



CHROMOSOMAL EFFECTS OF INFECTIONS IN MALNOURISHED AND EUTROPHIC CHILDREN OF GRAN LA PLATA

PADULAG^{A,B}, SEOANE^{A,B}

a- División Antropología. Facultad de Ciencias Naturales y Museo. U.N.L.P.

b- IGEVET. Facultad de Ciencias Veterinarias. U.N.L.P.

e-mail: giselpadula@yahoo.com.ar

ABSTRACT

The aim of this study was to assess the induction of structural chromosomal aberrations in peripheral blood lymphocytes of malnourished and eutrophic children with bacterial infections. Thirty-six infants concurrent to the Hospital Interzonal de Agudos y Crónicos Dr. Alejandro Korn, La Plata, Argentina were included in this analysis; 11 infected and malnourished (IM), 7 infected and eutrophic (IE) and 18 non-infected and eutrophic (NE).

Children aged 1-60 months. Anthropometric and clinic evaluation were performed to assess nutritional condition. We scored structural chromosome aberrations (SCA) in 100 metaphases per individual. Statistical analysis was performed by the Epi Dat 3.0 (OPS-OMS, 2003), through «Test de Diferencias entre dos proporciones muestrales» ($p < 0.05$). Total SCE frequency was five times higher in IM children than that of IE ones (15,1% vs. 3,33% $p < 0,001$) and two times greater in IE than in NE 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. It is difficult to discern whether structural chromosome aberrations are due to malnutrition per se, bacterial or viral infections, antibiotics or all of these factors acting on malnourished tissues. In conclusion, mutagenic factors cause chromosomal changes more easily in an altered environment.

Keywords: 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 protein-energy malnutrition (PEM). 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). 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). 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.

Chromosomal aberrations have been studied over a century and their importance in human health has been recognized (Natarajan et al., 2002). Cytogenetic analysis in peripheral blood lymphocytes has been widely used for monitoring human populations exposed to mutagenic agents (Carrano and Natarajan, 1988; Hagmar et al., 1998; Albertini et al., 2000).

The relation between PEM and genetic damage has been studied in human beings and laboratory animals obtaining contradictory evidences (Terreros et al., 1993). It has been found that children aged 1-60 months with severe PEM exhibited increased frequencies of chromosomal aberrations (dicentric chromosomes, gaps, isogaps and breaks) in peripheral lymphocytes and bone marrow cell cultures that persisted even after the children had attained normal height and weight (Armendares et al., 1971).

The purpose of this study was to assess the incidence of bacterial infectious diseases on the frequency of structural chromosomal aberrations (SCA) in peripheral blood lymphocytes of malnourished and eutrophic children.

Materials and Methods

Experimental Procedure

Thirty six 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. 11 were infected (with gastrointestinal or respiratory bacterial infections)

and malnourished (IM), 7 were infected and eutrophic (IE) and 18 were non-infected and eutrophic (NE). Children aged 1-60 months. Anthropometric and clinic evaluation were performed to assess nutritional condition. Prior to blood collection, we interviewed each individual parent to fill a semistructural questionnaire specifying age, dietary habits, viral or bacterial diseases, previous exposure to diagnostic x-rays and use of therapeutic drugs. We provided all participants with specific written information about the aims of the study; all participants gave their written informed consent (Ley Provincial N° 11044).

Cytogenetic Analysis

We used heparinised venous blood samples to obtain lymphocytes from the participants. We set up 2 replicate cultures, using 1 ml total blood in 9 ml Ham's F10 medium (Gibco-Invitrogen Buenos Aires, Argentina) containing 1% phytohemagglutinin (Gibco-Invitrogen), 100 IU penicillin, and 100 µg/ml streptomycin (Sigma, St. Louis, MO). We incubated these at 37°C and 5% CO₂ for 48 hours. Two hours before harvesting, we added colchicine at a final concentration of 0.1 µg/ml. We harvested the cells by centrifugation, hypotonic treatment with 0.075 potassium chloride for 20–22 minutes, and fixation with methanol:acetic acid (3:1 vol/vol) at least 3 times. We made slides by using routine cytogenetic procedures; we air-dried them and stained them with 5% Giemsa stain (Spectrum Chemicals, Gardena, CA). We scored structural chromosome aberrations in 100 metaphases per individual by using a blind analysis: one investigator numerically identified the samples, and another scored the aberrations. We carried out the identification of SCA 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 by the Epi Dat 3.0 (OPS-OMS, 2003), through «Test de Diferencias entre dos proporciones muestrales» ($p < 0.05$).

