ORIGINAL ARTICLE

Assessment of Nutritional Status: Vitamin A and Zinc in Patients With Common Variable Immunodeficiency

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Abstract

Background: Patients with common variable immunodeficiency (CVID) present with low antibody levels, impaired lymphocyte function, and chronic inflammation. Vitamin A and zinc are essential components of the immune system and can be redistributed in the body as a result of inflammation.
Objective: To compare levels of retinol, ß-carotene, and zinc in patients with CVID and healthy controls after evaluating a series of parameters for each participant.
Patients and Methods: We performed a cross-sectional study of CVID patients and healthy controls matched for age and gender. All participants underwent a nutritional and laboratory evaluation comprising a complete blood count and determination of levels of C-reactive protein (CRP), lipopolysaccharide (LPS), soluble CD14 (sCD14), retinol, ß-carotene, and serum and erythrocyte zinc.
Results: We included 17 patients (mean age, 28.54 years) and 17 controls. Mean (SD) retinol levels were lower in patients: 1.99 (0.67) µmol/L vs 2.72 (0.96) µmol/L. Median ß-carotene levels were similar in both groups (0.30 µmol/L). Median serum zinc levels were 50.0 µg/dL (50-100 µg/dL) in the patients and 100.0 µg/dL (50-150 µg/dL) in the controls. Mean levels of erythrocyte zinc were lower among patients: 37.32 (10.51) µgZn/gHb vs 44.91 (7.67) µgZn/gHb in the controls. Median CRP levels were significantly higher among patients: 4.99 (0.15-34.51) mg/L vs 0.55 (0.17-6.06) mg/L. No differences in translocation marker levels were observed between the groups.
Conclusions: CVID patients had lower levels of retinol and zinc than controls. Since micronutrient deficiency could aggravate their disease and contribute to chronic inflammation, micronutrient status should always be assessed in patients with primary immunodeficiency.

Key words: Antibody deficiency. Nutrition. Vitamin A. Zinc. Bacterial translocation.

Resumen

Antecedentes: Los pacientes con inmunodeficiencia variable común (CIVD) presentan un déficit de anticuerpos, una función alterada de linfocitos e inflamación crónica. La vitamina A y el zinc son elementos esenciales para el sistema inmune y pueden sufrir una redistribución en el organismo provocados por inflamación.
Objetivo: El objetivo de este estudio fue comparar los niveles de retinol, beta-caroteno, y zinc en pacientes con CIVD y controles sanos, habiendo evaluado todos los parámetros en cada sujeto.
Pacientes y métodos: Este estudio cruzado-seccional evaluó pacientes con CIVD de las consultas externas de una University Immunology Clinic y controles sanos emparejados por edad y género. A todos los pacientes se les realizó una evaluación nutricional y analítica mediante hemograma, proteína C reactiva (PCR), lipopolisacárido (LPS), CD14 soluble (CD14s), retinol, betacaroteno, suero y zinc en eritrocito.
Resultados: Diecisiete pacientes (edad media 28.54 años) y 17 controles fueron incluidos en el estudio. En cuanto a los resultados obtenidos, los niveles medios de retinol fueron más bajos en el grupo de pacientes comparados con los controles: 1.99 µmol/L (±0.67) y 2.72 µmol/L (±0.96), respectivamente. Los niveles medios de betacaroteno fueron similares en ambos grupos (0.30 µmol/L). Los niveles medios de zinc en suero fueron 50.0 µg/dL (50-100) en pacientes y 100.0 µg/dL (50-150) en el grupo control. Los niveles medios de zinc de eritrocito fueron más bajos en los pacientes comparados con los controles: 37.32 µgZn/gHb (±10.51) y 44.91 µgZn/gHb (±7.67) respectivamente. Los niveles medios de PCR fueron significativamente mayores en los pacientes comparados con los controles: 4.99 mg/L (0.15-34.51) y 0.55 mg/L (0.17-6.06) respectivamente. No se encontró diferencia en los niveles del marcador de translocación entre los grupos.
Conclusiones: Concluimos que los pacientes con CIVD presentaron niveles menores de retinol y de zinc comparados con controles. Dado que una deficiencia de micronutrientes puede agravar su enfermedad y contribuir a inflamación crónica, en los pacientes con inmunodeficiencia primaria debería siempre investigarse su estado de micronutrientes.

