

MN-RET FREQUENCY

The MN-RET mean frequency was $0.49 \pm 0.09\%$ for the WN group, which was significantly lower than that of the WNI group, with an average of $1.11 \pm 0.2\%$ ($p < 0.05$). These values indicate that the MN-RET frequency in the WNI group increased due to infection. The UNS group had a MN-RET higher frequency, with an average of $2.92 \pm 0.48\%$, compared to the UNM group, which had an average of $1.69 \pm 0.30\%$. However, this difference was not statistically significant. Moreover, the MN-RET percentage in the UNS group was significantly higher than that of the WNI group (2.92 and 1.11% , respectively; $p < 0.05$). When the MN-RET frequency was compared with those of the WN group and the UNM and UNS groups, both malnourished groups showed a statistically significant increase ($p < 0.05$ for both groups) (Fig. 2).

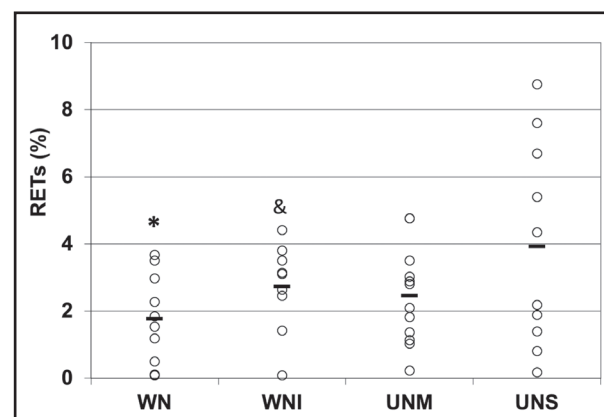


Figure 1.

Reticulocyte frequency in the peripheral blood samples from well-nourished children (WN), well-nourished infected children (WNI), moderately malnourished infected children (UNM) and severely malnourished children with infections (UNS). The data are presented as the average \pm standard error. Significant differences: *WN vs WNI, UNM, UNS and WNI vs UNS ($p < 0.05$).

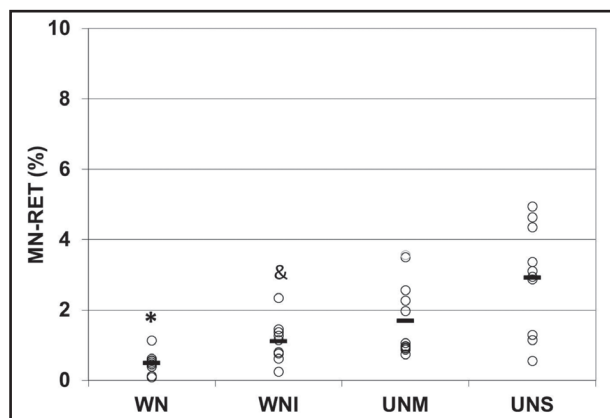


Figure 2.

Micronucleated reticulocyte frequency in the peripheral blood samples of well-nourished children (WN), well-nourished infected children (WNI), moderately malnourished infected children (UNM) and severely malnourished children with infections (UNS). The data are presented as the average \pm standard error. Significant differences: *WN vs WNI, UNM, UNS and WNI vs UNM and UNS ($p < 0.05$).

OXIDATIVE DAMAGE ANALYSIS IN THE ERYTHROCYTES FROM PERIPHERAL BLOOD

The data presented in figure 3 show that the UNS group significantly increased the hemolysis percentage, with an average of $119.19 \pm 31.41\%$ compared to the UNM group, which had an average of $87.96 \pm 21.37\%$. The hemolysis percentage was $78.63 \pm 11.01\%$ for the WN group, which was lower than that of the WNI group, with an average of $52.81 \pm 14.25\%$. The data showed that the hemolysis percentage of the UNM and UNS groups were significantly higher than that of the WNI group

