Abstract

Objective: to verify blood levels of vitamin D in patients with chronic cholestasis, and relate them to nutritional status, length of time since the onset of cholestasis and use of vitamin supplement.

Methods: controlled cross-sectional study with chronic cholestasis as study factor and blood levels of vitamin D as outcome. The study included patients aged between 4 months and 18 years, who were cared for at the Pediatric Gastroenterology Unit of Hospital de Clínicas de Porto Alegre. Controls were eutrophic children in the same age range. Blood was collected for radioimmunoassay. Anthropometric analyses were performed, as was determination of the length of time since the onset of cholestasis and use of vitamin supplement.

Results: twenty-two patients and 17 controls were evaluated. Average vitamin D level in patients was 13.7 ± 8.39 ng/ml, compared to 25.58 ± 16.73 ng/ml in controls (p = 0.007). Prevalence of hypovitaminosis D in patients was 36%. Median of period of time since the onset of cholestasis was 1 year with variation of 6 months to the 25th percentile and 3.9 years to the 75th percentile. Anthropometric evaluation (NCHS) showed 36% of malnutrition by weight and 41% by height. Anthropometric evaluation according to Z score showed 33.3% and 23.8% prevalence of malnutrition for the criteria height/age and weight/age, respectively. The evaluation of weight regarding height did not show values below two standard deviations. No relationship was found between nutritional state, use of oral vitamin supplement and blood levels of vitamin D.

Conclusions: blood levels of vitamin D in patients with cholestasis were lower than those of controls, but were not related to nutritional status, period of time since the onset of cholestasis or use of vitamin supplement.

Introduction

Cholestasis is a reduction in or an absence of the flow of bile in the duodenum which may be the result of the hepatocyte failing to secrete bile, an absence of the intrahepatic bile ducts or an obstruction of the extrahepatic ducts.1

The nutritional status of a child with chronic liver disease is one of the factors which interfere with survival, as much in the case of a patient awaiting a transplant as in the case of one who has already undergone transplant surgery.2,3

The absence of bile in the intestine causes steatorrhea with the signs and symptoms which result from malabsorption and which are4 diarrhea; malnutrition; reduced plasma proteins, night blindness and skin lesions due to vitamin A absorption deficiencies; osteopenia due to vitamin D malabsorption; bleeding and hematomas due to vitamin K malabsorption; neuromuscular weakening due to vitamin E malabsorption.
Fat soluble vitamins include A, D, E and K. They are absorbed in the gastrointestinal tract together with dietary fats. The organism is not capable of synthesizing them or synthesizes them in insufficient quantities and requires that they be supplied by food.

Considering its availability, metabolism and mechanism of action, it is more correct to consider vitamin D to be a hormonal steroid than a vitamin in the classical sense. This vitamin occurs naturally in animal derivative foods in the form of cholecalciferol. It is found in small and highly variable quantities in butter, cream, egg yolk and liver. Fish liver oil is considered the best source of vitamin D and has been historically recognized as a cure for rickets.5

Patients with chronic cholestasis present malabsorption of dietary vitamin D associated with lower levels of exposure to the sun due to their pathological condition. The primary manifestations of vitamin D deficiency are hypocalcemia, hypophosphatemia, tetany, osteomalacia and rickets.2

The ability to precisely measure metabolites of vitamin D in human serum or plasma has been perfected during recent decades, but, despite this progress, only the measurements of 25-OHD and 1.25 (OH2)D have proved useful clinically.6 A 25-OHD assay is the most apt manner in which to verify the real situation of plasma vitamin D levels since 1.25 (OH2)D levels are more indicative of the calcium situation and are not essential when monitoring vitamin D.7

