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

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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

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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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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

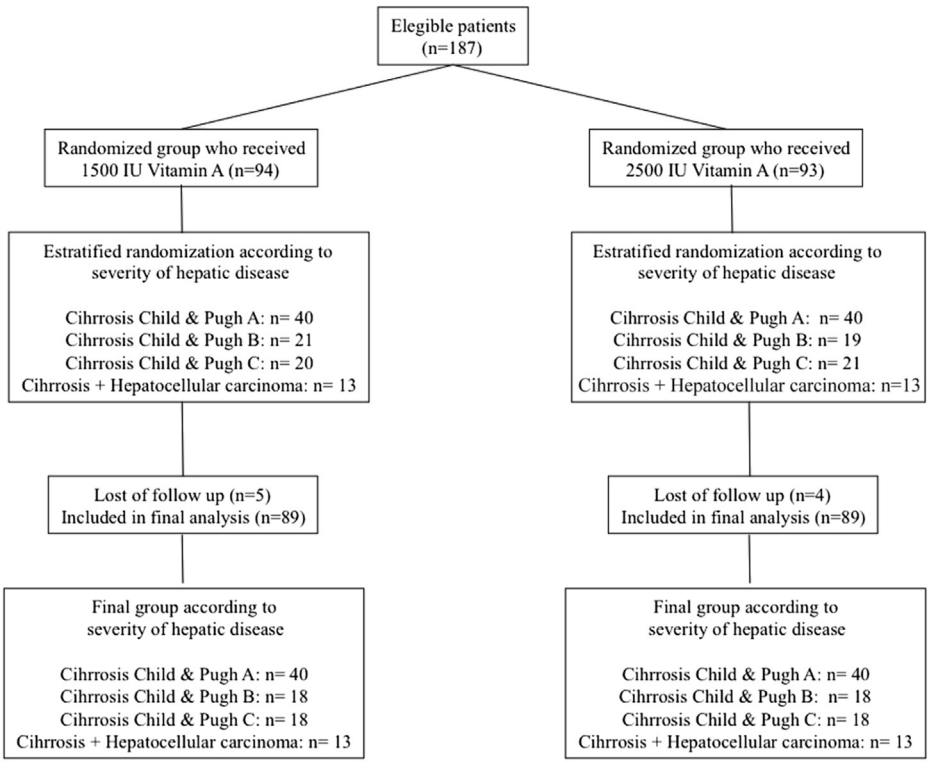

Fig. 1. CONSORT diagram of patients selection, treatment and analysis.

with retinol was classified as either adequate saturation (0.8–1.0 mol/mol), low saturation (<0.8 mol/mol) or low availability of RBP relative to retinol concentration (>1.0 mol/mol) [12].

Relative dose-response test

After taking the first blood sample, in a fasting state, a dose of 1500 IU or 2500 IU of retinyl palmitate was administered orally. Participants were then given a standard breakfast with an estimated total content of 9.7 g lipids to ensure retinyl palmitate absorption. Participants were instructed to drink only water. After 5 and 7 h, further blood samples were taken.

We calculated RDR using the following formula described by Loerch et al. [6]:

$$RDR(\%) = [(A_x - A_0)/A_x] \times 100$$

Where A_0 = basal retinol concentration; A_x = retinol concentration in the sample obtained 5 or 7 h after dose administered

A positive RDR test greater than or equal to 20% indicates inadequate vitamin A stores in the liver [13].

Further laboratory parameters

Laboratory parameters such as albumin, prothrombin time activity (PTA), total bilirubin, aspartate transaminase, alanine transaminase, and gamma-glutamyl transferase were measured by previously established standard laboratory methods.