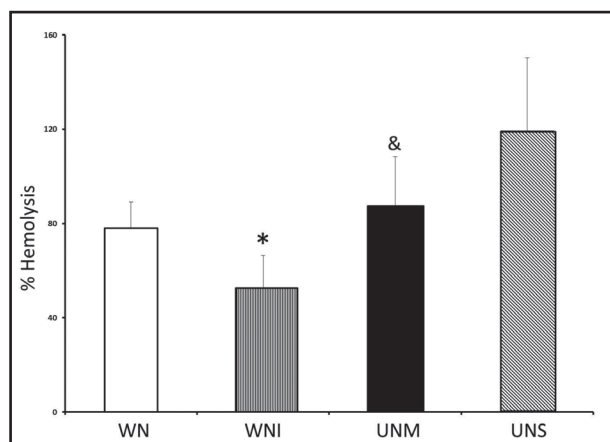


Figure 3.

Percent of hemolysis in the peripheral blood samples of well-nourished children (WN), well-nourished infected children (WNI), moderately malnourished infected children (UNM) and severely malnourished children with infections (UNS). The data are presented as the average \pm standard error. Significant differences: *WNI vs UNM and UNS; UNM vs UNS ($p < 0.05$).

($p < 0.05$). These results suggest that the hemolysis percentages in these two groups increased due to malnutrition. Moreover our results show a positive correlation between the MN-RET frequency and lipid peroxidation ($r = -0.973$) in severe malnourished children.

ANTIOXIDANT RESPONSE IN PERIPHERAL BLOOD

Superoxide dismutase activity (SOD)

Figure 4 shows that the BNI group had a higher SOD activity compared to the BN group (362 ± 69 USOD/ml of blood and 428 ± 38 USOD/ml, respectively). Nevertheless, this difference was not statistically significant. The SOD activity determined in the UNS group blood was 230 ± 26 USOD/ml. This result was, lower than the one obtained for the UNM group (300 ± 32 USOD/ml of blood). When the SOD activity was compared among the well-nourished groups (WN and WNI) and the malnourished groups (UNM and UNS), both malnourished groups showed a statistically significant decrease ($p < 0.05$ for both groups) (Fig. 4). These data suggest that the SOD activity in the malnourished groups decreased due to malnutrition.

Glutathione peroxidase activity (GPx)

As shown in figure 5, the GPx activity determined for the UNM and UNS groups ($12,480 \pm 339$ U/l and $11,727 \pm 3,354$ U/l of blood, respectively) significantly decreased compared to the WNI group ($14,337 \pm 1,957$ U/l of blood, $p < 0.05$). The data show that the BNI group had a similar SOD activity compared to the

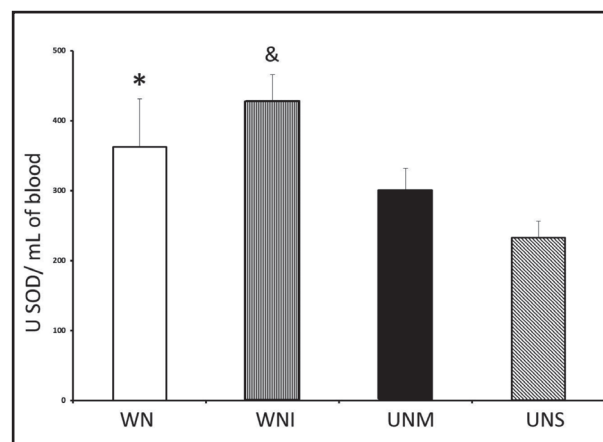


Figure 4.

SOD activity in the peripheral blood samples of well-nourished children (WN), well-nourished infected children (WNI), moderately malnourished infected children (UNM) and severely malnourished children with infections (UNS). The data are presented as the average \pm standard error. Significant differences: *WN vs UNM and UNS; WNI vs UNM and UNS ($p < 0.05$).