The methods of measuring 25-OHD described in literature are: “Competitive Protein Binding Assay”(CPBA), “High-Pressure Liquid Chromatography” (HPLC) and radioimmunoassay (RIA). The CBPA and HPLC techniques require deproteinization or extraction methods such as chromatographic preparation which require large sample volumes (1 to 5 ml). They are considered time-consuming and expensive techniques.8 Until a few years ago authors considered the radioimmunoassay technique to be unspecific and requiring chromatographic purification beforehand.6 More recent studies have used antisera which permit the performance of more specific assays which are simpler to execute and require lower volumes of plasma or serum.9

Vitamin D deficiency, according to studies by Heubi et al.,10 does not appear to be the principal cause of osteopenia in chronic cholestasis. They accept that the combination of alterations to mineral and vitamin D absorption associated with alterations to the protein matrix may contribute to the metabolic bone disease in such patients. It has been observed that patients with chronic cholestasis whose vitamin D status normalizes may continue to present evidence of metabolic bone disease and this reinforces the idea that there are other factors contributing to osteopenia in these patients.2

Plasma vitamin D tests are not performed routinely. No Brazilian studies of hypovitaminosis D in children with hepatopathy were found, but bone mineralization deficiency in children with chronic cholestasis, evaluated by densitometry, is already recognized in our environment.11

The objective of this study is to test the plasma vitamin D levels of a sample of children and adolescents with chronic cholestasis and relate this to the nutritional status, period of cholestasis and vitamin supplement usage.

Methods

A controlled transverse study was performed in which the factor studied was chronic cholestasis and the outcome measure plasma vitamin D levels. The population studied consisted of cholestatic children and adolescents who attended the clinic or were interned at the Pediatric Gastroenterology Unit of the Hospital de Clínicas de Porto Alegre (HCPA) in the period from December 2000 to April 2002. Well-nourished children and adolescents who were normal from a gastroenterological point of view and who attended the Pediatric Surgery clinic at the HCPA for elective surgery during the same period participated as controls.

The criteria used for the inclusion of patients were the following:

- Age of 4 months to 18 years.
- Evidence of cholestasis for a period greater than 3 months diagnosed by: jaundice, choluria, acholia or pruritis (itching) due to direct reaction hyperbilirubinemia greater than 30% of total bilirubin.

Exclusion criteria were the following:

- Chronic hepatopathy without evidence of cholestasis.
- The use of injected vitamin D during the previous 30 days.
- The use of total parenteral nutrition (TPN) during the previous 3 months.
- Failure of parents or guardians to sign the informed consent form.

Criteria for inclusion of the controls were the following:

- The same age group as the patients assessed (4 months to 18 years).
- Weight and stature between the 25th and 75th percentiles for age and sex according to the National Center of Health Statistics (NCHS) graph.

Criteria for exclusion of the controls were the following:

- Evidence of liver disease or gastrointestinal disease checked by means of medical history, physical examination and a review of medical records.
- Failure of parents or guardians to sign the informed consent form.

The sample size was calculated to give a statistical power of 80%, taking $\alpha = 0.05$ and an effect size of
approximately one standard deviation. In order to achieve this it was necessary to include, at least, 34 individuals between cases and controls.

After obtaining family consent, blood was taken in order to process the vitamin D assays. Blood was collected and put into flasks with EDTA and, after separation, the plasma was stored in a freezer at -80 °C for later vitamin assay. For the controls, in addition to the pre-operative exams that they needed, authorization was requested of their parents to take a blood sample for the vitamin assay. The technique used for the plasma assay of this vitamin was Radioimmunoassay using the 25-hydroxyvitamin D - Nichols Institute Diagnostics - Paris kit.

At the same time as blood was taken, a nutritional analysis was performed for the cholestatic patients. The analysis of anthropometric results was made based on the NCHS tables for percentiles and Z scores. Individuals whose measurements for weight and stature were two or more standard deviations below the average for their Z score or whose percentile on the NCHS standards was below 5.

A questionnaire was used to verify the use of fat soluble vitamin supplements which are prescribed for cholestatic patients and consist of Aderogil D3™ 12 drops twice a day (vitamin A 6,600 UI/dl and vitamin D 2,600 UI/dl); Ephynal™ 400mg every 4 days (vitamin E 100mg/day) and Kanakion™ once a month (Vitamin K 5mg/month).

