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RESEARCH ARTICLE

FREQUENCY OF IMMUNE ALTERATIONS AND MICRONUTRIENT DEFICIENCY IN SEVERELY MALNOURISHED CHILDREN

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ABSTRACT

Objective. To determine the frequency of cellular and humoral immune alterations and micronutrient deficiencies in severely malnourished patients and the relationship between these conditions.
Methods. Prospective, cross-sectional, analytical study of 30 severely malnourished 3-month-old to 5-year-old patients with measurements of vitamins B12, A, and E; folate; Zn, Cu, Se, and Fe; lymphocyte subpopulations; immunoglobulins; complement, lymphoproliferation; and 13 pneumococcal anti-polysaccharide antibodies.
Results. The study was conducted on 30 patients (15 female, 15 male): 28 had marasmus, and 2 had kwashiorkor. The mean age was 24.11 months; a small thymus was found in 15/30; and 3 patients had lymphopenia. A low number of CD19 lymphocytes were found in 27% of the patients; 24% did not produce antibodies against pneumococcal polysaccharides. The CD3, CD4, CD8, and CD16+56 lymphocytes were decreased in 11, 15, 8, and 6 patients, respectively; an inverted CD4/CD8 ratio was found in 40% of the patients. Abnormal lymphoproliferation was found in 7%; 30% had decreased C3; and 23% had decreased C4. Micronutrients were decreased in the follow percentages of patients: Fe 53%, Zn 17%, Cu 7%, and Se 13%. A statistically significant Pearson correlation was found between B12 and C4; between vitamin E and CD3, CD4, and CD19; between iron and CD3, CD8, and NK; and between Cu and C3 and IgG.
Conclusions: Previously unpublished data are presented in this study: decreased CD19 levels in 27% of the patients, abnormal response to polysaccharides in 24%, and abnormal lymphoproliferation in 7%.

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INTRODUCTION

In 2009, the World Health Organization (WHO) estimated that 27% of children under 5 years of age were malnourished. Approximately 178 million children (32% are from developing countries) suffer from chronic malnutrition (Rodriguez, 2011). In Mexico, according to the National Health and Nutrition Survey 2012 (ENSANUT: acronym in Spanish), 302,279 (2.8%) children under 5 years of age were underweight, 1,467,757 (13.6%) were low height, and approximately 171,982 (1.6%) were emaciated.

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The percentage of emaciated children found in ENSANUT 2012 was not lower than the values found for the surveys conducted in 1988, 1999, and 2006 (Gutiérrez, 2012). In 2010, the Mexican Health Foundation (FUNSALUD: acronym in Spanish) estimated that 1.8 million children under 5 years of age had malnutrition (Melendez, 2011). Malnutrition is the leading global cause of acquired immunodeficiency and has been correlated with a decrease in the proportion of T lymphocytes, a reduction in mature T lymphocytes, lymphocyte depletion, and inhibition of the proliferative response. There is an inversion of the normal helper/suppressor (CD4:CD8) lymphocyte ratio. The number of B lymphocytes is not altered, and although there may be an increase in immunoglobulins. The total hemolytic complement levels are decreased (Ramos, 1969; Waterlow, 1996; Suskind, 1990;

Frenk, 1957; Amati, 2003; Cegielski, 2004; Christi, 2009; Ritz; 2006; Solon, 2006; Hughes, 2006; Keusch, 2003 and Rice, 2000). Micronutrient deficiencies have been shown to produce immune alterations (Ramos, 1969; Waterlow, 1996; Suskind, 1990; Bhaskaram, 2002; Wintergerst, 2007; Savino, 2010; Zulfiqar, 1999 and Wapnir, 2000). Malnourished patients may suffer from a deficiency in at least one micronutrient, thus further compromising an immune system that is already impaired by malnutrition itself. The aim of this study is to describe the alterations in cellular and humoral immunity and the micronutrient deficiencies that affect severely malnourished patients and to identify the correlation between micronutrient deficiency and immune response.

METHODS

A prospective, transversal, observational, and analytical study was conducted and was approved by the Ethics and Research Committees of the National Institute of Pediatrics under number 106/2012. Infants and preschool children from 3 months to 5 years of age were included from March 2014 to December 2015, without a discrimination based on gender. The patients were severely malnourished and treated at the Department of Gastroenterology and Nutrition of the National Institute of Pediatrics, and the parents or guardian had signed an informed consent. Patients with primary or secondary immunodeficiency diagnoses, those with intestinal malabsorption syndromes, and those who had taken vitamins and/or minerals were excluded. Severe malnutrition was defined in children under 2 years of age according to the weight/age indicator (-3 standard deviations (SD)). Children 1 to 5 years of age were classified by weight/height (-3 SD). If the clinical picture corresponded to kwashiorkor, the case was included without regard to weight/age or weight/height ratios, and the mid-upper arm circumference (MUAC) was used with a cutoff of -110 mm (Waterlow, 1996 and Puntis, 2010). Patients who met the selection criteria were tested for cellular and humoral immune responses and to determine the serum levels of vitamins (A, E, B12, and folate) and minerals (Zn, Cu, Fe, and Se).

