Antioxidant Vitamins and Brain Dysfunction in Alcoholics

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Abstract — Aims: Alcohol induces cytokine secretion by Kupffer cells, which may exert also deleterious effects on distant organs, mediated in part by cytokine-derived increased production of reactive oxygen species (ROS). It is therefore important to assess antioxidant levels. The objective of this study is to analyse the relation of antioxidant vitamins with brain atrophy and cognitive dysfunction. Methods: In 77 alcoholic patients admitted for withdrawal syndrome, subjected to brain computed tomography (CT), and 19 controls, we determined antioxidant vitamin levels and analysed their relationships with data of brain atrophy and dysfunction. Searching for causes of altered vitamin levels, we also assessed liver function, nutritional status, eating habits, alcohol intake, proinflammatory cytokine (TNF-α, IL-6, IL-8) levels and malondialdehyde (MDA) levels. Results: Both retinol (vitamin A) and tocopherol (vitamin E) levels were decreased in alcoholics, the former in relation with liver failure, and the latter in relation with triglyceride levels and fat mass. Both were related to data of brain atrophy and cerebellar shrinkage (to which also IL-6 was significantly related). Conclusion: Among alcoholics, liver function impairment leads to altered serum vitamin A levels, which are related to brain alterations. Vitamin E levels are also decreased, but although in relation with liver function impairment, its decrease seems to be more dependent on nutritional status and irregular eating habits. Both vitamins are lower in patients with cerebellar atrophy and other features related to brain atrophy.

INTRODUCTION

Ethanol increases intestinal wall permeability, allowing gram-negative bacteria to reach the liver via the portal system (Su et al., 2002). These bacteria activate Kupffer cells, triggering an inflammatory response, in which macrophage-derived cytokines, especially TNF-α, IL-1β and IL-6 play outstanding roles (Fujimoto et al., 2000; McClain et al., 2004). These cytokines can cross the blood–brain barrier, and activate brain microgial cells, endothelial cells and vagal afferents, leading to neuroinflammation (Crews et al., 2006). In fact, in experimental conditions, microglia can be activated after a single intraperitoneal lipopolysaccharide injection (Qin et al., 2007), with a marked and protracted increase in TNF-α local production (Qin et al., 2008) and increased oxidative stress (Qin and Crews, 2012), which leads to neuronal damage and brain atrophy. Indeed, any inflammatory response is accompanied by an increased production of reactive oxygen species (ROS). This may be especially dangerous in the alcoholic, given the reduced activity of glutathione peroxidase and superoxide dismutase, as well as the decreased levels of antioxidant micronutrients such as retinol, ascorbic acid and α-tocopherol (Fauzallah et al., 1986; Van de Casteele et al., 2002), selenium and zinc (Menzano and Carlen, 1994; Gueguen et al., 2003; González-Reimers et al., 2008) described in these patients, although normal antioxidant vitamin levels have also been reported (Fernández-Solà et al., 1998). Since oxidative stress is related to a proinflammatory cytokine response, and, as mentioned before, it may lead to neuronal damage and brain atrophy, it is important to assess the behaviour of antioxidant vitamins in alcoholic patients with brain dysfunction, to discern which factor(s) may be involved in their (eventually) altered levels, and which are their relations with brain atrophy and cognitive impairment.

Based on these facts, in the present study we analyse the relationships of serum levels of vitamins A, C and E and the intensity of brain atrophy and globally assessed cognitive impairment. As a secondary objective, we also try to discern the relative roles of liver function derangement, dietary habits, ethanol intake and nutritional status, on altered vitamin levels.

PATIENTS AND METHODS

The study protocol was approved by the local ethical committee of our Hospital (number 2012-01), and conforms to the ethical guidelines of the 1975 Declaration of Helsinki. Included patients gave informed consent to the work.

We included a total of 77 alcoholics (69 men and 8 women), drinkers of more than 150 g ethanol/day for a long time (Table 1), aged 52.47 ± 11.06 years, and compared them with 19 age- (47.16 ± 12.23 years, t = 1.83) and sex-matched controls (3 women; P = 0.45). The patients were consecutively admitted with major symptoms of withdrawal syndrome, and were included in the study if a brain computed tomography (CT) was necessary for medical reasons. None of them was affected by any other organic or psychiatric disorder, and they did not report addiction to any other illicit drug. Based on clinical examination and ultrasonography (liver morphology, splenomegaly, collateral circulation) patients were classified in cirrhotics and non-cirrhotics. Also, in order to get a global assessment of liver function, all the patients were classified according to Child–Pugh’s scoring system (Child and Turcotte, 1964).