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 child less than 2 years old 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 includes for 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

Tables 1 and 2 show results of anthropometric evaluation. Table 3 shows results of cytogenetic analysis. Total SCE frequency was five times higher in IM children than that of IE ones (15,1% vs. 3,33% $p < 0,001$) and two times greater in IE than in NE children (3,33% vs. 1,88% $p < 0,05$)

When detailed analysis was carried out, IM children showed higher frequencies of gaps, breaks, fragments, dicentric chromosomes and telomeric associations than IE. These differences were significant for gaps ($p < 0,01$), monochromatid breaks ($p < 0,001$), fragments ($p < 0,05$) and telomeric associations ($p < 0,01$) frequencies. In addition, IE children showed higher, non significant differences with respect to NE ones.

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

	< 2 aged									> 2 aged					
	Desnutrid			Desnutrid and Stunted			Wasted			Wasted and Stunted			Stunted		
	D1	D2	D3	D1	D2	D3	D1	D2	D3	D1	D2	D3	D1	D2	D3
Female	1	1	1	1	1	0	1	1	0	1	0	1	0	0	0
Male	0	0	1	1	0	0	0	0	0	0	1	0	1	0	1
Total	1	1	2	2	0	0	1	1	0	1	1	1	1	0	1

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

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

	MUAC//A			TS//A			SS//A		
	E	LR	VLR	E	LR	VLR	E	LR	VLR
	Female	0	1	6	1	6	0	1	4
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 2. Anthropometric evaluation of malnourished children by using MUAC//A, TS//A, SS//A indexes.

Structural Chromosome aberrations	Infected malnourished children		Infected euthrophic children		Non-infected euthrophic children	
	Absolute frequencies	Percent frequencies	Absolute frequencies	Percent frequencies	Absolute frequencies	Percent frequencies
chtg	45	4.41	8	0.52	9	0.52
chrg	8	0.78	0	0.23	4	0.23
chtb	35	3.43	3	0.23	4	0.23
chrb	4	0.39	1	0	0	0
ace	21	2.06	3	0.17	3	0.17
dic	5	0.49	0	0	0	0
r	0	0	0	0	0	0
tas	36	3.53	5	0.64	11	0.64
Total SCA	154	15.1	20	1.80	31	1.80

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

Table 3. Structural chromosome aberrations in 36 children.

Discussion

Results presented here showed an increase frequency of SCA not only in relation with malnutrition but also with the presence of bacterial infections. These results are similar to those published by some 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), who 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 severe infection and malnutrition 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 aminoacids 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 aminoacids 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. The repair processes involve many steps and enzymes and interference with any step in the repair process will lead to increased biological effects, including chromosome aberrations (Natarajan, 2002). González and coworkers (2002) found a reduction in the ability to repair DNA damage in lymphocytes of malnourished children over the controls. 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 their 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. In conclusion, mutagenic factors cause chromosomal changes more easily in an altered environment. The damage observed could be attributed to a deficiency of essential nutrients required in the synthesis of proteins associated with the integrity of DNA, the deterioration of repair mechanisms allowing the persistence of an abnormally high number of SCA and/or the lack of molecules that are necessary to protect the cell against oxidative DNA damage.

Development of procedures that can be used to identify health hazards in malnourished children is important to establish programs for disease prevention. 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.

References

- Albertini RJ, Anderson D, Douglas GR, Hagmar L, Hemminki K, Merlo F, Natarajan AT, Norppa H, Shuker DE, Tice R, Waters MD, Aitio A (2000). IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. International Programme on Chemical Safety. *Mutat Res* 463:111-72.
- Archer PG, Bender M, Bloom AD, Brewen JG, Carrano AV, Preston RJ. Guidelines for cytogenetic studies in mutagen-exposed human populations. In: Bloom AD, ed. *Guidelines for Studies on Human Populations Exposed to Mutagenic and Reproductive Hazards*. White Plains, NY: March of Dimes Birth Defects Foundation; 1981:1-35.