Palabras clave: Déficit de anticueropos. Nutrición. Vitamina A. Zinc. Translocación bacteriana.

Introduction

The term common variable immunodeficiency (CVID) encompasses a very diverse group of diseases characterized by low antibody levels and impaired lymphocyte function [1]. A significant number of CVID patients have gastrointestinal symptoms such as malabsorption, chronic diarrhea, and small bowel inflammation [2].

Micronutrients, including vitamin A and zinc, are essential for immune function. Retinol, retinaldehyde, and retinoic acid are active forms of vitamin A with an important role in the immune system, including mucosal immunity [3,4].

Some studies have shown lower levels of retinol in CVID patients than in healthy controls [5] and an inverse correlation between vitamin levels and immune activation markers [6]. Recent studies suggested that retinoic acid has an important effect on T lymphocytes by providing a mechanism for self-regulation of proinflammatory functions and anti-inflammatory functions in the gastrointestinal tract [7,8].

ß-Carotene has a major role in the immune system [9], and patients with secondary immunodeficiency frequently have ß-carotene deficiency [10].

Zinc deficiency causes severe thymic atrophy, which compromises the T lymphocyte response [11] and causes B-cell depletion, mainly through apoptosis [12]. Serum levels of zinc are considerably lower in CVID patients than in healthy individuals because of malabsorption and chronic inflammatory processes [13].

Considering the importance of these micronutrients in the immune system and their redistribution in the organism as a result of inflammation, the aim of this study was to compare levels of retinol, ß-carotene, and zinc between CVID patients and healthy controls, since these elements have not been previously evaluated together.

Patients and Methods

Patients and Study Design

We performed a cross-sectional study from December 2008 to October 2009 with patients followed at the immunology outpatient clinic of the Federal University of Sao Paulo (UNIFESP) and a control group. The inclusion criteria for patients were a diagnosis of CVID according to the European Society for Immunodeficiencies and regular treatment with intravenous immunoglobulin (IVIG). Controls had to be in good health and of the same gender and age as the patients (zinc levels differ in childhood, adolescence, and adulthood). The exclusion criteria were smoking, acute infection, and vitamin supplementation.

The local ethics committee approved the study.

Data Collection

Clinical data were collected, and all participants underwent physical examination. Anthropometric data were also recorded [14], and a socioeconomic questionnaire developed by the Brazilian Association of Research Companies was administered [15]. Nutritional status was evaluated using body mass index, as previously described [16].

Laboratory Assessment

Blood samples were collected using pyrogen-free tubes after an 8-hour fast. All samples were analyzed in duplicate. Patients were under regular IVIG treatment (every 4 weeks) and blood was always collected before the next infusion (on the same day). We evaluated the following parameters:

- White blood cell count and differential (as markers of infection).
- C-reactive protein (CRP) (as a marker of inflammation and infection [17]).
- Serum LPS and soluble CD14 (sCD14) (as markers of bacterial translocation: reference values <50 pg/mL for LPS and <2.4 µg/mL for sCD14 [18]).
- Retinol and ß-carotene: determined using high-performance liquid chromatography [19].
- Serum and erythrocyte zinc levels: determined by atomic absorption spectroscopy [20].

Statistical Analysis

Statistical analyses were conducted using Minitab version 15.1 (Minitab Inc) and BioEstat version 5.0 [21]. Statistical significance was set at a P value of <.05. Normally distributed quantitative data were compared using the t test; nonnormally distributed data were compared using the Mann-Whitney test. Qualitative variables were analyzed using the χ^2 test or Fisher exact test.