Following a previously reported protocol (Santolaria et al., 2000), we recorded the eating habits of the patients, asking them where do they usually eat (at home or in bars or taverns), how many times a day and what they eat (sandwiches or snacks, or normal dishes) classifying them in three categories (normal, irregular habits (loss of some meals) and poor eating habits).


habits (usually in bars or taverns, in form of sandwiches or snacks, once or twice daily)). Since only few patients fell in this third category, we further grouped the alcoholics included in this study in only two groups (normal vs irregular eating habits).

Whole body densitometric composition

After informed consent, patients and controls underwent densitometric evaluation with a Lunar Prodigy Advance device (General Electric, Piscataway, NJ, USA). We performed a whole body densitometric analysis, recording fat and lean mass at different parts of the body, such as upper limbs, ribs, pelvis, lower limbs, spine and total body. Total lean mass and total fat mass were used in this study in the assessment of nutritional status. Body mass index (as weight (in kg)/height$^2$ (in m)) was also recorded.

Biochemical assessment

Blood samples were taken at 8.00 a.m. in fasting conditions, 1–2 days before hospital discharge (about 10–15 days after admission), when patients were already stable from clinical point of view. Blood samples were immediately frozen at −20°C. In addition to routine laboratory evaluation (which included, among other variables, serum triglycerides, cholesterol, bilirubin, prothrombin activity and albumin), we performed the following biochemical determinations:

- Serum vitamin A (retinol) and Vitamin E (α-tocopherol), by high performance liquid chromatography (HPLC) Loinc® (Urbanek et al., 2006); serum ascorbic acid, by spectrophotometry (Loinc®); serum tumour necrosis factor (TNF)-α by immunometric chemiluminiscent assay (intra-assay variation coefficient ranging 4.6–6.5%; interassay variation coefficient ranging 2.6–3.6%; recovery 92–112%; Diagnostic Products Corporation (DPC), Los Angeles, CA, USA); interleukin (IL)-6, by chemiluminiscent assay (interassay variation coefficient ranging 5.3–7.5%; recovery = 85–104%; DPC, Los Angeles, CA, USA); and IL-8, by chemiluminiscent assay (interassay variation coefficient ranging 5.3–7.5%; DPC, Los Angeles, CA, USA; Berthier et al., 1999). As shown in Table 1, some vitamins and cytokines were not determined to all cases.

Lipid peroxidation products

Serum malondialdehyde (MDA) levels, referred to as thiobarbituric acid-reactive substance (TBARS), were measured according to the method described by Kikugawa et al. (1992). A volume sample of 0.2 ml of plasma was added to 0.2 ml of H$_3$PO$_4$ (0.2 M) and the colour reaction was initiated by the addition of 25 µl of 0.11 M thiobarbituric acid (TBA) solution. Samples were placed in a 90°C heating block for 45 min. After the samples were cooled, the TBARS (pink complex colour) were extracted with 0.4 ml of n-butanol. Butanol phase was separated by centrifugation at 6000×g for 10 min. Aliquots of the n-butanol phase were placed in a 96-well plate and read at 535 nm in a microlate spectrophotometer reader (Benchmark Plus, Bio-Rad, Hercules, CA, USA). The calibration curve was prepared with authentic MDA standards ranging from 0 to 20 µM. The intra- and inter-assay variation coefficients were 1.82 and 4.01, respectively.

Assessment of brain atrophy

Patients underwent a whole brain CT scan due to medical reasons (mostly because of traumatism, convulsion, confusion). Besides evaluation by a neuroradiologist, who recorded the presence or not of cortical atrophy and cerebellar atrophy, the following parameters (Meese et al., 1980) were determined (Figs 1 and 2):

- BF/BF1= Bifrontal index
- B/B1= bicaudate index
- B/E= Evan’s Index
- B/BF= ventricular index

Maximum width of the anterior horns of the lateral ventricles (HLV) in relation to the inner skull width at the same level (Bifrontal index).

Minimum width of the lateral ventricles (MLV) in relation to the inner skull at the same level (Bicaudate index).