Statistical analysis

Statistical analysis was performed using SPSS software (version 17.0: SPSS Inc. Chicago, IL, USA), with a significance level of 5%. The distribution of the values was identified as not normal, except for age. The Mann-Whitney test was used for between-group comparison. Associations were performed by Pearson's chi-square test and Spearman's correlation coefficient. The two-way repeated measures ANOVA test was used to evaluate how retinol and RBP behaved between supplementation groups at the three intervals when blood was drawn. For this analysis, data were log transformed to improve normality. The Bonferroni correction was used to identify which intervals were significantly different for each group. We performed logistic regression to evaluate the simultaneous influence of the study variables (laboratorial parameter, severity of disease, age, ascites, encephalopathy) on the failure to respond to the RDR test. Stepwise elimination of non-significant variables led to a preferred model with eight predictors: Severity of disease, ascites, age, retinol, RBP, albumin, total bilirubin, PTA.

Results

Serum retinol and RBP concentration

One hundred and seventy-eight patients were enrolled, 69 (62.4%) of them were male. The mean age of the study participants was 58.0 ± 9.3 y, and 44.4% of the sample group was 60 y old or older. No relationship was found between age and serum retinol concentration ($P = 0.41$). Serum retinol did not differ according to sex ($P = 0.29$).

Participant median baseline retinol (n = 178) was 0.89 μmol/L (min–max; 0.06–2.34), revealing 65.5% of the sample group to suffer from VAD. VAD frequency according to the stage of liver disease was 42.5% for patients with Child & Pugh class A cirrhosis, 86.1% for those with Child & Pugh class B cirrhosis, 89.7% for those with Child & Pugh class C cirrhosis, and 69.2% for those with HCC-associated liver cirrhosis. There was a progressive and statistically significant drop in serum retinol levels according to liver disease severity ($P < 0.001$). This study noted a greater prevalence of severe VAD in Child & Pugh class C cirrhosis (52.8%). Of those with Child & Pugh class A and HCC cirrhosis, no cases of severe VAD were found (Table 1).

Participant median baseline RBP was 0.52 μmol/L (0.05–1.88). There was a progressive and significant drop in serum RBP according to liver disease severity. We found levels to be significantly lower in patients with Child class C cirrhosis (0.33 μmol/L) compared with the Child class A (0.69 μmol/L) and B (0.56 μmol/L) ($P = 0.002$) cases.

The median relative saturation of RBP with retinol was 1.65 ± 1.39 mol/mol. Median distribution according to disease severity was: 1.43, 0.97, 0.92, 1.85, 1.24 mol/mol for Child & Pugh class A, B, C cirrhosis and HCC-related cirrhosis, respectively.

This study noted a very low prevalence (1.9%) of adequate saturation in the sample group, and 50% of the patients presented a low RBP availability relative to retinol concentration (>1.0 mol/mol).

Relative dose-response test

RDR testing showed liver vitamin A reserves to be inadequate in 50.3% of the patients. A significant association was found between vitamin A reserves in the liver and baseline serum retinol ($P < 0.0001$). However, nearly half (49.3%) of the patients with low serum retinol levels were shown to have adequate levels of retinol in the liver. Additionally, these patients had significantly lower levels of RBP ($P = 0.001$).

Biochemical parameters

We found a significant correlation between RBP and such markers of liver function as albumin ($r = 0.436$; $P < 0.001$), total bilirubin ($r = -0.407$; $P < 0.001$), and PTA ($r = -0.516$; $P < 0.001$), as well as with the markers of liver damage aspartate transaminase ($r = -0.354$; $P < 0.001$) and alanine transaminase ($r = -0.189$; $P = 0.048$).

Serum retinol and RBP behavior after vitamin A supplementation

In assessing the behavior of serum retinol and serum RBP in relation to time in both intervention groups, we found there was a significant variation (time effect) in retinol only in the group supplemented with 2500 IU ($P = 0.016$), where a significant drop in retinol from T5 to T7 was identified. In the group supplemented with 1500 IU there were no significant differences between the three blood-collection intervals. Considering that in the 2500 IU group there was a significant drop in retinol concentration over the 5 h after supplementation, we did not include T7 in our assessment when determining the adequacy of the RDR test.

