Nutrition 31 (2015) 664–668

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

Vitamin A and retinol-binding protein deficiency among chronic liver disease patients

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ARTICLE INFO

Article history:
Received 1 July 2014
Accepted 27 October 2014

Keywords:
Vitamin A nutritional status
Retinol-binding protein
Liver disease
RDR test

ABSTRACT

Objective: Vitamin A deficiency (VAD) is associated with the progression of chronic liver disease (CLD). The aim in this study was to assess levels of serum retinol and retinol-binding protein (RBP) as well as liver vitamin A stores in the presence of liver cirrhosis and hepatocellular carcinoma.
Methods: We ascertained the serum retinol and RBP levels of randomly selected CLD patients divided into two groups, one given 1500 UI (n = 89) and the other receiving 2500 UI (n = 89) doses of retinyl palmitate for the relative dose response test. Blood samples were collected in a fasting state and 5 and 7 h after supplementation.
Results: The prevalence of VAD was 62.4%. There was a progressive drop in serum retinol ($P < 0.001$) and RBP ($P = 0.002$) according to the severity of the liver disease, and a greater prevalence of severe VAD was noted in cirrhosis Child & Pugh C (52.8%). Fifty percent of the patients presented a low availability of RBP relative to retinol concentration, and there was no peak in RBP levels regardless of the dose of retinyl palmitate administered.
Conclusions: Our findings suggest serum retinol and RBP are relevant as indicators of vitamin A nutritional status in the presence of CLD. Liver vitamin A store cannot be evaluated using the RDR test because CLD causes a reduction in RBP synthesis and interferes with the mobilization of endogenous vitamin A. Considering how the patients already showed a drop in RBP relative to retinol concentrations, it is reasonable to assume vitamin A supplementation may trigger harmful effects in CLD patients.

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Introduction

Vitamin A deficiency (VAD) is one of the world's greatest malnutrition problems. According to the World Health Organization, Brazil is classified as having a major prevalence of subclinical micronutrient deficiency [1]. Vitamin A provides significant antioxidant action and enhances the detoxifying enzymes that combat the harmful effects of reactive oxygen species [2]. Research has found an association between oxidative stress and the progression of liver disease. It has been reported that reactive oxygen species activates hepatic stellate cells, which then leads to hepatic fibrosis and the progression of liver disease. Past studies report that lower levels of serum retinol may promote hepatocarcinogenesis in cirrhotic patients, suggesting that vitamin A may suppress tumor growth and progression [3]. Therefore, an accurate assessment of vitamin A nutritional status (VANS) in the presence of chronic liver disease (CLD) is essential for identifying those at risk of oxidative stress and VAD. Liver vitamin A stores should thus be the best early indicator of vitamin A status, because over 90% of all of the vitamin A in the body is stored in the liver [4,5].

Consequently, the relative dose response test (RDR) can be considered a good functional reference method and is based on the principle that, when a small dose of retinol is orally

This research was supported by Brazilian National Council for Scientific and Technological Development. All the authors declare there is no conflict of interest.

All of the authors conceived and coordinated the study and carried out the biochemical and statistical analyses. All authors contributed to the writing and reviewing of the paper and approved the final version.

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http://dx.doi.org/10.1016/j.nut.2014.10.016
0899-9007/Published by Elsevier Inc.

administered, it binds to retinol-binding protein (RBP) that is released into the bloodstream. Thus, serum retinol levels rise rapidly and are sustained for on average 5 h. By the same token, when VANS is adequate, the newly absorbed vitamin A is stored in the liver and the serum vitamin A concentration remains unchanged [6,7]. In fact, previous studies have demonstrated a modified oral RDR test is the most effective means of identifying vitamin A deficiency in children with chronic liver disease [4].

However, considering the possibility that evaluation of time and/or dose of vitamin A administered may be inadequate, given how the test was designed for the groups traditionally at risk of VAD [5], it is important to test two different relative dose-response-test protocols in CLD patients. Considering how a full evaluation of biochemical indicators may be needed to assess vitamin A nutritional status at different stages of CLD, the aim of this study was to evaluate serum retinol levels, plasma retinol-binding protein concentration and liver vitamin A stores using two different relative dose-response-test protocols in patients with liver cirrhosis or hepatocellular carcinoma (HCC)-related cirrhosis.

Materials and methods

Study design and participants

This is a randomized, single-blind clinical trial in which patients were stratified by severity of liver disease (Child-Pugh-class A, B, C, or HCC-related cirrhosis), with equal randomization (1:1), and comprised 178 patients, 89 in each group. The patients enlisted were randomly assigned to one of two groups by computer randomization procedures. Nine of the participants did not complete the three blood samples, having been deemed clinically incapable, and were excluded from the study (Fig. 1).