Width of both cellae media in relation to the inner skull at the same level, which corresponds to the maximum inner skull diameter (MISD) (Cella media index)

Evan’s index (= HLV/MISD)

Ventricular index (= MLV/HLV)

| Table 1. Differences in vitamins, cytokines and MDA among patients and controls |
|----------------|----------------|----------------|
| Patients | Controls | $n$ | Mean ± SD | $n$ | Mean ± SD | $T$ | $P$ |
| Vitamin A (mg/l) | 73 | 0.258 ± 0.232 | 18 | 0.467 ± 0.135 | $T = 3.67; P < 0.001$ |
| Vitamin E (µg/ml) | 67 | 8.775 ± 4.718 | 18 | 13.983 ± 5.303 | $T = 4.04; P < 0.001$ |
| Vitamin C (µg/dl) | 58 | 0.986 ± 0.598 | 19 | 1.248 ± 0.575 | $T = 1.67; NS$ |
| Age (years) | 77 | 52.01 ± 11.19 | 19 | 47.16 ± 12.23 | $T = 1.66; NS$ |
| MDA (µmol/l) | 50 | 6.94 ± 6.1 | 5.53 (2.29–7.37) | 19 | 1.39 ± 0.89 | 0.959 (0.742–2.206) | $Z = 5.04; P < 0.001$ |
| TNF-α (pg/ml) | 46 | 13.01 ± 9.03 | 11.00 (7.73–16.58) | 19 | 5.75 ± 1.85 | 5.10 (5.00–6.70) | $Z = 4.74; P < 0.001$ |
| IL-6 (pg/ml) | 46 | 21.72 ± 46.95 | 7.23 (4.98–19.50) | 19 | 5.97 ± 1.62 | 5.00 (5.00–6.00) | $Z = 1.30; NS$ |
| IL-8 (pg/ml) | 40 | 41.20 ± 55.91 | 23.3 (12.00–44.78) | 17 | 6.71 ± 1.82 | 6.80 (5.00–7.75) | $Z = 4.46; P < 0.001$ |
Assessment of cognitive functions

This was performed by means of the minimental test (Folstein et al., 1975), to 51 individuals at the day at which blood was extracted.

Statistics

The Kolmogorov–Smirnov test was used to test normality, a condition not fulfilled by several variables. Therefore, non-parametric tests, such as Mann–Whitney’s U test and Kruskal–Wallis were used to analyse between-group differences in these variables. Spearman’s correlation analysis was used to compare quantitative variables. Otherwise, Student’s t-test, ANOVA and eventually Pearson’s correlation analysis were used with the normally distributed parameters, and \( \chi^2 \) test to analyse the association between two or more qualitative variables.

RESULTS

Antioxidant vitamins in patients and controls

Marked differences were observed between patients and controls regarding vitamin A and E (lower in patients, \( t = 3.67 \) and \( t = 4.04 \), respectively; \( P < 0.001 \) in both cases), whereas a trend was observed for ascorbic acid levels, higher among controls (\( t = 1.67 \), Table 1). Significant correlations were observed between Vitamin A and Vitamin E (\( \rho = 0.46; P < 0.001 \)) and between Vitamin E and vitamin C levels (\( \rho = 0.28; P = 0.026 \)).

Table 2. Serum vitamins in cirrhotics and non-cirrhotics

<table>
<thead>
<tr>
<th></th>
<th>Cirrhotics</th>
<th>Non-cirrhotics</th>
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<tbody>
<tr>
<td></td>
<td>( n )</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Vitamin A  (mg/l)</td>
<td>48</td>
<td>0.183 ± 0.151</td>
</tr>
<tr>
<td>Vitamin E  (µg/ml)</td>
<td>44</td>
<td>7.659 ± 3.881</td>
</tr>
<tr>
<td>Vitamin C  (mg/dl)</td>
<td>38</td>
<td>0.928 ± 0.542</td>
</tr>
</tbody>
</table>

Relationships of altered vitamin levels and clinical features

Relationships with liver function

Vitamins A (\( t = 3.65 \)) and E (\( t = 2.89; P < 0.001 \) in both cases) were lower in cirrhotics, whereas no differences were observed regarding ascorbic acid (Table 2). In addition, vitamin A and vitamin E levels were significantly related to liver function impairment (assessed by prothrombin, albumin and bilirubin). Vitamin A was related to prothrombin activity (\( r = 0.70 \)), albumin (\( r = 0.56 \)) and, inversely, with bilirubin (\( r = -0.59 \)). Vitamin E was related to prothrombin activity (\( r = 0.39; P = 0.001 \)) and albumin (\( r = 0.36; P = 0.002 \)), whereas no relations were observed between ascorbic acid and liver function impairment. Patients with ascites showed lower vitamin A values (\( t = 2.73; P = 0.008 \)), whereas patients with encephalopathy showed both lower vitamin A (\( t = 3.13; P = 0.003 \)) and vitamin E (\( t = 3.03; P = 0.004 \)) levels. Classifying all the patients according to Child’s score (which includes bilirubin, albumin and prothrombin activity, and the presence and characteristics of ascites and encephalopathy), both vitamin A and vitamin E were significantly lower among Child’s B and C patients than among Child’s A patients (Figs 3 and 4). No relationship was observed between ascorbic acid and liver function.