Once the patient was selected for inclusion to assess the immune response (Volker, 2011; Alkhater, 2009; Oliveira, 2010; Norosky, 1998; Sewell, 2006; Cooper, 2003 and Slatter, 2008), the leukocytes and lymphocytes were measured using a blood count with adjustment for age (Stiehm, 2004). Immunoglobulin levels (IgM, IgA, IgG, and IgE) were measured for humoral immunity and adjusted for age (Kjellman, 1979; Jolliff, 1982 and Zetterstrom, 1981). For a more accurate assessment of the functionality of B lymphocytes, antibody quantification was performed against 13 pneumococcal polysaccharides (WHO, 2012; Balloch , 2010). To study the cellular response (T lymphocytes), a chest X-ray was obtained to assess the presence and approximate size of the thymus. A T-lymphocyte count was then performed using differential flow cytometry (Ramírez, 2004), mainly for CD3, CD4, and CD8 cells, CD19 B lymphocytes, and CD16+56 natural killer cells; the results were later adjusted for age (36-37). Lymphoproliferation was performed to assess the T-cell response (Quah, 2007). Complement was assessed by measuring the C3 and C4 fractions and was adjusted for age (Jolliff, 1982). The minerals (Zn, Cu, Se, and Fe) were measured by inductively argon-coupled plasma-mass spectrometry (ICP-MS). Vitamins were measured in the patient´s serum.

Statistical analysis

The variables were analyzed using SPSS software version 20.0, and the database of the obtained results was captured. For quantitative variables, descriptive statistics were used: the central tendency and dispersion measures and normality tests. For categorical variables, frequencies and percentages were used. For the variables that did not meet the normality assumption (IgA, IgE, and IgM), the serum levels were log-transformed (natural log). Pearson correlation was performed to examine the association between micronutrients and cellular and humoral immunity.

RESULTS

A total of 768 malnourished children were studied, with 480 mild cases, 192 moderate, and 96 severe. Only 65 met the inclusion criteria, and 35 were excluded because of the previous intake of multivitamin supplements, malabsorption diseases, cancer, and other conditions.

Demographic characteristics

The study included 30 patients (15 female, 15 male) diagnosed with severe malnutrition: 28 with marasmus and 2 with kwashiorkor. The mean values were age 24.11 months (SD 15.21, 95% confidence interval (CI) 18.21-30.01); weight 5.51 kg (SD 2.14, 95% CI 4.67-6.34); and height 69.03 cm (SD 12.29, 95% CI 64.27-73.80). Z score: mean weight/age -5.67 (SD 1.35, 95% CI -6.20 to -5.15); mean weight/height -4.18 (SD .937, 95% CI -4.55 to -3.82); and mean height/age -5.35 (SD 2.28, 95% CI -6.24 to -4.27).

Vitamins and minerals

Table 1 shows the mean, SD, and 95% confidence interval (95% CI) of micronutrient levels, which were classified as low, normal, or high and were adjusted for age. We found decreased serum iron levels in 53% of the patients, zinc in 17%, copper in 7%, and selenium in 13%. In contrast, for vitamins, the serum levels of vitamin E were low in 17% of the patients, and the folate levels were low in 3%.

Immunity

On admission, a chest X-ray was performed to study the thymus image, which was reduced in size in 15/30 of the patients. The mean white blood cell count was 8,877 (SD 3,858, 95% CI 7,537-10,418). The mean lymphocyte count was 4,047 (SD 2,029, 95% CI 3,289-4,804), with the count being below 1500 for 3 cases. Regarding humoral immunity, the mean CD19 B lymphocyte count measured by flow cytometry was 561.90 (SD 451.06, 95% CI 393.47 - 730.43), with 22/30 normal and 8/30 (27%) being low. The results of the measurements of immunoglobulins A, G, E, and M and their age-adjusted interpretation are shown in Table 2. An antibody response against the 13 pneumococcal polysaccharide subtypes was present in 22 children; there was no response in 7 (24%), and the test could not be performed in 1 case. Table 3 shows the cellular immunity results with the T-cell subpopulations measured by flow cytometry and adjusted for age. A T-cell count below 1500 was found in 3/30 cases. The CD4/CD8 ratio was normal in 18/30 of the patients and inverted in 12/30 (40%). The lymphoproliferation was normal in 28/30 of the patients and abnormal in 2/30 (7%).