Results

Twenty patients with a confirmed diagnosis of CVID who fulfilled the inclusion criteria were invited to participate in the study; the final sample comprised 17 patients. Two patients did not attend the scheduled visits, and 1 refused to participate.

Both the study and the control groups included 1 child (aged 9 years), 3 adolescents (from 13 to 18 years of age), and 13 adults.

Mean age was 28.54 years. No differences were observed between patients and controls in nutritional and socioeconomic status (Table 1).

No differences were observed between the groups in LPS and sCD14 levels. In 11 patients (64.70%) and 5 controls (29.41%), sCD14 levels were higher than the reference values (P=.105). One of 17 patients (5.88%) and none of the controls showed high levels of LPS. CRP levels were elevated (>1.1 mg/L) in 73.33% of the patients (11/15) and in 29.41% of the controls (5/17); median CRP levels were significantly higher in the patient group (Table 2). CRP results were not available for 2 patients.

Mean retinol levels were significantly lower in patients. ß-Carotene levels did not differ between the groups. Serum and erythrocyte zinc levels were lower in the patient group (Table 2).

No significant correlations were observed between sCD14, LPS, and CRP with zinc, retinol, and ß-carotene. No correlations were observed between zinc and retinol levels.

Table 1. Characteristics and Nutritional Status of Patients With Common Variable Immunodeficiency and Controls

	Patients	Controls	*P* Value
Mean (SD) age, y	28.54 (11.29)	28.07 (10.35)	.901[a]
Male, No. (%)	8/17 (47.06)	8/17 (47.06)	>.999[b]
Socioeconomic class A, No. (%)	2/17 (11.76)	7/17 (41.18)	.118[c]
Socioeconomic classes B and C, No. (%)	15/17 (88.24)	10/17 (58.82)	
Body mass index, kg/m^2	19.3 (17.3-28.9)	22.2 (17.5-34)	.053[d]
Malnourished, No. (%)	3/17 (17.65)	1/17 (5.88)[e]	.601[c]
Well-nourished or overweight, No. (%)	14/17 (82.35)	16/17 (91.12)	

[a]*t* test.
[b]χ^2 test.
[c]Fisher exact test.
[d]Mann-Whitney test.
[e]Adequate according to body fat percentage and abdominal circumference [41].

Table 2. Levels of Biological Markers in Patients With Common Variable Immunodeficiency and Controls[a]

	Patients	Controls	*P* Value
Lipopolysaccharide, pg/mL	17.99 (13.68-65.44)	20.21 (13.13-41.68)	.630[b]
Soluble CD14, μg/mL	2.60 (0.59)	2.31 (0.39)	.105[c]
C-reactive protein, μg/L	4.99 (0.15-36.22)	0.55 (0.17-6.06)	.004[b]
Retinol, μmol/L	1.99 (0.67)	2.73 (0.96)	.014[c]
ß-Carotene, μmol/L	0.30 (0.10-1.20)	0.30 (0.16-0.70)	.861[b]
Serum zinc, μg/dL	50 (50-100)	100 (50-150)	.020[b]
Erythrocyte zinc, μgZn/gHb	37.32 (10.51)	44.91 (7.67)	.045[c]

[a]Values are expressed as median (range) or mean (SD).
[b]Mann-Whitney test.
[c]*t* test.

Moderate-to-severe gastrointestinal symptoms (chronic and/or bloody diarrhea and weight loss) were recorded in 4 out of 17 patients; the remaining 13 presented mild symptoms (epigastric pain or occasional diarrhea) or no symptoms. Levels of ß-carotene, serum zinc, erythrocyte zinc, LPS, and sCD14 were similar between the patients with moderate-to-severe gastrointestinal symptoms and those with no or mild gastrointestinal symptoms ($P>.05$). CRP levels could not be compared in these groups, as results were available for only 3 of the patients with severe gastrointestinal symptoms.