Relationships with nutritional parameters

Both vitamin A and vitamin E showed significant relationships with triglyceride (\( r = 0.29; P = 0.014 \) and \( r = 0.44; P < 0.001 \), respectively) and cholesterol levels (\( r = 0.42; P < 0.001 \) and \( r = 0.44; P = 0.001 \), respectively).

Vitamin E was inversely correlated with total fat (\( r = -0.31; P = 0.018 \)) and, especially, with trunk fat (\( r = -0.34; P = 0.008 \)), whereas no relationships were observed between vitamin C and fat parameters. A significant correlation was also observed between vitamin A and right arm lean mass (\( r = 0.29; P = 0.018 \)). Vitamin A also showed weak, inverse correlations with trunk fat (\( r = -0.27; P = 0.026 \)), and total fat mass (\( r = -0.24; P = 0.051 \)). Patients with irregular eating habits showed lower vitamin E levels (\( Z = 2.07; P = 0.039 \)) and a non-significant trend to lower vitamin C values.

Relationships with ethanol intake and related parameters

No relations were observed between vitamin E, A or C and intensity of ethanol consumption, duration of drinking habit, MCV (mean corpuscular volume) or GGT (gamma glutamyltransferase).

Relationship with cytokines and MDA

Vitamin A showed significant, inverse relationships with TNF-\( \alpha \) (\( \rho = -0.29; P = 0.045 \)), IL-6 (\( \rho = -0.47; P = 0.001 \)),
IL-8 ($\rho = -0.50; P = 0.001$) and MDA ($\rho = -0.33; P = 0.028$). Vitamin E levels also showed negative correlations with TNF-α ($\rho = -0.47; P = 0.001$), IL-6 ($\rho = -0.37; P = 0.011$), but not with IL-8 ($\rho = -0.09$) or MDA ($\rho = -0.27$).

Factors involved in decreased levels of vitamin A and E. In order to discern which of the analysed variables was independently related to vitamin levels, we performed stepwise multiple correlation analyses, introducing liver function tests (such as prothrombin, albumin and bilirubin), nutritional parameters (lean mass, trunk fat mass, cholesterol, triglyceride levels), eating habits and daily drinking amount. We found that serum triglyceride levels and trunk fat mass (inverse correlation) displaced liver function parameters from their correlation with vitamin E. On the contrary, in the case of vitamin A, prothrombin activity was the first—and only—variable that entered the multiple regression analysis.

Relationships between antioxidant vitamins and brain alterations

Antioxidant vitamins and brain CT alterations

Vitamin E showed a significant correlation with ventricular index ($r = 0.31; P = 0.013$) and also with bicaudate index ($r = 0.29; P = 0.019$). Using Spearman’s analysis, a correlation was also observed vitamin A and ventricular index ($\rho = 0.24; P = 0.049$) and between vitamin C and Evan’s index. Patients with cerebellar atrophy showed lower levels of both vitamin A ($r = 2.41; P = 0.019$) and vitamin E ($r = 2.05, P = 0.045$) (Table 3). Brain atrophy data showed no relationship with MDA, liver function tests, TNF-α, IL-8 or nutritional parameters, but several relationships were observed with IL-6: patients with cerebellar atrophy showed higher values of IL-6 ($Z = 2.60; P = 0.009$), and IL-6 was directly correlated with Evans’ index ($\rho = 0.43; P = 0.003$) and bifrontal index ($\rho = 0.31; P = 0.035$). By logistic regression analysis, introducing IL-6, serum vitamins and liver function tests, only vitamin A was independently related to cerebellar atrophy, but vitamin A was displaced by the variable age when this last was also introduced in the analysis.