Table 1. Microelements values and adjusted for age

Data				Adjusted for age		
Microelement	Mean	SD	95% CI	Low n/%	Normal n/%	High n/%
B12 pg/mL	769.23	279.94	664.70-973.77	0/0	30/100	0/0
Folate ng/mL	16.73	6.98	14.12-19.32	1/3	29/96	0
A mmol/L	4,32	2.72	3.29-5.36	0/0	13/45	15/55
E mmol/L	24.60	9.55	20.96-28.23	5/17	23/80	1/3
Fe ug/dL	59.30	30.40	47.97-70.65	16/53	14/47	0/0
Zn ug/dL	75.53	20.46	67.89-83.18	5/17	25/83	0/0
Cu ug/dL	126.73	38.58	112.33-141.14	2/7	25/83	3/10
Se ug/dL	96.97	28.17	86.45-107.49	4/13	25/84	1/3

SD: standard deviation, CI: confidence interval, n/%: patient number/percentage

Table 2. Measures of immunoglobulins A, G, E and M and their age-adjustment

	Data			Age adjusted		
Immunoglobulin	Media	DE	IC 95%	Low n/%	Normal n/%	High n/%
IgA Tr	4.43	0.60	4.21-4.66	2/7	18/60	10/33
IgG Tr	6.70	0.44	6.53-6.86	2/7	21/70	7/23
IgE Tr	3.08	1.35	2.57-3.58	0/0	14/47	16/53
IgM Tr	4.79	0.597	4.57-5.02	2/7	22/73	6/20

Tr = transform in natural log. SD: standard deviation, CI: Confidence interval, n/%: patients number/percentage

Table 3. T-cell subpopulations and adjusted for age

	Data			Adjusted for age		
T-cell subpopulations	Media	SD	95% CI	Low n/%	Normal n/%	High n/%
CD3	1858.70	1123.18	1401.96-2315.44	11/37	17/56	2/7
CD4	884.60	616.64	654.34-1114.86	15/50	15/50	0/0
CD8	850.20	678.49	596.85-1103.55	8/27	19/63	3/10
CD16+56 (NK)	532.67	561.61	322.96-742.38	6/20	21/70	3/10

SD: standard deviation, CI: Confidence interval, n/%: patients number/percentage

Table 4. Pearson´s correlation between micronutrient serum levels and immunoglobulins, T cell subpopulations, and C3 and C4 levels

Micronutrient	Correlation	Correlation coefficient	P
B12	C4	0.380	0.038
Vitamin E	CD3	0.368	0.050
	CD8	0.379	0.043
	CD19	0.467	0.011
Iron	CD3	0.437	0.016
	CD8	0.420	0.021
	NK	0.492	0.006
Copper	IgG	0.402	0.028
	C3	0.477	0.008
	Albumin	0.526	0.003

The mean serum C3 levels were 96.45 (SD 22.68, 95% CI 87.98-104.91), and the mean C4 levels were 20.99 (SD 8.90, 95% CI 17.67-24.32). After adjustments for age, reduced C3 and C4 levels were found in 9/30 (30%) and 7/30 (23%), respectively, of the patients. Associated infections were found for 11/30 (37%); of these, 3 were gastrointestinal, 5 were respiratory, and 3 were urinary. An abnormal complete urine examination was found in 5 (17%) of the patients, although only 2 urine cultures were positive. The blood and stool cultures were negative in all cases. The mean serum albumin was 3.2 (SD .81, 95% CI 3.01-3.62). Strikingly, the albumin levels in the 2 patients with kwashiorkor were 1 and 1.2. A Pearson correlation was performed to determine whether there is a relationship between micronutrient serum levels and immunoglobulins, T cell subpopulations, and C3 and C4 levels, and the results are shown in Table 4.

DISCUSSION

Micronutrient deficiencies and immune alterations that result from malnutrition have a very profound impact on the progression of severely malnourished patients. The vicious cycle between malnutrition and infection increases the risk of mortality in this group of patients. In 2009, Christi *et al*. conducted a systematic review of the mortality risk according to the degree of malnutrition and found that the OR is 1.2 to 36.5 (lower limit CI 0.6-5.7; upper limit 2.0-22.4) for moderate malnutrition and 2.9 to 121.2 (lower limit CI 2.0-13.1; upper limit 4.1-55.7) for severe malnutrition (Christi, 2009).

