Vitamin D, Vascular Calcification and Mortality among Alcoholics

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(Received 10 June 2014; first review notified 1 September 2014; in revised form 20 September 2014; accepted 2 October 2014)

Abstract — Aims: To analyze the relationship between low vitamin D levels and mortality among alcoholics. Methods: One hundred twenty-eight alcoholic patients admitted to our hospital were followed up as outpatients. Nutritional status was evaluated measuring percentages of fat and lean mass in different body compartments. Results: Lower vitamin D levels were observed in patients with worse liver function. Vitamin D was lower in patients with lower total lean mass (Z = 2.8, P = 0.005), but it was not related to fat mass. There was a significant trend to higher long-term mortality among non-cirrhotics with vitamin D levels below 30 ng/ml, although Cox’s regression model revealed that only Child score and age were independently related to mortality. Conclusion: Vitamin D deficiency is common among alcoholic patients and is associated with low lean mass and liver dysfunction. Among non-cirrhotics, serum vitamin D levels below 30 ng/ml are associated with a greater long-term mortality.

INTRODUCTION

Over the past two decades the effects of alcohol intake on morbidity and mortality have been extensively studied (O’Keefe et al., 2007; Costanzo et al., 2010). While labeled a myth by some (Hansel et al., 2012), moderate levels of alcohol consumption have been associated with lower cardiovascular mortality. This is illustrated by a U or J shaped curve that has been reproduced in several epidemiologic studies (Di Castelnuovo et al., 2006). However, while moderate drinkers may have a more favorable lipid profile than non-drinkers with higher HDL cholesterol and lower LDL cholesterol and apolipoprotein(a) levels which may contribute to improved cardiovascular outcomes (De Oliveira et al., 2000), these beneficial effects are offset by the toxic effects of heavy drinking that lead to increased mortality.

Several factors may explain this increased mortality. Ethanol leads to important derangements of key organs such as liver, heart, immune system, central nervous system, all of them able to cause increased mortality (Rehm et al., 2003). In addition, ethanol may affect the metabolism of some intermediary factors which may lead to higher mortality. One of these factors is vitamin D, low levels of which have been associated with increased mortality. Indeed, vitamin D not only has an important role in bone metabolism but it also has a role in determining muscle strength and in the development of cardiovascular disease and cancer, so that low vitamin D levels are related to all-cause mortality (Wang et al., 2008; Chowdhury et al., 2014). Since ethanol consumption is associated with low vitamin D levels and hypovitaminosis D is in turn associated with increased mortality, we sought to analyze vitamin D and its relationship with mortality among patients with alcoholism.

METHODS

Patients
We included 128 alcoholic patients admitted to an Internal Medicine ward, aged 50.8 ± 10.98 (median = 49). We included patients who consumed at least 80 g of ethanol per day for a minimum of 5 years. Eighty-eight percent of patients were men and most of them were heavy drinkers of >100 g of ethanol per day (194.5 ± 83 g/day) for a period of 29.5 ± 10.23 years. Patients were followed up as outpatients during a mean of 40 months [interquartile range (IQ) 27.25–88 months].

Liver function
Patients were classified as having cirrhosis or not according to clinical grounds and ultrasound findings. Forty-eight percent of patients had liver cirrhosis at the time of inclusion in the study while the remaining 52% showed either hepatic steatosis or a normal liver morphology on abdominal ultrasound and had no clinical evidence of cirrhosis. Most cirrhotics (34/61; 55.7%) were admitted with complications of liver disease (ascites, encephalopathy, gastrointestinal bleeding), and 15 more, with a diagnosis of withdrawal syndrome. On the contrary, the vast majority (63%, 42/67) of non-cirrhotics had a diagnosis on admission of withdrawal syndrome; 12% of these had a diagnosis of sepsis; only two of them were diagnosed with upper gastrointestinal bleeding or complications of liver disease; the remaining patients had pancreatitis or other problems. The severity of cirrhosis was then assessed using Child’s classification which is based on serum albumin, bilirubin, prothrombin activity, ascites and encephalopathy. Ascites was assessed by physical examination whereas the presence of encephalopathy was established using the West Haven classification system (18.8% had hepatic encephalopathy and 11.7, 4.7 and 2.3% had grade 2, 3 and 4 encephalopathy, respectively). Using the Child score, 16.4% of patients were classified as Child A (less severe), 44.3% as Child B (moderately severe) and 32.8% as Child C (most severe). While Child score was originally designed to predict cirrhosis mortality, few patients in our study had a histological diagnosis of cirrhosis. In order to provide a global assessment of liver function (to be compared with vitamin D levels, mortality, etc.), we applied the Child’s score to the entire population, even in patients without cirrhosis.