Antioxidant vitamins and cognitive dysfunction

A trend was observed between minimental test and bicaudate index ($\rho = -0.30, P = 0.055$), and also, nearly significant

### Table 3. Vitamins and interleukin-6 (the only interleukin which showed differences) among patients with or without cerebellar or frontal atrophy

<table>
<thead>
<tr>
<th></th>
<th>Cerebellar atrophy</th>
<th>Non-cerebellar atrophy</th>
<th>T, P</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± SD</td>
<td>n</td>
</tr>
<tr>
<td>Vitamin A (mg/l)</td>
<td>55</td>
<td>0.222 ± 0.185</td>
<td>18</td>
</tr>
<tr>
<td>Vitamin E (μg/ml)</td>
<td>49</td>
<td>7.659 ± 3.881</td>
<td>18</td>
</tr>
<tr>
<td>Vitamin C (mg/dl)</td>
<td>47</td>
<td>1.010 ± 0.627</td>
<td>11</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58</td>
<td>54.00 ± 10.97</td>
<td>19</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>35</td>
<td>26.39 ± 52.91 9.12 (5.00–22.40)</td>
<td>11</td>
</tr>
<tr>
<td>Frontal atrophy</td>
<td>n</td>
<td>Mean ± SD</td>
<td>n</td>
</tr>
<tr>
<td>Vitamin A (mg/l)</td>
<td>58</td>
<td>0.243 ± 0.226</td>
<td>16</td>
</tr>
<tr>
<td>Vitamin E (μg/ml)</td>
<td>53</td>
<td>8.435 ± 4.577</td>
<td>14</td>
</tr>
<tr>
<td>Vitamin C (mg/dl)</td>
<td>47</td>
<td>0.990 ± 0.635</td>
<td>11</td>
</tr>
<tr>
<td>Age (years)</td>
<td>61</td>
<td>53.28 ± 11.15</td>
<td>16</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>37</td>
<td>23.76 ± 51.93 7.84 (5.00–19.05)</td>
<td>9</td>
</tr>
</tbody>
</table>
relationships with ventricular index ($\rho = -0.27$) and bifrontal index ($\rho = -0.26, 0.1 > P > 0.05$ in both cases). However, no relationships were observed between serum vitamins and cognitive function (assessed by minimental test). Also, no relationships were observed between minimental test, cytokines, MDA or liver function.

DISCUSSION

This study shows that serum levels of vitamin E and vitamin A are lower in alcoholics than in controls. This finding is in accordance with the results obtained by most (Bjørneboe et al., 1987), but not all the authors (Fernández-Solá et al., 1998) who have analysed this item. In alcoholics diverse mechanisms may cause a decrease in antioxidant vitamins, such as poor diet (Gueguen et al., 2003), malnutrition (Leo et al., 1993), malabsorption (Krasner et al., 1976), liver failure (Van de Casteele et al., 2002), inflammation (Santolaria et al., 2000) or even increased urinary excretion (Fai zal l et al., 1986). Our results suggest that different mechanisms may be involved. Both serum α-tocopherol and vitamin A levels were significantly lower among cirrhotics. The relation between both vitamins and liver function was also underscored by the strong correlations observed between vitamin A and prothrombin activity, serum albumin, serum bilirubin and Child’s score. However, alcoholics are also malnourished, and malnourishment could account, theoretically, for low vitamin levels. Indeed, both vitamin A (especially with parameters related to lean mass) and vitamin E (with parameters related to fat mass) were related to nutritional variables. In the case of vitamin A, liver function parameters displaced nutritional ones by stepwise multivariate analysis, suggesting an outstanding importance of liver function on vitamin A levels, a result in accordance with others reported (Van de Casteele et al., 2002). On the contrary, nutritional parameters—trunk fat and triglyceride levels—displaced liver function in the case of vitamin E, suggesting a greater importance of nutritional changes on vitamin E levels. In this last case it is also noteworthy that vitamin E levels were lower among those who ate irregularly, suggesting that dietary deficiency may also account for low vitamin E levels, in accordance with other authors who also reported a relation between tocopherol levels and altered intake (Gueguen et al., 2003), altered absorption (Krasner et al., 1976) or poor nutrition in general (Leo et al., 1993; Santolaria et al., 2000). Therefore, the mechanisms leading to deficiency in retinol and α-tocopherol may differ among alcoholics.

However, it is important to consider that the relation of vitamin E (and A) with fat parameters were inverse ones; i.e. both vitamins showed higher levels when trunk fat mass was lower. Although explanation of these results may be problematic, it is possible that a greater fat mass in a setting of increased lipid peroxidation may also lead to an enhanced consumption of antioxidant vitamins. However, this is speculative, since, for instance, MDA did not show any correlation with fat parameters ($\rho = 0.12; P > 0.40$).

