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Chapter 5

GENOTOXIC EFFECTS OF MALNUTRITION AND INFECTIONS IN ARGENTINEAN CHILDREN

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ABSTRACT

The purpose of this study was to evaluate the relationship between malnutrition, bacterial infections and citogenetic damage. In order to obtain the frequencies of structural chromosomal aberrations (SCAs) in peripheral blood lymphocytes, we sample malnourished and eutrophic children with and without bacterial infections. Fifty infants concurrent to the Hospital Interzonal de Agudos y Crónicos Dr. Alejandro Korn, La Plata, Argentina were included in this study; 14 malnourished and non-infected (MN), 11 malnourished and infected (MI), 18 eutrophic and non-infected (EN) and 7 eutrophic and infected (EI). Children aged 1-60 months. Anthropometric and clinic evaluation were performed to assess nutritional condition. Before blood collection, we interviewed each individual's parent to complete a semi structural survey specifying age,

dietary habits, bacterial diseases; previous exposure to diagnostic x-rays; and use of therapeutic drugs. After 48 hours, 100 cultured lymphocytes were analyzed per individual. Statistical analysis was performed by the Epi Info 6.0, through the Pearson $\chi 2$ Test ($p<0.05$). No significant differences were found in total SCA frequency between MN and MI (14.2% vs. 15.1%). In MN children the total SCA frequency was four times higher than that of EI ones (14.2% vs. 3.33% $p<0.001$), and eight times higher than that of EN ones (14.2 vs. 1.8 <0.001). As well, in MI children the total SCA frequency was five times higher than that of EI ones (15.1% vs. 3.33% $p<0.001$) and eight times higher than that of EN ones (15.1 vs. 1.8 <0.001). Meanwhile the total SCA frequency was two times greater in EI than in EN children (3.33% vs. 1.88% $p<0.05$). Results presented here showed an increase frequency of SCA not only in relation with malnutrition but also with the presence of bacterial infections. However the effect of malnutrition was more important than the other. It is difficult to discern whether structural chromosome aberrations are due to malnutrition per se, bacterial or viral infections, or all of these factors acting on malnourished tissues. DNA damage could be attributed to several reasons: severe deficiency of essential nutrients (i.e. zinc, iron, and vitamin A) required in the synthesis of DNA maintenances factors; decline of repair mechanisms; and/or the absence of specific substances needed to protect the cell against oxidative DNA damage. In conclusion, mutagenic agents cause chromosomal changes more easily in an altered environment.

Keywords: Primary Protein Energy Malnutrition, Bacterial Infections, Structural Chromosome Aberrations, Children

INTRODUCTION

Growth was defined as a process resulting from the continuous and complex interaction of inheritance and environment (Tanner, 1986). In developed countries, genetic factors are responsible of most of growth retardations. On the contrary, in developing countries, nutritional deficit is frequently the cause of growth retardations. Malnutrition occurs when there is a cellular imbalance between the supply of nutrients and energy and the body's demand for them to ensure growth, maintenance, and specific functions (Waterlow, 1996). In this sense the term protein-energy malnutrition applies to a group of related disorders that develop in children and adults whose consumption of protein and energy (measured by calories) is insufficient to

satisfy the body's nutritional needs (primary PEM). PEM may also occur in persons who are unable to absorb vital nutrients or convert them to energy essential for healthy tissue formation and organ function (secondary PEM) (Más Vida, 2002; Padula et al., 2009). PEM results from food insufficiency as well as from poor social and economic conditions, children with PEM lose their resistance to infectious diseases due to immune system disorders (Dulger et al., 2002).

In addition, malnutrition has been associated with high levels of DNA damage as a result of various factors, including the possibility that the cells of malnourished children are more susceptible to environmental damage (Gonzalez et al., 2002; Padula et al ., 2004; Padula et al., 2009). In this regard, some authors (Betancourt et al., 1995; Ortiz et al., 1997; Gonzalez et al. 2002a and b) reported an increase in DNA damage in malnourished children with bacterial infections with respect to eutrophic children with bacterial infections.

The purpose of this chapter was to evaluate the relationship between malnutrition, bacterial infections and citogenetic damage, in children peripheral blood lymphocytes.

MATERIALS AND METHODS

Experimental Procedure

Fifty primary malnourished infants concurrent to the Consultorio del Niño Sano of the Hospital Interzonal de Agudos y Crónicos Dr. Alejandro Korn, La Plata, Argentina were included in this analysis; 14 malnourished and non-infected (MN), 11 malnourished and infected (MI) (with gastrointestinal or respiratory bacterial infections), 18 eutrophic and non-infected (EN) and 7 eutrophic and infected (EI). Children were aged 1 to 60 months. Anthropometric and clinic evaluation were performed to assess nutritional condition. Before blood collection, we interviewed each individual's parent to complete a semi structural survey specifying age, dietary habits, bacterial diseases; previous exposure to diagnostic x-rays; and use of therapeutic drugs for 1 month before the study. Also, children with anemia or signs of vitamin deficiency were not included in this study. Specific written information about the aims of the study was provided to all participants.Written informed consent was obtained from the participants' parents (Ley Provincial Nº 11044).