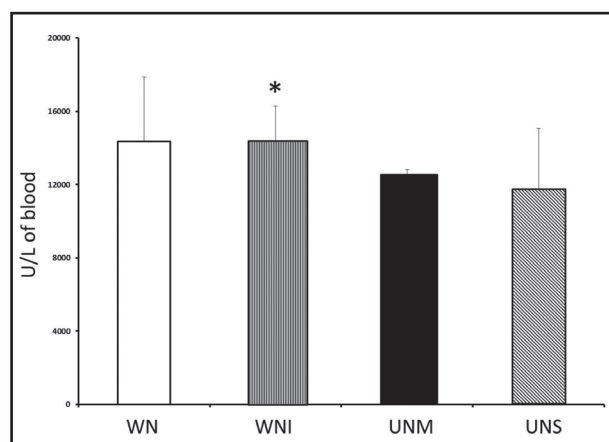


Figure 5.

GPx activity in the peripheral blood samples of well-nourished children (WN), well-nourished infected children (WNI), moderately malnourished infected children (UNM) and severely malnourished children with infections (UNS). The data are presented as the average \pm standard error. Significant differences: *WNI vs UNM and UNS ($p < 0.05$).

BN group ($14,354 \pm 3,509$ U/L of blood), suggesting that GPx activity decreased due to malnutrition.

Negative correlations were obtained between lipid peroxidation and SOD ($r = -0.762$) and between GPx and lipid peroxidation ($r = -0.700$) in severe malnourished children, supporting the fact that an energy-deficient state may result in enhanced lipid peroxidation and decreased antioxidant enzyme activities.

DISCUSSION

Protein energy malnutrition is one of the major health problems in developing countries, since the harmful effects produced by the insufficient intake of nutrients can negatively impact the organism growth and development. Recent data from Cervantes et al. (3) showed a higher MN frequency in reticulocytes from the peripheral blood of moderate and severely malnourished children compared to well-nourished infected children. Furthermore, it has been shown that malnourished rats have a high MN-RETs frequency, pointing towards an increased genetic damage during malnourishment (1). These and other studies have reported genetic damage in malnourished humans and animal models, but the essential mechanisms remain unclear.

Therefore, our aim was to correlate the DNA damage (MN-RET frequency) with the activity of antioxidant enzymes and lipid oxidative damage in well-nourished infected and malnourished infected children.

The average RET percentage for the WNI group was significantly higher than that of the WN group, suggesting that infection may increase the RETs frequency. These data are in agreement with previous reports showing that children with gastrointestinal or respiratory infections exhibit an increased RET percentage (3).

In relation to children with malnutrition and infection, the UNM average is similar to that of the WNI group, implying that moderate malnutrition does not affect circulating RETs number. However, the severely malnourished group had an increase in RETs average when compared to the WN and WNI groups, suggesting that children with severe malnutrition have hematologic alterations. An early study also reported that anemic individuals increased the circulating RETs content (19), and it is also known that anemia is often associated with malnutrition (20).

On the other hand, the 0.49% MN-RET frequency determined in well-nourished children concurs with what has been previously reported (3,21). At the same time, the MN-RETs higher frequency of WNI when compared to the WN group also coincides with previous reports (3).

Epidemiological studies have shown that most bacterial gastrointestinal infections in children from developing countries are caused by *Streptococcus pneumonia* and *Escherichia coli* (22). Therefore, the augmented frequency of MN in the WNI group may be due to molecules released by infectious agents that provoked genetic damage (23). Indeed, it has been shown that *E. coli* produces cytotoxic enterotoxins that affect protein synthesis and inhibit DNA repair (24). Moreover, it has been demonstrated that *S. pneumonia* produces a DNA recombinase, which destabilizes the host DNA and renders it susceptible to single strand breaks (25).

Our data showed a significant increase in the two malnourished infected groups compared to the WN and WNI groups. The inclusion of an undernourished uninfected child group was not possible because undernourished children frequently suffer from infections. Hence, we assumed that the comparison between the WN and WNI groups shows the changes associated with the infection, and that the comparison between the WNI and malnourished groups shows the alterations related to malnutrition.

The higher frequency of MN-RETs (DNA damage) in malnourished children cells may be attributed to several causes, such as micronutrients malabsorption (e.g., folate) and/or oxidative stress. It is known that folic acid deficiency promotes genetic damage via DNA breakage (26). Therefore, the higher MN-RET frequency observed in malnourished children might be related to a folate deficiency. Further studies will be necessary to address the relationship between levels of specific micronutrients, oxidant stress and genome stability, and additionally, to support the relation between oxidant stress and different types of malnutrition.