Discussion

The elevated CRP levels found in the patients indicate a chronic inflammatory process [17], as observed elsewhere in patients with CVID [2]. On the other hand, the similar levels of LPS and sCD14 in both patients and controls suggest the absence of bacterial translocation and of anti-inflammatory activity of monocytes [18], respectively. Such findings indicate that the chronic inflammatory process in patients is localized and, even if bacterial translocation does occur, it could be controlled using IVIG.

Although levels of sCD14 were similar in both groups, elevated levels were found in more patients than controls. Unlike the results of previous works [18,22], this finding points to a nonexclusive relationship between LPS and sCD14. Indeed, a recent work showed that the CD14 molecule is a cofactor not only for toll-like receptor 4, which recognizes LPS, but also for other toll-like receptors (eg, 7 and 9) [23].

Plasma levels of retinol were lower in the patients, a finding that is consistent with those of some authors [7,24] and different from those of others [25]. The function of retinol in mucosal immunity is well established [26]. Nevertheless, as previously reported [27,28], we found no differences in retinol levels in our patients, regardless of the severity of gastrointestinal symptoms. Similarly, no significant differences were found for ß-carotene, serum zinc, erythrocyte zinc, LPS, and sCD14 levels in patients according to the severity of gastrointestinal symptoms. The lack of statistical significance

in this case is probably due to the small number of patients presenting with more severe symptoms.

Lower levels of retinol in patients with chronic inflammation may be the result of low food intake, impaired absorption [23], redistribution caused by inflammation [7,29,30], and repeated infections [31]. Therefore, the presence of repeated infections in our and other CVID patients [1,2] may contribute to the low levels of retinol observed. Moreover, the concomitant presence of zinc deficiency has been shown to negatively affect retinol mobilization in the bowel [32]. Consequently, vitamin A supplementation in CVID patients with vitamin A deficiency could improve immune function [5], although further studies are needed to test this hypothesis.

Unlike retinol, ß-carotene levels were similar in patients and controls. Decreased retinol levels have been reported to be associated with normal ß-carotene levels in chronic inflammation [10], partly owing to the concomitant presence of zinc deficiency, which negatively affects retinol mobilization in the bowel [32], and the negative impact of the inflammatory response on retinol levels [33].

Zinc deficiency was previously demonstrated in patients with secondary immunodeficiency [34]. The lower serum zinc levels we detected in the patients may reflect acute depletion, while lower erythrocyte zinc levels reflect chronic deficiency states [35]. Thus, chronic inflammation and repeated infections, both of which are common in CVID patients [1,2], are likely to play an important role in depletion.

Similar to retinol, low serum zinc levels may be due to redistribution toward sites of higher use through inflammation, thus indicating a possibly lower response to zinc supplementation in patients with increased levels of inflammatory markers [36]. In the present study, findings for nutritional state regarding zinc probably result from an association between redistribution and chronic inflammation.

CVID patients have lower memory B-cell counts [37,38]. Given the importance of zinc in bowel absorption, chronicity of inflammatory processes, and lymphocyte differentiation, maturation, and survival, together with the fact that zinc deficiency results in increased B-cell apoptosis [12], low levels of this micronutrient may affect disease progression [11,13].

Despite lower levels of serum and erythrocyte zinc, it is difficult to propose a supplementation strategy, for the following reasons: *(a)* Available recommendations target individuals not affected by chronic diseases [39]; *(b)* Patients with lower zinc levels have an altered inflammatory status; and *(c)* The number of available biochemical markers for a broader assessment of zinc levels is limited [40].

In conclusion, we found lower levels of retinol and zinc in CVID patients than in healthy controls. Since micronutrient deficiency could aggravate disease and contribute to chronic inflammation, micronutrient status should always be investigated in patients with primary immunodeficiency.

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