Nutritional status
Nutritional status was evaluated objectively assessing whole body composition using a HOLOGIC QDR-2000 (Waltham,
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Sixty-one patients had cirrhosis and 67 were non-cirrhotics. Serum vitamin D levels did not differ significantly in both groups \((Z = 0.62, P = 0.19; Table 1)\). There was a significant correlation between serum albumin and serum vitamin D levels \((\rho = 0.30, P = 0.01)\) and an inverse one between bilirubin and vitamin D levels \((\rho = -0.18, P = 0.038)\). However, there was no correlation between prothrombin activity and vitamin D and we also failed to find differences regarding vitamin D levels and the presence of ascites or encephalopathy (Table 1). However, there was a trend \((K-W = 5.51, P = 0.064)\) to lower vitamin D levels in patients with worse liver function assessed using the Child classification (Table 1). These differences were statistically significant when patients classified as Child A were compared with those classified as Child C \((Z = 2.31, P = 0.021)\).

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These patients were followed up during a median period of 40 months \((IQR = 27.25–88)\) with a minimum follow-up time of 1 day to a maximum time of 100 months. During this time 54 patients died and 62 survived; the remaining 12 were lost. There was no relationship between the presence of vascular calcifications and mortality. There was a trend \((P = 0.056\) using Fisher’s exact test) towards higher mortality in those with vascular calcifications in abdominal plain film. However, when calcification was assessed by means of computed tomography (CT) scan, there was no relationship between calcium and mortality. Considering CT scan as the gold standard for the detection of vascular calcifications we found that the sensitivity of abdominal plain film for the detection of vascular calcifications was low \((16\%)\).

There was a trend to lower vitamin D levels among those who died \((Z = 1.86, P = 0.06)\). A similar trend was observed when patients with vitamin D levels over the median were compared with those with vitamin D below the median \((\chi^2 = 2.81, P = 0.09)\) or vitamin D higher or lower than 30 ng/ml \((\chi^2 = 2.64, P = 0.10)\). Whereas no association was found between low vitamin D levels and mortality among cirrhotics \((below\ the\ median, \chi^2 = 0.39, NS; below\ 30\ ng/ml, \chi^2 = 0.02, NS)\), there was an association between low serum vitamin D

RESULTS

We included a total of 128 patients, 113 men and 15 women. Vitamin D levels were relatively low \((median\ 26.15\ ng/ml, interquartile\ (IQ)\ range\ 19–39.5\ ng/ml)\). Indeed, following the usually recommended cutoff points to define insufficiency \((<30\ ng/ml)\) and deficiency \((<20\ ng/ml, Kennel et al., 2010)\), 73 patients were classified as having vitamin D insufficiency while 36 of them had vitamin D deficiency. Moreover, for comparative purposes we provide in Table 1 the data derived from 22 healthy hospital workers \((median\ age = 42.5; IQ = 31–47\ years; 7\ women, 15\ men)\), who showed median serum vitamin D values of 84.49 ng/ml and an IQ range = 69.02–109.88 ng/ml.

Serum vitamin D levels were similar among men and women, as is shown in Table 1 \((Z = -0.44, P = 0.66, NS)\). There was no association between age and vitamin D levels \((\rho = 0.01, P = 0.91)\). We did not find any significant correlation between vitamin D levels and the amount of ethanol consumed \((\rho = 0.03, P = 0.76)\), duration of alcohol consumption \((\rho = 0.05, P = 0.62)\), of number of pack-years \((\rho = 0.16, P = 0.14)\).

Vitamin D and liver function

We collected blood samples after overnight fast. Samples were stored at -80°C until the following biochemical markers were determined. We determined serum vitamin D, albumin and bilirubin levels in all patients. We also measured prothrombin activity.

Statistics

The Kolmogorov–Smirnov test was used to test for normal distribution, a condition not fulfilled by vitamin D, lean mass in different body compartments, and BMI. Mann–Whitney’s U-test and Kruskall–Wallis test were used to analyze differences in these parameters between groups. Spearman’s rho was used in correlations of non-parametric variables. We used a level of significance of \(\alpha = 0.05\) for all tests. Survival was assessed by Kaplan–Meier curves and Cox regression model. Statistical analyses were performed using SPSS software (Chicago, IL, USA).

Ethics committee

The study was approved by the ethic committee of our Hospital (2014_11), and every patient provided written informed consent.

MA, USA) dual-energy X-ray densitometer. Body mass index (BMI) was calculated in all patients. However, due to the fact that total weight (including the weight of extracellular fluid in patients with ascites and pleural effusion) was used to calculate BMI, percentages and absolute amounts of fat and lean mass in different body compartments were measured by the densitometer.