The sample group consisted of patients ≥19 y of age with CLD of various etiologies. The diagnosis of liver cirrhosis was based on clinical manifestations and laboratorial testing, as well as on ultrasonographic imaging and histologic evaluation whenever necessary. Diagnosis of HCC was based on computerized tomography and/or magnetic resonance findings and serum α-fetoprotein. Severity of liver failure was classified according to Child & Pugh score [8]. Data were collected prospectively from October 2008 to December 2009 at the University Hospital of the Federal University of Rio de Janeiro, Brazil. All participants signed an informed consent agreement, and the study was approved by the Ethics Committee (Institutional Review Board, protocol 068/01). The criteria for exclusion were malabsorption syndrome, diabetes mellitus, cancer except HCC, cholestasis, chronic kidney disease, amyloidosis, pregnancy, respiratory or cardiovascular disease, chronic alcoholism, use of supplements containing vitamin A during the last 6 mo, and patients with C-reactive protein levels higher than 5 mg/L.

Protocol

Patients underwent three blood sample collections to determine serum retinol and RBP. The first collection was performed after a 12-h fast (T0). Patients were then divided into two groups according to the concentration of retinyl palmitate administered: 1500 IU (450 μg vitamin A) or 2500 IU (750 μg vitamin A) (UNICEF, Batch, 948 R.P. Schem Pty, Co., Melbourne, Australia). Only the researcher in charge (GVC) was aware of the dose administered to each patient.

Five and seven hours after supplementation, additional blood samples were collected (T5 and T7, respectively). No adverse reactions to the tests were recorded. This trial was registered at clinicaltrials.gov under trial number NCT01634698.

Biochemical indicators

Serum retinol and RBP

As previously reported [9], the levels of serum retinol were determined by online solid-phase extraction high-performance liquid chromatography coupled with tandem mass spectrometry. We performed mass spectrometry using a Micromass-Waters (Wythenshawe, UK) Quattro LC triple-stage quadrupole. VAD was classified as either severe deficiency (<0.35 mol/L), moderate deficiency (0.35 mol/L < 0.70 mol/L) or mild deficiency (0.70 mol/L < 1.05 mol/L) [10].

We determined plasma RBP concentration for a subsample of 112 patients. To equally select this subsample into the two intervention groups and into severity of liver disease, patients were randomly stratified by computer randomization procedures. For each intervention group, we determined the RBP levels of 20, 18, 9, and 13 patients with Child & Pugh A, B, C cirrhosis, and HCC-related cirrhosis, respectively. RBP quantification was carried out using commercially available enzyme-linked immunosorbent assay kits (Human Retinol BP ELISA, Immunology Consultants Laboratory, Inc., Newberg, OR, USA).We calculated RBP molar concentration at a molecular weight of 21 000 g/mol [11]. The saturation of RBP

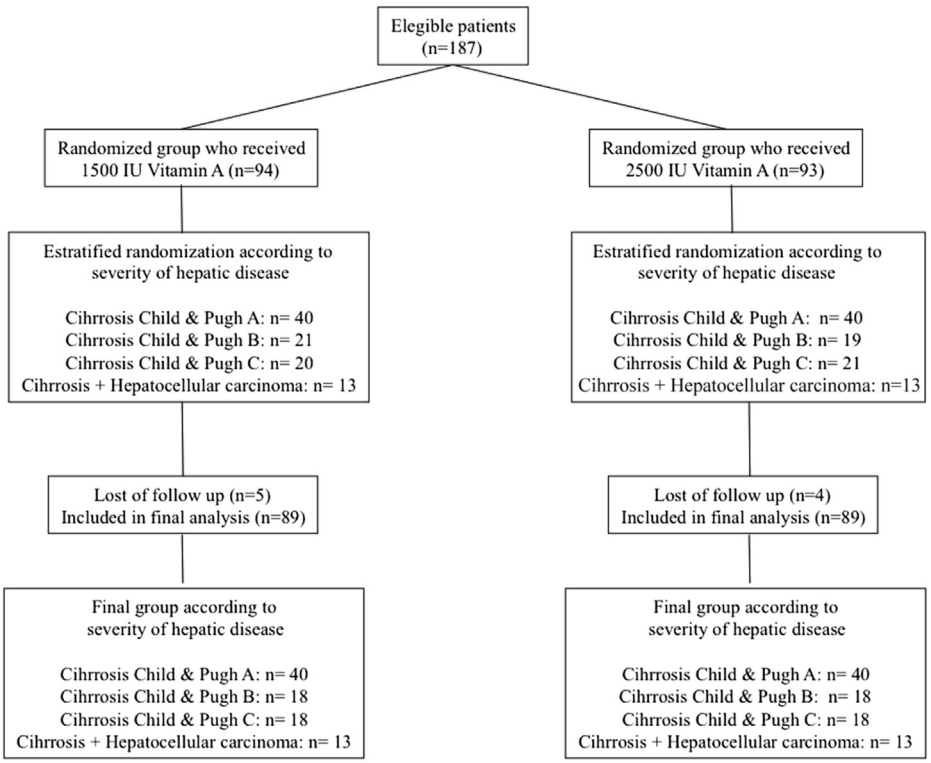

Fig. 1. CONSORT diagram of patients selection, treatment and analysis.