Micronutrients

The most common etiology in the patients analyzed in this sample was malnutrition secondary to neurological damage and swallowing disorders. Only in 1 marasmus case was primary etiology. The main reasons for exclusion were prior vitamin intake, malabsorption diseases, and/or immunocompromised conditions. Most patients included in the study had marasmus, and only 2 had kwashiorkor. However, the patients with kwashiorkor had the lowest albumin levels (1 and 1.2), whereas the albumin level was higher than 3.5 in 16 patients of the patients with marasmus and was lower than 3.5

in 12 of these patients. Although the albumin levels in this sample were lower for the patients with kwashiorkor, this finding may be have been influenced by sample size. However, according to Golden and Gopalan (Ahmed, 2009), neither albumin nor diet type correlates with the type of malnutrition. According to the studies reported by Ciliberto (Osorio, 2011), the difference in the clinical presentation of these disease variants is due to the specific deficiency of micronutrients, mainly those with antioxidant properties (vitamin E, riboflavin, selenium, and N-acetylcysteine). However, in a study where these microelements were used for prevention, the researchers failed to substantiate this difference. This result coincides with our findings for the 2 patients with kwashiorkor: one patient presented with deficiencies in folate, iron, and zinc, and the other had deficiencies in vitamin E, zinc, copper, and selenium. Srikantia reported that these patients had increased ferritin levels, which caused an antidiuretic effect, and demonstrated both a reduction in the levels of amino acid-metabolizing enzymes in the liver and the presence of abnormal urinary metabolites (Golden, 2010). However, the etiology of kwashiorkor is still uncertain.

As reported in the world literature, WHO estimates that iron deficiency occurs in 47.4% of preschool children and zinc deficiency in 17.3% of the population (Bailey, 2015).

In this sample, iron deficiency was the most prevalent at 53% (16/30) of the patients, followed by zinc deficiency in 17% (5/30). This finding is consistent with previous results found worldwide; however, the result disagrees with the article published by Shamah-Levy *et al*. in 2012 (Shamah-Levy, 2011), based on ENSANUT 2006, which reported iron deficiency in 26% (calculated through serum ferritin) and zinc deficiency in 27.5% of children under 5 years. Shamah-Levy *et al*. also reported copper deficiency in 30.6% of children 1-10 years of age (Shamah, 2011), which is striking because copper deficiency was only found in 7% (2/30) of the patients in our sample. There are still no standard levels of selenium for all populations. Carmona-Fonseca analyzed the studies published between 1972 and 2009 in different populations and found that for Latin America and for children under 15 years of age, the level was 93.25 ± 39.2 (Carmona, 2010). With the information from this reference, we found a deficiency in this mineral in 13% (4/30) of the patients in our sample. According to WHO data, 250-500 million children have blindness secondary to vitamin A deficiency; of these, half will die within the first year of having lost vision (Bailey, 2015). In the present study, no patient had vitamin A deficiency because this vitamin is administered routinely along with vaccines during vaccination campaigns (bi-annual), as recommended by WHO. In contrast, the vitamin E levels were low in 17% (5/30) of the patients: 4 had marasmus, and 1 had kwashiorkor. Shamah-Levy *et al*. reported folate deficiency in 3.2% of children under 5 years of age (Shamah-Levy, 2011), and we found only 1 patient with decreased levels, which is equivalent to 3%. Shamah-Levy *et al*. reported vitamin B12 deficiency in 7.7% of these children, whereas no patient in our sample was found deficient in this vitamin.

Immune response

Malnourished patients have a decreased immune response, causing the suppression of immune functions against pathogens, which causes an increase in susceptibility to infections. Malnutrition causes atrophy of lymphoid tissues (Calder, 2013). Thus, in our patients, we found that 50% had a decreased thymus size. Although leucopenia has been classically reported to be present in these patients, studies performed by Najera (Nájera, 2007) and later by Paes *et al*. (2015) did not find leucopenia or lymphopenia in the blood count (Paes-Silva, 2005). In our sample, lymphopenia with a count below 1500 was found in 10% (3/30) of the patients. When flow cytometry was performed to count different lymphocyte subpopulations, decreased CD19 B lymphocyte levels were found in 27% (8/30) of the patients, which contrasts with the data published worldwide reporting no alteration in the number of B lymphocytes (Calder, 2013). However, the total CD3 T lymphocytes were decreased in 37% (11/30) of the patients, with decreased differential CD4 in 50% (15/30), decreased CD8 in 27% (8/30), and an inverted CD4/CD8 ratio in 40% (12/30) of the patients. CD16+56 natural killer cells were decreased in 20%, which is consistent with the value reported in the world literature (Savino, 2010 and Calder, 2013). When qualitative tests were used to assess the immune response, we found that only 2 patients (7%) had no lymphoproliferative response to 2 mitogens. This result contrasts with globally reported finding in the sense that the proliferative capacity of T lymphocytes is reduced (Ramos, 1969; Waterlow, 1996; Rice, 2000; Bhaskaram, 2002; Savino, 2010; Calder, 2013).