As for the effect of dosage variation, no significant difference was found in the progression of retinol between the two groups ($P = 0.39$) over the course of the experiment; that is, the groups progressed similarly regardless of the amount of retinyl palmitate administered.

Table 1
Distribution of Vitamin A deficiency considering all stages of chronic liver disease and both intervention groups

Liver disease stages	Normal levels ≥1.05 μmol/L	Mild deficiency <1.05–0.70 μmol/L	Moderate deficiency 0.69–0.35 μmol/L	Severe deficiency < 0.35 μmol/L	*P* value
Cirrhosis Child & Pugh A n = 80	57.6%	28.8%	13.8%	–	0.0001
Cirrhosis Child & Pugh B n = 36	13.9%	13.9%	47.2%	25.0%	
Cirrhosis Child & Pugh C n = 36	8.3%	8.3%	30.6%	52.8%	
Cirrhosis + HCC n = 26	30.8%	34.6%	34.6%	–	

HCC, hepatocellular carcinoma

Likewise, no significant difference was found in the progression of RBP between the two intervention groups ($P = 0.570$); i.e., RBP behaves the same regardless of retinyl palmitate dosage. Furthermore, no significant variation in serum RBP was found for any of the three test intervals (time effect), as shown in Table 2.

Vitamin A nutritional status: Association between RDR test and baseline retinol

RDR testing for T5 showed liver vitamin A reserves to be inadequate in 29.4% of the sample group studied, regardless of the dose administered. We found a significant association between liver vitamin A reserves and baseline serum retinol ($P < 0.001$). In contrast, of the patients with low serum retinol levels, 49.3%, that is, nearly half of the patient studies, were shown to have adequate levels of retinol in the liver. Considering a drop in serum retinol only occurs once liver vitamin A reserves have been depleted, we assumed these patients did not respond to the RDR test.

When we divided the patients by test response at T5, regardless of the retinyl palmitate dosage administered, the median retinol, RBP and albumin levels were significantly lower in the participants who did not respond to the test, and median total bilirubin and PTA were significantly higher in those self-same patients. As for categorical variables, disease severity and the presence of ascites were the only variables associated with failure to respond to the RDR test (Table 3). Assessing the simultaneous influence of variables on failed test responses, RBP was the only independent variable we could find to explain this failure (relative risk = 1.21; 95% confidence interval 0.17–1.86; $P = 0.035$). The other variables showed no independent contribution at the 5% level.

Discussion

In our research we found a high prevalence of VAD, corroborating several prior studies addressing VANS in patients suffering from liver disease [3,9,14,15]. This is the first study to our knowledge investigating vitamin A nutritional status according to serum retinol levels, plasma retinol-binding protein concentration, and liver vitamin A stores using two different relative dose-response-test protocols in patients with CLD.

VAD prevalence was 65.5%, which is consistent with the findings of Peres et al. [9] and Paula et al. [15] in studying CLD patients. It is noteworthy that only the Child C group bore median serum retinol values lower than the 0.35 μmol/L cut-off point, demonstrating the lowest vitamin A values are found during the more advanced stages of liver cirrhosis. Our results confirm there is a progressive decrease in serum retinol with an increase in CLD severity, corroborating prior studies [9,14]. This progressive drop in serum vitamin A levels could be a consequence of decreases in vitamin A in the hepatic stellate cells because of activation by reactive oxygen species [16]. Other factors associated with VAD in CLD, such as reduced hepatic RBP synthesis, inflammation, and malabsorption of fat-soluble vitamins can be speculated on [17]; however, cholestasis and CRP levels over 5.0 mg/L were a criterion for exclusion in this research. In this study, there was a significant drop in serum RBP concentration at the most severe stage of the disease, demonstrating diminished ability to synthesize and/or release holo-RBP. We found a significant correlation between RBP and liver function tests. This finding corroborates prior studies involving RBP4 and insulin resistance in CLD patients [18,19]. To our knowledge, ours is the first study to show that liver cirrhosis lowers the liver's ability to synthesize RBP, hindering the RDR test for assessing liver vitamin A stores in cirrhotic patients.