- Armendares S, Salamanca F y Frenk S (1971). Chromosome abnormalities in severe protein calorie malnutrition. *Nature* 232: 271-273.
- Betancourt M, Ortíz R, González C, Pérez P, Cortés L, Rodríguez L, Villasenor L (1995). Assessment of DNA damage in leukocytes from infected and malnourished children by single cell gel electrophoresis/comet assay. *Mutat Res.* 331(1):65-77.
- Carrano AV, Natarajan AT (1988). International Commission for Protection Against Environmental Mutagens and Carcinogens. ICPEMC publication no. 14. Considerations for population monitoring using cytogenetic techniques. *Mutat Res* 204:379-406.
- Dulger H, Arik M, Sekeroglu MR, Tarakcioglu M, Noyan T, Cesur Y, Balahoroglu R (2002). Pro-inflammatory cytokines in Turkish children with protein-energy malnutrition. *Mediators Inflamm.* 11(6):363-365.
- Epi Info. CDC / OMS. Procesador de texto, Base de datos y Estadística, para la Salud Pública. Versión 6.04d. 2001.
- Epidat. OPS / OMS. Programa para análisis epidemiológico de datos tabulados. Versión 3.0. Xunta de Galicia. 2003.
- González C, Najera O, Cortes E, Toledo G, Lopez L, Betancourt M, Ortiz R (2002b). Susceptibility to DNA damage induced by antibiotics in lymphocytes from malnourished children. *Teratog Carcinog Mutagen.* 22(2):147-158.
- González C, Najera O, Cortés E, Toledo G, López L, Betancourt M, Ortíz R (2002a). Hydrogen peroxide-induced DNA damage and DNA repair in lymphocytes from malnourished children, *Environ Mol Mutagen.* 39(1):33-42.
- Guide to short-term tests for detecting mutagenic, carcinogenic chemicals. In: *Environmental Health Criteria* 51. Geneva, Switzerland. World Health Organization; 1985:57-67.
- Hagmar L, Bonassi S, Stromberg U, Brogger A, Knudsen LE, Norppa H, Reuterwall C (1998). Chromosomal aberrations in lymphocytes predict cancer: a report from the European Study Group on Cytogenetic Biomarkers and Health (ESCH), *Cancer Res.* 58: 4117-4121.
- International System for Human Cytogenetic Nomenclature (1995).
- Jackson AA y Golden MHN (1983). Whole body protein turnover and nitrogen balance in young children at intakes of protein and energy in the region of maintenance. *Human Nutrition: Clinical Nutrition* 37C: 433-446.
- Khouri FP y McLaren DS (1973). Cytogenetic studies in protein-calorie malnutrition. *Am J Hum Genet* 25: 465-470.
- Krishnaswamy K (1989). Drug metabolism and pharmacokinetics in malnourished children. *Clinical Pharmacokinetics* 17 (1): 68-88.
- Murthy PB, Bhaskaram P y Srikantia SG (1980). Sister chromatid exchanges in protein-energy malnutrition. *Hum Genet* 55: 404-406.
- Natarajan AT (2002). Chromosome aberrations: past, present and future. *Mutat Res.* 504: 3-16.
- NCHS/OMS. Growth curves for children. Birth-18 years. Vital and Health Statistics. US Department of Health, Education, and Welfare. Public Health Service. Series 11:165.
- Ortiz R, Cortes L, Gonzalez C, Lopez L, Perez P, Cortes E, Betancourt M (1997). Analysis of mitomycin C-induced micronuclei in lymphocytes from malnourished infected children. *Environ Mol Mutagen.* 30(4):363-370.
- Padula G, Salceda SA, Prozzi M, Fernández A, Seoane A (2004). Alteraciones Cromosómicas en niños con Malnutrición Proteico-Energética: resultados preliminares. *Ludovica Pediátrica. Publicación Científica del Hospital Sor María Ludovica de La Plata.* ISSN 1514-5654. Vol VI (2):43-50.
- Programa Más Vida (2002). Guía Normativa sobre desnutrición.
- Tanner JM. *El Hombre antes del Hombre. El crecimiento físico desde la concepción hasta la madurez.* México: Fondo de Cultura Económica; 1986.
- Terreros MC, De Luca JC y Dulout FN (1993). The effect of a Hypoproteic Diet and Ethanol Consumption on the Yield of Chromosomal Damage Detected in Bone Marrow Cells of Mice. *J Vet Med Sci* 55 (2):191-194.

- Torun B y Chew F (1994). Protein Energy Malnutrition. En: Modern Nutrition in health and disease. Shils ME, Olson JA y Shike M (eds.). Lea & Febiger (editorial). 8° Edición. Vol 2.
- Upadhyaya KC, Uerma IC y Ghai OP (1975). Chromosomal aberrations in protein-calorie malnutrition. *Lancet* 2: 704.
- Upadhyaya KC, Uerma IC y Ghai OP (1975). Chromosome aberrations in protein-calorie malnutrition. *Indian Pediatr* 12: 857-863.
- Waterlow JC, Buzina R, Keller W, Lane JM, Nichman MZ y Tanner JM (1977). The presentation and use of the Weight and Height data for comparing the nutritional status of groups of children under the age of 10 years. *Bull WHO* 55: 489.
- Waterlow JC. Malnutrición proteico-energética. Organización Panamericana de la Salud. Organización Mundial de la Salud. Publicación Científica Nro. 555. Washington. D.C.; 1996.
- Zeiger E, Tennant RW (1986). Mutagenesis, clastogenesis, carcinogenesis: expectations, correlations and relations. *Prog Clin Biol Res* 209:75-84.