Regarding the function of B lymphocytes, the literature reports (Ramos, 1969; Waterlow, 1996; Rice, 2000; Bhaskaram, 2002; Savino, 2010; Calder, 2013) that immunoglobulins remain high or normal. In this sample, the total IgA, IgG, and IgE levels were decreased in 7% of the patients (2/30 in each case). When the response against 13 subtypes of pneumococcal polysaccharides was evaluated, we found that no response in 24% (7/30) of the patients. All patients received at least 1 dose of pneumococcal polyvalent vaccine. We could not find a single report in the world literature about this phenomenon. For the patients with a reported response to the vaccine, a relationship with the number of vaccine doses could not be established. Regarding complement, C3 was decreased in 30% (9/30) of cases and C4 in 23% (7/30), which is consistent with the worldwide literature (Ramos, 1969; Waterlow, 1996; Rice, 2000 and Bhaskaram, 2002). There were 11/30 (37%) cases of associated infections, with 3 being gastrointestinal, 5 respiratory, and 3 urinary. A positive urinary culture was confirmed in only 2 cases, whereas the blood and stool cultures were negative in all cases.

Micronutrients and immunity

Although the relationship between different micronutrient deficiencies and immunity is well known, in this study, we aimed to demonstrate a direct relationship between these factors in malnourished patients because malnutrition alone is known to alter the immune response. Additionally, the response of these patients is likely to worsen if a micronutrient deficiency is also present. However, when evaluating the correlation between micronutrients and the immunological response, the only relationship found was that between a decreased serum iron concentration and the levels of CD3 (r 0.437, p 0.016), CD8 (r 0.420, p 0.021) and natural killer cells (r 0.492, p 0.006). As reported in the literature, iron deficiency causes a reduction in NK cell activity and T-cell proliferation in addition to reductions in the delayed hypersensitivity response, macrophage bactericidal activity, and the CD4:CD8 ratio with a corresponding increase in CD8 (4–6,16–20,42,45).

The inversion of the CD4/CD8 ratio was also demonstrated in this study; however, when the model was run, this association was not verified. Zinc deficiency is associated with multiple immune alterations, such as increased stress and oxidative DNA damage. This deficiency suppresses the Th1 response and decreases TNF a and IL2 levels, NK cell activity, macrophage activity, cytolytic T-cell activity, and the delayed hypersensitivity response. This deficiency also increases the apoptosis of B and pre-T cells, thus producing lymphopenia and thymic atrophy. In our sample, we found that 17% of the children had low serum zinc levels; however, a correlation with the immune alterations described above could not be explained maybe to the sample size. Copper deficiency has been linked to decreased T-cell proliferation and increased numbers of circulating B cells, reduced neutrophil phagocytic activity and NK activity, and decreased complement function (Amati, 2003 and Wintergerst, 2007). In our patients, we found that copper levels correlated with the C3 (r 0.477, p 0.008) and IgG (r 0.402, p 0.028) levels. The serum vitamin E levels correlated with CD3 (r 0.368, p 0.050), CD8 (r 0.379, p 0.043), and CD19 (r 0.467, p 0.011) lymphocytes. The antioxidant effect of vitamin E and its role as an oxidative stress protector of lipid membranes are well known. Vitamin E also reduces the production of immunosuppressive factors such as prostaglandin E2 in macrophages and boosts Th1 immune response, and vitamin E deficiency decreases the T-cell response and delayed hypersensitivity.

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Conclusion

Some of the immune alterations already described in the literature were verified in this study. However, this study provides valuable information about the immunological behavior of malnourished patients with such findings as decreased CD19 levels in 27% of the patients, an abnormal response to polysaccharides in 24%, and abnormal lymphoproliferation in 7%. There is a clear association between cellular immunity and iron deficiency and between copper and complement. However, this type of relationship was not confirmed for the other micronutrients, which could be due to the size of the sample, thus requiring further research with a larger number of cases.

Key messages

Malnutrition affects both cellular and humoral immunity, causes leucopenia and lymphopenia. A decrease in the number of B lymphocytes (27%) and an abnormal antibody response against pneumococcal polysaccharides (24%) was found in the severely malnourished children.

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