We found, in a high proportion of patients, that serum retinol did not increase by more than 20% after supplementation, possibly leading to a false diagnosis of adequate liver reserves. Peres et al. [5], having had similar results, proposed two lines of explanation: One based on the presence of factors that could lead to diminished RBP synthesis, and another suggesting the RDR procedures should be modified to account for time and the dose

Table 2
Response of the serum RBP (mol/L) according to the treatment group

Group	Time	Mean ± SD	Median (min–max)	*P* value*	*P* value†
1500 IU (n = 56)	Basal (T0)	0.556 ± 0.328	0.470 (0.15–1.70)	0.24	0.57
	T5	0.551 ± 0.321	0.465 (0.15–2.36)		
	T7	0.585 ± 0.400	0.510 (0.19–2.24)		
2500 IU (n = 56)	Basal (T0)	0.620 ± 0.371	0.575 (0.13–1.88)	0.54	
	T5	0.596 ± 0.313	0.530 (0.14–1.63)		
	T7	0.619 ± 0.347	0.510 (0.16–1.69)		

Max, maximum; Min, minimum; RBP, retinol-binding protein; SD, standard deviation

* The two-way repeated measures ANOVA test within each group (time effect).

† The two-way repeated measures ANOVA test between the two groups (interaction effect: Group × time).

Table 3
Univariate analysis between the groups that responded and failed in the response to the RDR test

Variables	Adequate answer (n = 141)		Failure in the response (n = 37)		*P* value*
Numerical variables	Median (min–max)		Median (min–max)		
Age (y)	59.0 (34.0–81.0)		53.0 (30.0–74.0)		0.002
Retinol (mol/L)	1.00 (0.04–2.24)		0.46 (0.02–0.63)		<0.001
RBP (mol/L)	0.58 (0.12–1.88)		0.38 (0.05–1.00)		0.001
Albumin (g/dL)	3.4 (1.7–4.5)		3.0 (1.2–4.2)		0.002
Total bilirubin (mg/dL)	1.1 (0.2–5.6)		1.9 (0.6–7.8)		0.001
PTAs	2.9 (0.0–14.0)		4.3 (0.0–14.6)		<0.001
Aspartate transaminase (U/L)	67.0 (15.0–335.0)		86.0 (25.0–381.0)		0.119
Alanine transaminase (U/L)	66.6 (11.0–327.0)		63.0 (14.0–282.0)		0.657
Gamma-glutamyl transferase (U/L)	96.0 (15.0–986.0)		84.0 (19.0–1189.0)		0.793
Categorical variables	n	%	n	%	
Disease severity					0.001
Cirrhosis Child & Pugh A	74	92.5	06	7.5	
Cirrhosis Child & Pugh B	26	72.2	10	27.8	
Cirrhosis Child & Pugh C	22	61.1	14	38.9	
Cirrhosis + HCC	19	73.0	7	27.0	
Presence of ascites					0.046
Absence	96	83.5	19	16.5	
Presence	45	71.4	18	28.6	
Liver encephalopathy					0.238
Absence	117	81.3	27	18.8	
Presence	24	70.6	10	29.4	
Hypertensive gastropathy					0.234
Absence	36	85.7	6	14.3	
Presence	52	74.3	18	25.7	
Administered dose of vitamin A					0.500
1500 IU	71	79.8	18	20.2	
2500 IU	70	78.7	19	21.3	
Nutritional status					0.843
Eutrophy	93	80.2	23	19.8	
Protein-calorie malnutrition	41	78.8	11	21.2	

HCC, hepatocellular carcinoma; IU, international unit; Max, maximum; Min, minimum; PTAs, Prothrombin time activity (measured in s over the control); RDR, relative dose response

* Mann-Whitney test was applied to numerical variables and the chi-square (X^2) test for categorical variables.