Assessment of vascular calcifications

The presence of vascular calcifications was established by examining plain chest and abdominal films. Seventy-eight percent of the patients studied underwent computed tomography scans (by clinical grounds, i.e., pancreatitis, study of hepatomegaly, tumor suspicion, etc.), so vascular calcifications were also assessed by this method.

Biochemical parameters

We included a total of 128 patients, 113 men and 15 women. Vitamin D levels were similar among men and women, as is shown in Table 1 \((Z = -0.44, P = 0.66, NS)\). There was no association between age and vitamin D levels \((\rho = 0.01, P = 0.91)\). We did not find any significant correlation between vitamin D levels and the amount of ethanol consumed \((\rho = 0.03, P = 0.76)\), duration of alcohol consumption \((\rho = 0.05, P = 0.62)\), or number of pack-years \((\rho = 0.16, P = 0.14)\).

Vitamin D and nutritional status

In order to group patients into those with lower and higher lean mass, we calculated the median total lean mass \((50618.8 g)\), trunk lean mass \((25459.55 g)\), right arm lean mass \((2629.5 g)\), right leg lean mass \((7293.1 g)\), left leg lean mass \((6995.35 g)\) and left arm lean mass \((2573.3 g)\). As shown in Table 2, Vitamin D was significantly lower in patients with lower trunk lean mass \((Z = 2.86, P = 0.004)\) and total lean mass \((Z = 2.80, P = 0.005)\). We also found that vitamin D levels were nearly significantly lower in patients with lower left arm lean mass \((Z = -1.94, P = 0.052)\). There were also non-significant trends to lower vitamin D levels among those below the median right arm, left leg and right leg lean mass (Table 2). In contrast, no differences were observed when vitamin D levels were compared among fat mass below or above the median at different parts of the body (Table 2). We found lower vitamin D levels among patients with BMI values below the median \((Z = 2.3, P = 0.021)\).

Statistics

The Kolmogorov–Smirnov test was used to test for normal distribution, a condition not fulfilled by vitamin D, lean mass in different body compartments, and BMI. Mann–Whitney’s U-test and Kruskall–Wallis test were used to analyze differences in these parameters between groups. Spearman’s rho was used in correlations of non-parametric variables. We used a level of significance of \(\alpha = 0.05\) for all tests. Survival was assessed by Kaplan–Meier curves and Cox regression model. Statistical analyses were performed using SPSS software (Chicago, IL, USA).

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levels and mortality among non-cirrhotics [only 3 out of 26 non-cirrhotic patients with serum vitamin D levels over 30 ng/ml died versus 14 out of 37 with vitamin D levels below 30 ng/ml (χ² = 4.11, P = 0.043 after Yates correction; relative risk = 1.65; 95% confidence interval = 1.15–2.37)], and only 4 out of 30 non-cirrhotics with vitamin D levels over the median died versus 13 out of 33 with vitamin D levels below the median (χ² = 4.18, P = 0.041 after Yates correction; relative risk = 1.76 (95% confidence interval = 1.15–2.18)).

Similar results were observed when survival was analyzed by means of Kaplan–Meier curves (Figs 1 and 2). There were significant differences in survival among non-cirrhotics with vitamin D levels below the median (log rank = 4.19; P = 0.041), but not among cirrhotics or considering the whole population (log rank = 1.38; P = 0.240).

However, when mortality was assessed by using the Cox regression model, vitamin D levels were displaced: only Child score and age were independently associated with mortality (Table 3).

### DISCUSSION

In this study we found an association between low vitamin D levels and low lean mass but we failed to find a correlation with fat mass. The relationship between vitamin D and muscle mass and function has been disentangled in the last decade, especially after the identification of a specific vitamin D receptor in muscle fibers (Bischoff et al., 2001; Girgis et al., 2013). This explains the common observation of proximal muscle...
atrophy in patients affected by celiac disease, other malabsorptive conditions and osteomalacia (Hall, 1968; Byrne et al., 2002; Glerup et al., 2000). In addition, it has been shown that experimental animals lacking a vitamin D receptor showed reduced muscle mass and fiber size compared with wild-type animals (Endo et al., 2003). These results are in accordance with the observation performed by our group in which vitamin D levels were related to type IIa muscle fiber area (González-Reimers et al., 2010). The results obtained regarding vitamin D levels and lean mass in the present study are full in accordance with these observations. In this sense it is interesting to point out that there was no correlation between vitamin D levels and the amount of fat mass, something which would not have happened if vitamin D deficiency and reduced lean mass were due to malabsorption. In this case, fat mass would have also been reduced and we would have expected to find a correlation with vitamin D levels.