Cytogenetic Analysis

Heparinized venous blood samples were used to obtain lymphocytes from the participants and set up 2 replicate cultures, using 1 ml total blood in 9 ml RPMI 1640 medium (Gibco-Invitrogen Buenos Aires, Argentina) containing 1% phytohemagglutinin (Gibco-Invitrogen), 100 IU penicillin, and 100 μg/ml streptomycin (Sigma, St. Louis, MO). The cultures were incubated at 37°C and 5% CO^2 for 48 hours. Two hours before harvesting, colchicine was added at a final concentration of 0.1 g/ml. The cells were harvested by centrifugation. Metaphases were obtained by routine protocols and stained with 5% Giemsa, (Spectrum Chemicals, Gardena, CA). Structural chromosome aberrations were scored in 100 metaphases per individual by using a blind analysis: one investigator numerically identified the samples, and another scored the aberrations. Only metaphases with 46 chromosomes were considered. The identification of SCA was carried out by following the criteria recommended by Archer and coworkers (1981) and the World Health Organization (1985). Mitotic Index (MI) was calculated. Statistical analysis was performed using the Epi Dat 3.0 (OPS-OMS, 2003), through «Test de Diferencias entre dos proporciones muestrales». The differences were considered significant if the probability values were less than .05.

Anthropometric Evaluation

Height (H), weight (W), mid upper arm circumference (MUAC), triceps (TS) and subscapular skinfold (SS) were measured. The height for age index (H//A), the weight for age index (W//A), the weight for height index (W//H), the mid upper arm circumference for age index (MUAC//A), the triceps skinfold for age index (TS//A) and the subscapular skinfold for age index (SS//A) were calculated. Variables were introduced into the EpiInfo 6.04 program (CDC-OMS, 2001) and transformed into z-scores, using the NCHS anthropometric standards as reference (1977). A z-score of less than –1.1 was the cut-off point to determine the prevalence of stunting, underweight and wasting respectively. Malnutrition degrees were established according to Torun and Chew (1994). For children younger than 2 years, the H//A and W//A, stunting and underweight indicators, were used. H//A and W//H, stunting and wasting indicators modified from Waterlow (1976), were applied for the others. These classification include the following classes: 1) normal:

W//H adequate with normal stature, 2) stunting: W//H adequate with low stature, 3) wasting: W//H low with normal stature, 4) stunting and wasting: W//H low with low stature.

RESULTS

The anthropometric classification of the malnourished children is shown in Tables 1 and 2. Table 3 shows the results of cytogenetic analysis. No significant differences were found in total SCA frequency between MN and MI (14.2% vs. 15.1%). In MN children the total SCA frequency was four times higher than that of EI ones (14.2% vs. 3.33% $p<0.001$), and eight times higher than that of EN ones (14.2 vs. 1.8 <0.001). As well, in MI children the total SCA frequency was five times higher than that of EI ones (15.1% vs. 3.33% $p<0.001$) and eight times higher than that of EN ones (15.1 vs. 1.8 <0.001). Meanwhile the total SCA frequency was two times greater in EI than in EN children (3.33% vs. 1.88% $p<0.05$).

Table 1. Anthropometric evaluation of malnourished children using H//A, W//A, W//H indexes

	<2 y old					>2 y old						
	Underweight		Underweight and Stunting			Wasting		Wasting and Stunting			Stunting	
Sex	D1	D2	D1	D2	D3	D1	D2	D1	D2	D3	D1	D2
Female	1	1	1	1	0	1	1	0	1	0	0	0
Male	0	0	1	1	0	0	0	0	0	1	0	1
Total	1	1	2	2	0	1	1	0	1	1	o	1

D1: degree 1; D2: degree 2; D3: degree 3.

Table 2. Anthropometric evaluation of malnourished children using mid upper arm circumference for age index (MUAC//A), triceps skinfold for age index (TS//A) and subscapular skinfold for age index (SS//A)

	MUAC//A			TS//A			SS//A		
Sex	E	LR	VLR	E	LR	VLR	E	LR	VLR
Female	0	1	6	1	6	0	1	4	2
Male	0	0	4	1	1	2	1	0	3
Total	0	1	10	2	7	2	2	4	5

E: euthrophic; LR: low reserve; VLR: very low reserve.