In relation to oxidative stress, it is known that infectious agents (e.g., bacteria) trigger an immune response in the host, which increases free radicals generation (27). Moreover, several studies have shown that serum antioxidants concentration is significantly lower in severely malnourished children (15,28) as well as increased lipid peroxidation (29), but there are no reports relating infections and oxidative stress in malnourished children.

Our data showed increased lipid peroxidation in malnourished children peripheral blood compared to WN and WNI children. This is an important factor that correlates with our findings of increased MN-RETs and DNA damage in malnourished children.

Infections in malnourished children may activate the production of free radicals, such as superoxide anions, hydrogen peroxide

and myeloperoxidase. Leukocytes and other phagocytic cells fight bacteria, parasites, and virus-infected cells by destroying them with NO, O₂, and H₂O₂ (30,31). These molecules contribute to lipids, proteins and nucleic acids oxidation. Some studies of children with kwashiorkor have demonstrated decreased concentrations of antioxidant vitamins, such as vitamin A and vitamin E, and elevated lipid peroxidation levels (32).

However, contradictory evidence has been reported by Partadiredja et al. (33), who found that malnutrition during the gestation and pre-weaning period in mice had no significant effect on lipid peroxidation. In contrast, other studies have reported an increase in lipid peroxidation following diet restriction (34-36).

Okunade et al. (37) showed that lipid peroxidation in kwashiorkor children erythrocytes was higher than the lipid peroxidation found in the well-nourished infants' erythrocytes. Lenhart et al. (38) also confirmed excessive lipid peroxidation in kwashiorkor children. Our results agree with these results since lipid peroxidation in the UNM and UNS groups were significantly higher than in the WNI group.

Previous studies have shown that lipid peroxidation end products binding to DNA bases may create mutagenic lesions (39), supporting the relationship between DNA damage and elevated lipid peroxidation.

Oxidative damage in malnourished children may be related to impaired antioxidant system function. Our data showed an increase in SOD activity in the WNI group compared to the WN group. The SOD activity for the UNS group was lower (1.3-fold decrease) than in the UNM group. When the SOD activity was compared between the WN-WNI groups and UNM-UNS groups, both malnourished groups showed a statistically significant decrease compared to the well-nourished groups.

The same was observed in relation to GPX activity, which was significantly reduced in moderate and severely malnourished children in comparison to well-nourished infected children. Similar data have also been identified in other studies where GSH levels in malnourished children were lower when compared with the controls (15,32,40).

Moreover, Partadiredja et al. (33) reported that the changes in the GSH levels are an important indication of the anti-oxidant defense mechanisms during early life malnutrition.

Boşnak et al. (29) found that chronic inflammation may promote an imbalance between oxidant and antioxidant mechanisms in malnutrition because malnourished children are prone to developing frequent infections. They did not find a significant difference between malnourished infected children and well-nourished infected children. In contrast, in the present study, a significant difference between both groups was detected (WNI vs UNM and UNS). Therefore, malnutrition might be a cause of the elevated oxidant stress, which may provoke oxidative damage and increased MN formation. The damage observed may be due to the deficiency of several essential nutrients required for protein synthesis that are associated with DNA integrity, impaired DNA repair mechanisms, or unavailability of molecules necessary to protect cells against DNA oxidative damage.

In conclusion, the data from this study support that moderate and severe malnutrition in children is associated with increased

MN-RET frequency. Negative correlations were obtained between lipid peroxidation and SOD-GPx activities in severe malnourished children, supporting the fact that an energy-deficient state may result in enhanced lipid peroxidation and decreased antioxidant enzyme activities. Hence, malnutrition may be considered as a pro-oxidant condition because it increases the levels of lipid peroxidation and decreases antioxidant activity.

Therefore, increased oxidative stress may represent a mechanistic link between malnutrition and genotoxicity.

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