of retinyl palmitate supplementation, as the RDR test has been validated for classic risk groups and may be insufficient to offset the limited absorption and liver changes experienced by some cirrhosis patients. In fact, previous studies have noted a drop in postprandial clearance of retinyl esters in the circulation of the elderly, and recommend a 7-h RDR duration for those over 60 y of age [20,21]. However, in our study, neither time nor the adjusted dosage corresponded with an adequate test response. By separating participants with inadequate test responses from those with adequate test responses, we could observe that RBP was the only variable independently associated with failed test response. Furthermore, age was not a determinant in the inability of the test to quantify liver vitamin A reserves in cirrhosis sufferers. These results reinforce the hypothesis that liver dysfunction is most responsible for failure to respond to the RDR test. We can thus draw the conclusion that the drop in RBP synthesis caused by advanced liver disease—especially at the most severe stages of the disease—is a factor that limits the applicability of the RDR test.

Liver vitamin A reserves have been shown to rise while RBP levels drop, and this may lead to liver damage and the progression of liver disease [22,23]. The toxicity caused by excessive vitamin A intake may be the result of a systemic increase in the concentration of free retinol, whether non-esterified or bound to carrier proteins, leading to a subsequent increase in concentrations of its retinoic acid metabolite. As both free retinol and its retinoic acid metabolite are fat soluble, they can interpose between the lipids in the cell membrane, thereby destroying the membrane and leading to cell lysis [23]. Furthermore, excess free retinoic acid also enhances stimulus to gene expression in retinoic acid-responsive genes, causing aberrations in cell differentiation and inducing cellular apoptosis [24]. After vitamin A supplementation, oxidative retinol and such retinoic acid metabolites as 4-oxo-retinol and 13-cis-4-oxo-retinoic acid are found in the bloodstream. Considering how the participants in this study already showed a decrease in RBP relative to retinol concentrations even before vitamin A supplementation, it is reasonable to assume that vitamin A supplementation may trigger harmful effects in liver cirrhosis sufferers. One of the limitations of this study was we did not take liver biopsies to compare our findings with standard-RDR-test results gleaned directly from liver tissue.

In conclusion, we found a high prevalence of VAD in CLD, and the serum retinol and RBP levels proved to be a good parameter for evaluating VANS in cirrhotic and HCC patients, for its association with disease severity. Furthermore, by modifying the RDR test to have longer time intervals and/or different dosages we were unable to improve test response for our research. Therefore, so far this test cannot be considered appropriate for assessing liver vitamin A stores in this sample group. Disease severity seems to be the main factor limiting the capacity of the RDR test to predict liver retinol reserves in CLD patients.