The principal objective of this study was to analyse if there was a relationship between brain atrophy and antioxidant vitamin levels. We did find that low levels of vitamin A and vitamin E are related to several parameters that indicate brain alteration. These results are in agreement with other observations. In experimental setting, the addition of α-tocopherol increases brain glutathione levels (Bondy et al., 1996); an acute ethanol load produces an increase in lipid peroxidation in the rat cerebellum, together with a decrease in ascorbate and alpha-tocopherol levels (Rouach et al., 1987), and, in other studies, deficiency in antioxidant vitamins was related to brain alterations, which reverted after vitamin E supplementation (Shirpo or et al., 2009). However, evidence of a beneficial effect of vitamin E supplementation on brain alterations in human beings is lacking (Isaac et al., 2008), and it also remains unclear whether antioxidant supplements are useful or not in other forms of alcohol-mediated damage, such as liver disease (Bjelakovic et al., 2010).

Cerebellar atrophy is a common finding among alcoholics (Nicolás et al., 2000). We observed that patients with cerebellar atrophy showed lower values of serum vitamin A and tocopherol. Other authors also report a relation between vitamin E deficiency and cerebellar atrophy (Battisti et al., 1998). However, in this study, vitamin A, but not tocopherol, was the biochemical parameter which showed an independent relation with cerebellar atrophy, only displaced by age. Following the same reasoning as other authors (Rouach et al., 1987), this result could be interpreted as related to increased oxidative stress: the more intensely decreased levels of both retinol and tocopherol in those with cerebellar atrophy possibly reflect the consumption of these antioxidants trying to counteract an excessive ROS production. Raised MDA levels reported in this study lend support to this hypothesis. Several other drugs, which consumption is frequently associated with ethanol addiction in some groups of alcoholics, may also lead to increased ROS production and neurotoxicity (Gutowicz et al., 2006; Vitcheva, 2012). However, this was not the case in the patients included in this study, most of them inhabitants of rural areas, who solely reported ingestion of ethanol.

We also found in this study inverse relations between proinflammatory cytokines and tocopherol and vitamin A, especially with this last. Raised levels of these proinflammatory cytokines have been well described in the alcoholics (Fujimoto et al., 2000; Taieb et al., 2000; Su et al., 2002; McClain et al., 2004). It seems that the initial event in cytokine secretion is stimulation of Kupffer cells by gram-negative bacteria reaching the liver in the context of ethanol-mediated increased intestinal permeability (Crews et al., 2006). As commented, these cytokines may be also involved in oxidative stress and neuroinflammation (Qin and Crews, 2012). We failed to find direct relationships with TNF, IL-8 or MDA and brain alterations, but indeed between IL-6 and cerebellar atrophy, Evan’s index and bifrontal index, suggesting the existence of a relation between this cytokine on brain alterations observed in alcoholics. Interestingly, we also found significant, inverse relations of IL-6 with both vitamin A ($\rho = -0.47$) and vitamin E ($\rho = -0.37$) levels, i.e. inverse relationship between increased neuroinflammation and decreased antioxidant levels. Other authors have also reported an inverse relation of IL-6 and age-related brain atrophy (Jefferson et al., 2007), supporting the view that IL-6 may be a factor involved in the development of brain atrophy in different settings, not only in alcoholics.

Therefore, we conclude that among alcoholics, liver function impairment is related to altered serum vitamin A levels. Vitamin E levels are also decreased, but although related to liver function impairment, its decrease seems to be more dependent on nutritional status and irregular eating habits. Both
vitamins are lower in patients with cerebellar atrophy and other brain alterations, and show inverse correlations with proinflammatory cytokines, especially with IL-6, which is also related to brain alterations.

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Conflict of interest statement. Authors declare that there are no conflicts of interest.

AUTHORS’ CONTRIBUTIONS

E.G.-R. designed the study, performed statistical analysis and wrote the manuscript. M.C.M.-G., C.M.F.-R., I.H.-B., O.E.-C. collected the data and analysed the brain CT. P.A.-G. performed MDA analysis. M.J.V.-P. performed cytokine analysis and F.S.-F. participated in the study design and revised the manuscript. All the authors approved the final version of the manuscript and state that the manuscript, including related data, figures and tables, has not been previously published and is not under consideration for publication elsewhere.

REFERENCES