The period of cholestasis was defined as the date of diagnosis of the primary disease identified by clinical history and a review of the patient’s medical record.

The research project “Serum levels of vitamin D in children and adolescents with chronic cholestasis” was approved by the Commission for Research and Ethics in Health of the HCPA along with the interview questionnaire and the consent forms for the patients and controls.

The statistical package used was the Statistical Package for Social Science (SPSS). A descriptive analysis was performed in order to characterize the population being studied with mean percentage, median, standard deviation and percentiles.

The parametric T test was used to evaluate results with normal behavior between the two groups (cholestatic patients and controls): age and vitamin D plasma level. The remaining tests were non-parametric as there was no normal distribution of the variables of interest. The chi-square test was used for comparisons between the qualitative variables gender, group, nutritional analysis and use of oral supplements. The Mann-Whitney U test was used for the quantitative variables (age, plasma levels, period of cholestasis). Correlations between plasma levels and the period of cholestasis were analyzed with the Spearman coefficient. For all of the above tests a value of p < 0.05 was considered significant.

Results

Twenty-two children and adolescents with chronic cholestasis participated in the study, of whom 13 were female, (59.1%) and nine male (40.9%). The median of their ages was 3.6 years with variations between 10 months for the 25th percentile and 12 years for the 75th percentile. Seventeen normal children participated as controls, 13 of whom were male (76.5%) and four female (23.5%). The median of their ages was 4.3 years with variations from 2 years at the 25th percentile and 10.8 years at the 75th. There was no statistical difference between the cholestatic patients and the control group in terms of age (p = 0.235) or gender (p = 0.42).

In Table 1 we find the clinical and laboratorial characteristics of each of the patients studied. In the anthropometric analysis by NCHS tables, we observe a prevalence of 36% malnutrition by weight and 41% by stature in relation to age and sex. In the anthropometric analysis by Z score, we obtained malnourishment prevalence for the criteria height/age and weight/age of 33.3% and 23.8%, respectively. When we evaluated weight in relation to height we did not observe values below 2 standard deviations.

The regular use of fat soluble oral vitamin supplements was observed in 14 patients (63%). The remaining eight patients (36.4%) did not use them or used the medication prescribed in an irregular manner.

The median of the periods of cholestasis was 1 year with variations from 6 months for the 25th percentile and 3.9 years for the 75th percentile. When the period of cholestasis was related to nutritional state based on the Z score (H/A) we observed that the well-nourished patients had a period of cholestasis which varied from 3 months to 7.7 years with a median at 6 months whereas the malnourished patients had a period of cholestasis which varied from 4 months to 16 years with a median at 12 months. These differences are not statistically significant (p = 0.17).

The mean average value for vitamin D among the cholestatic patients was 13.07 ± 8.39 ng/ml and among the controls it was 25.58 ± 16.73 ng/ml. Figure 1 demonstrates that there was a statistically significant difference. Among the cholestatic individuals we observed a prevalence of 36% of vitamin D deficit (values lower than 9 ng/ml). In the control group no deficiency of this vitamin was found.

The median of the vitamin D values among the well-nourished individuals was 10.47 ng/ml and for those that were malnourished it was 13.63 ng/ml which is not a statistically significant difference as Figure 2 demonstrates. The correlation between period of cholestasis and plasma vitamin D levels was also not significant, as we observe a Spearmann correlation coefficient of 0.09 with a p value of 0.67.