References

[1] World Health Organization, Global prevalence of vitamin A deficiency. Indicators for assessing vitamin A deficiency and their application in monitoring and evaluating intervention programmes. Geneva: Switzerland: Micronutrient series WHO/NUT10; 1996.
[2] Namiduru ES, Namiduru M, Tarakçioglu M, Tanriverdi M. Levels of malondialdehyde, myeloperoxidase and nitrotyrosine in patients with chronic viral hepatitis B and C. Adv Clin Exp Med 2012;21:47–53.
[3] Clemente C, Elba S, Buongiorno G, Berloco P, Guerra V, Di Leo A. Serum retinol and risk of hepatocellular carcinoma in patients with child-Pugh class A cirrhosis. Cancer Lett 2002;178:123–9.
[4] Feranchak AP, Gralla J, King R. Comparison of indices of vitamin A status in children with chronic liver disease. Hepatology 2005;42:782–92.
[5] Peres WAF, Chaves GV, Gonçalves JC, Ramalho A, Coelho HSM. Assessment of the relative dose-response test as indicators of hepatic vitamin A stores in various stages of chronic liver disease. Nutr Clin Pract 2013;28:95–100.
[6] Loerch JD, Underwood BA, Lewis KC. Response of plasma levels of vitamin A to a dose of vitamin A as an indicator of hepatic vitamin A reserves in rats. J Nutr 1979;109:778–86.
[7] Flores H, Campos F, Araujo RC, Underwood BA. Assessment of marginal vitamin A deficiency in Brazilian children using the relative dose response procedure. Am J Clin Nutr 1984;40:128–89.
[8] Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973;60:646–69.
[9] Peres WAF, Chaves GV, Gonçalves JC, Ramalho A, Coelho HSM. Vitamin A deficiency in patients with hepatitis C vírus-related chronic liver disease. Br J Nutr 2011;106:1724–31.
[10] International Vitamin A Consultative Group. Improving the vitamin A status of populations. USAID: ILSI; 2003.
[11] Colantuoni V, Romano V, Bensi G, Santoro C, Constanzo F, Raugei G, et al. Cloning and sequencing of a full length cDNA coding for human retinol-binding protein. Nucleic Acids Res 1983;11:7769–76.
[12] Mourey MS, Siegenthaler G, Amedee-Manesme O. Regulation of metabolism of retinol-binding protein by vitamin A status in children with biliary atresia. Am J Clin Nutr 1990;51:638–43.
[13] Flores H, Araújo CR, Campos FA, Underwood B. Importance of the early diagnoses of vitamin A deficiency at the epidemiologic level. Int J Vitam Nutr Res 1983;24:23–4.
[14] Newsome PN, Beldon I, Moussa Y, Delahooke TE, Poulopoulos G, Hayes PC, et al. Low serum retinol levels are associated with hepatocellular carcinoma in patients with chronic liver disease. Aliment Pharmacol Ther 2000;14:1295–301.
[15] De Paula TP, Ramalho A, Braulio VB. The effectiveness of relative dose response to retinol intake as an evaluation of vitamin A status of cirrhotic patients. J Hum Nutr Diet 2010;23:583–9.
[16] Casu A, Bassi AM, Canepa C, Maloberti G, Nanni G. Thioacetamide impairs retinol storage and dolichol content in rat liver cells in vivo. Biochim Biophys Acta 2002;1583:266–72.
[17] Russell RM, Iber FL, Krasinski SD, Miller P. Protein-energy malnutrition and liver dysfunction limit the usefulness of the relative dose response (RDR) test for predicting vitamin A deficiency. Hum Nutr Clin Nutr 1983;37:361–71.
[18] Yagmur E, Weiskirchen R, Gressner AM, Trautwein C, Tacke F. Insulin resistance in liver cirrhosis is not associated with circulating retinol-binding protein. Diabetes Care 2007;30:1168–72.
[19] Kwon JH, Park ST, Kim GD, You CR, Kim JD, Woo HY, et al. The value of serum retinol-binding protein 4 levels for determining disease severity in patients with chronic liver diseases. Korean J Hepatol 2009;15:59–69.
[20] Bulux J, Carranza E, Castañeda C, Solomons NW, Sokoll LJ, Morrow FD, et al. Studies on the application of the relative-dose-response test for assessing vitamin A status in older adults. Am J Clin Nutr 1992;56:543–7.
[21] Ribaya-Mercado JD, Mazariegos M, Tang G, Romero-Abal ME, Mena I, Solomons NW, et al. Assessment of total body stores of vitamin A in Guatemalan elderly by the deuterated-retinol-dilution method. Am J Clin Nutr 1999;69:278–84.
[22] Dan Z, Popov Y, Patsenker E, Preimel D, Liu C, Wang XD, et al. Hepatotoxicity of alcohol-induced polar retinol metabolites involves apoptosis via loss of mitochondrial membrane potential. FASEB J 2005;19:845–7.
[23] Ross AC, Russel RM, Miller SA, Munro IC, Rodricks JV, Yetley EA, et al. Application of a key events dose-response analysis to nutrients: A case study with vitamin A (retinol). Crit Rev Food Sci Nutr 2009;49:708–17.
[24] Yin WH, Raffelsberger W, Gronemeyer H. Retinoic acid determines life span of leukemic cells by inducing antagonistic apoptosis-regulatory programs. Int J Biochem Cell Biol 2005;37:1696–708.