We also found an association between vitamin D levels and liver function. Liver dysfunction may be associated with varying degrees of malabsorption, not only related to the consumption of ethanol itself but also to portal hypertension secondary to liver cirrhosis (Hayashi et al., 2000). Therefore it is not surprising that deranged liver function is accompanied by low levels of liposoluble vitamins, the absorption of which requires the presence of bile salts (Fisher, 2009). Several studies report an association between liver disease and vitamin D deficiency, both in alcoholic and in non-alcoholic liver disease. Some authors have found an independent relationship between low 25-hydroxy vitamin D and non-alcoholic fatty liver disease (Eliades et al., 2013), while other authors report low levels of vitamin D in alcoholic liver dysfunction (Song and Rockey, 2013). The findings of some authors suggest that vitamin D deficiency may be involved in progressive liver fibrous tissue deposition (Abramovitch et al., 2011; Nobili et al., 2014). However, this finding is not shared by other authors and some have failed to find any relationship between liver dysfunction and vitamin D deficiency (Skaaby et al., 2013). In any case, the pathogenetic mechanisms have not been identified. The possible explanation of impaired absorption due to bile salt alteration mentioned above is merely speculative but is a theoretical possibility.

In accordance with studies performed on the general population (Zitterman et al., 2009; Bielakovic et al., 2014), we found a higher mortality among non-cirrhotic alcoholic patients with low vitamin D levels. Recent research has shown that due to the vast distribution of its receptor (VDR), vitamin D deficiency can have protean manifestations (Bouillon et al., 2008). As mentioned earlier, by incompletely understood mechanisms, vitamin D deficiency may be involved in increased cardiovascular risk (Elamin et al., 2011; McGreevy and Williams, 2011; Brundum-Jacobsen et al., 2012; Liss and Frishman, 2012; Siadat et al., 2012; Tomson et al., 2013) and perhaps in carcinogenesis (Giovannucci, 2005, 2009; Pilz et al., 2013). Some authors have shown that vitamin D levels below 10 ng/ml in older adults are associated with an increased risk of all-cause mortality [HR (95% confidence

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient ($\beta$)</th>
<th>Wald</th>
<th>$P$-value</th>
<th>Odds ratio</th>
</tr>
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<tbody>
<tr>
<td>Step 1: Global Child score$^a$</td>
<td>0.885</td>
<td>23.698</td>
<td>0.000</td>
<td>2.422</td>
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<tr>
<td>Step 2: Age</td>
<td>0.34</td>
<td>6.946</td>
<td>0.008</td>
<td>1.035</td>
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<tr>
<td>Global Child score$^a$</td>
<td>0.894</td>
<td>23.240</td>
<td>0.000</td>
<td>2.445</td>
</tr>
</tbody>
</table>

$^a$Note: We applied the Child score to the entire population, even in patients without cirrhosis, in order to provide a global assessment of liver function.
interval [CI] 2.27 (1.59–3.24)] (Kritchevsky et al., 2012). In our series, although median values of vitamin D levels were low, and a high proportion of patients showed vitamin D values below 30 ng/ml, only three alcoholics showed values below 10 ng/ml, precluding statistical analysis.

It is noteworthy that low vitamin D levels were related to mortality only in the subgroup of non-cirrhotic patients. Although the reason is unclear, it could be speculated that, perhaps, the presence among cirrhotics of several potentially lethal conditions, such as bleeding tendency, liver failure, ascites with possible renal impairment or variceal bleeding, may obscure the eventual importance of low vitamin D levels regarding mortality. In any case, Cox regression analysis reveals that only classical parameters, namely Child score and age, were independently associated with mortality. This is in contrast with the results of a recent study on 2649 patients. In that study vitamin D was found to be an independent prognostic factor, and the authors failed to find a relationship between altered liver enzyme levels and mortality associated with low vitamin D levels (Skåaby et al., 2013). However, that was a population-based study.

A recent meta-analysis of observational studies and randomized controlled trials showed that supplementation with vitamin D significantly reduces overall mortality among older adults (Chowdhury et al., 2014). Other reviews suggest similar results but the validity of the results has been put into question and more randomized controlled trials may be needed (Bielakovic et al., 2014; Theodoratou et al., 2014).

Studies also point out that low vitamin D levels, related to sarcopenia and increased mortality, could be considered a marker of frailty (Bischoff-Ferrari et al., 2004, 2009; Kim et al., 2011). Therefore the association with mortality would not be a direct effect of vitamin D deficiency but it would be a consequence of the fact that vitamin D deficiency itself is a marker of frailty.

In any case we conclude that vitamin D deficiency is common in alcoholic patients and it is associated with liver dysfunction and low lean mass but not with low fat mass. In addition, low vitamin D values are associated with long-term mortality among non-cirrhotic alcoholics, as assessed using Kaplan–Meier curves, although the only variables independently associated with mortality using Cox regression are Child score and age.

Conflict of interest statement. None declared.

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