Table 3. Structural chromosome aberrations (SCA) in malnourished and non-infected (MN), malnourished and infected (MI), eutrophic and non-infected (EN) and eutrophic and infected (EI) children

	MN		MI		EN		EI	
SCA	Absolute	Percentage	Absolute	Percentage	Absolute	Percentage	Absolute	Percentage
Chtg	49	3.83	45	4.41	9	0.52	8	1.33
Chrg	14	1.09	8	0.78	4	0.23	0	0
Chtb	48	3.75	35	3.43	4	0.23	3	0.5
Chrb	6	0.47	4	0.39	0	0	1	0.17
Ace	36	2.81	21	2.06	3	0.17	3	0.5
Dic	4	0.31	5	0.49	0	0	0	0
r	0	0	0	0	0	0	0	0
Tas	25	1.95	36	3.53	11	0.64	5	0.83
Total SCA	182	14.2	154	15.1	31	1.8	20	3.33
Total Cells	1280	85.8	1020	84.9	1720	98.2	600	96.67

chtg: monochromatid gaps; chrg: isochromatid gaps; chtb: monochromatid breaks; chrb: isochromatid breaks; ace: fragments; dic: dicentric chromosomes; r: rings; tas: telomeric associations.

When detailed analysis was carried out, MN children showed similar frequencies of gaps, breaks, fragments and dicentric chromosomes than MI, only telomeric associatios was significantly lower in MN ($p<0.05$). On the other hand, MN children showed higher percentages of gaps, breaks, fragments, dicentric chromosomes and telomeric associations than EI and EN. These differences were significant for gaps ($p<0.01$), monochromatid breaks ($p<0.001$), fragments ($p<0.05$) and telomeric associations ($p<0.01$, only MN vs. EN) frequencies. Comparable results were found for MI vs. EI and EN. In addition, EI children showed higher rates of gaps, breaks, fragments and telomeric associations in compare to EN ones.

Children exposition to potential genotoxic agents, just as medication, radiation, pesticides and industrial residues, showed no significant differences between groups.

DISCUSSION

Our results showed an increase frequency of SCA not only in relation with malnutrition but also with the presence of bacterial infections. Nevertheles,

malnutrition induced damage was higher than the other. In the same way, others authors (Armendares et al., 1971; Khouri y McLaren, 1973; Betancourt et al., 1974; Upadhyaya et al., 1975a y b; Ortiz et al., 1997; Murthy et al., 1980; Betancourt et al., 1995), observed increase DNA damage in healthy infected versus healthy non-infected children and in malnourished infected versus healthy infected children. Results reported by Gonzalez and colleagues (2002a and b), agree with those above mentioned and confirmed that malnutrition and severe infections are two factors clearly associated with DNA damage increase.

In addition, Gonzalez and coworkers (2002b) observed significant increments of DNA damage assessed by comet assay in malnourished infected versus healthy infected children under medical treatment, suggesting that malnutrition enhance drug susceptibility inducing DNA damage. In malnutrition, it is likely to be impaired drug metabolism alterations because of the delay in absorption, reducing linkage protein, changes in the distribution volume, redox changes in the liver and reducing of the renal clearance. Krishnaswamy (1989) has reported that gentamicin half life is 25% higher and depuration 50% lower in malnourished versus eutrophic children. Drugs plasma concentrations were higher in malnutrition than in healthy state (Waterlow, 1996). It must be considered that energy and amino acids are requirements for proper protein synthesis process. In children fed protein-low diets, protein synthesis decreases by 25% (Jackson et al., 1983). Malnutrition means a decrease in the availability of energy or essential amino acids and it can cause a decrease in protein synthesis affecting the production of enzymes required for DNA repair. Induced DNA lesions are repaired efficiently by the cellular repair processes. This process involves many steps and interference with any of them will lead to negative biological effects, including chromosome aberrations (Natarajan, 2002). González and coworkers (2002) found a reduction in the repair ability in lymphocytes of malnourished children. The damage observed in malnourished children could be due to the deficiency of several essential nutrients required to protein synthesis that are associated with DNA integrity, impaired DNA repair mechanisms, and/or to the unavailability of molecules necessary to protect the cells against DNA oxidative damage. A cell with diminished ability to repair is likely to mutate more easily triggering processes of mutagenesis, carcinogenesis, teratogenesis, early senescence or even apoptosis (Zeiger and Tennant, 1986; Hagmar et al., 1998; Natarajan, 2002). For the foregoing, it is difficult to discern whether structural chromosome aberrations are due to malnutrition per se, bacterial and viral infections, antibiotics or all of these factors acting on malnourished

tissues. Chromosomal damage could be attributed to several factors: a severe deficiency of essential nutrients and energy required for the synthesis of DNA maintenance factors; deterioration of repair mechanisms allowing the persistence of an abnormally high number of SCA; and/or the lack of specific factors that are needed to protect the cell against oxidative DNA damage. Further studies will be necessary involving a large number of patients to address the relationship between levels of DNA damage and specific kinds of infection, drug treatments, and the type and severity of malnutrition.

CONCLUSIONS

Severe infection would be associated with chromosomal damage and would have a more important effect in malnourished children. This fact could be explained by the alteration of DNA repair mechanisms.

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