When we compared plasma vitamin D levels of patients who regularly used vitamin supplements with those of patients who did not we found that there was no statistically significant difference as Figure 3 demonstrates.
### Table 1 - Clinic and laboratory characteristics of children with cholestasis

<table>
<thead>
<tr>
<th>ID</th>
<th>Age (months)</th>
<th>Weight (grams)</th>
<th>Height (cm)</th>
<th>Cholestasis period (months)</th>
<th>AST (U/I)</th>
<th>ALT (U/I)</th>
<th>TB/DB (mg/ml)</th>
<th>GGT (U/I)</th>
<th>AP (U/I)</th>
<th>ALB (g/dl)</th>
<th>VIT. D (ng/ml)</th>
<th>Primary disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>7,020</td>
<td>66</td>
<td>9</td>
<td>191</td>
<td>237</td>
<td>3.1 / 1.6</td>
<td>317</td>
<td>858</td>
<td>3.9</td>
<td>17.821</td>
<td>EHBA</td>
</tr>
<tr>
<td>2</td>
<td>62</td>
<td>19,500</td>
<td>105.5</td>
<td>10</td>
<td>224</td>
<td>53</td>
<td>8.4 / 3.9</td>
<td>53</td>
<td>1062</td>
<td>2.6</td>
<td>11.174</td>
<td>EHBA</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>7,900</td>
<td>68</td>
<td>21</td>
<td>137</td>
<td>84</td>
<td>11.5 / 6.5</td>
<td>130</td>
<td>459</td>
<td>2.4</td>
<td>15.413</td>
<td>EHBA</td>
</tr>
<tr>
<td>4</td>
<td>156</td>
<td>34,200</td>
<td>‡</td>
<td>69</td>
<td>154</td>
<td>58</td>
<td>8.1 / 4.9</td>
<td>850</td>
<td>1204</td>
<td>3.5</td>
<td>15.884</td>
<td>PTCH</td>
</tr>
<tr>
<td>5</td>
<td>21</td>
<td>11,300</td>
<td>86</td>
<td>225</td>
<td>229</td>
<td>14.5 / 7.9</td>
<td>215</td>
<td>2,305</td>
<td>2,7</td>
<td>10.748</td>
<td>N/D</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>6,680</td>
<td>§</td>
<td>67.5</td>
<td>9</td>
<td>225</td>
<td>14.5 / 7.9</td>
<td>215</td>
<td>2,305</td>
<td>2,7</td>
<td>10.748</td>
<td>N/D</td>
</tr>
<tr>
<td>7</td>
<td>167</td>
<td>61,500</td>
<td>158</td>
<td>13</td>
<td>338</td>
<td>190</td>
<td>5.3 / 2.5</td>
<td>33</td>
<td>617</td>
<td>2.2</td>
<td>4.1055</td>
<td>N/D</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>5,200†</td>
<td>†§</td>
<td>60</td>
<td>5</td>
<td>152</td>
<td>1.6 / 0.7</td>
<td>105</td>
<td>1682</td>
<td>3.2</td>
<td>28.033</td>
<td>EHBA</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>6,850†</td>
<td>62§</td>
<td>105</td>
<td>30</td>
<td>38 / 19.2</td>
<td>38</td>
<td>156</td>
<td>93</td>
<td>3.4</td>
<td>6.9592</td>
<td>CHF</td>
</tr>
<tr>
<td>10</td>
<td>41</td>
<td>16,000</td>
<td>93</td>
<td>12</td>
<td>125</td>
<td>72</td>
<td>1.6 / 0.7</td>
<td>105</td>
<td>1682</td>
<td>3.2</td>
<td>28.033</td>
<td>N/D</td>
</tr>
<tr>
<td>11</td>
<td>93</td>
<td>17,000</td>
<td>110§</td>
<td>83</td>
<td>111</td>
<td>1.8</td>
<td>341</td>
<td>1303</td>
<td>4</td>
<td>21.007</td>
<td>Al. syn.</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>15</td>
<td>9,500</td>
<td>73.5</td>
<td>15</td>
<td>154</td>
<td>68</td>
<td>29.5 / 22.6</td>
<td>180</td>
<td>1205</td>
<td>4.3446</td>
<td>Al. syn.</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>216</td>
<td>60,300</td>
<td>163</td>
<td>104</td>
<td>187</td>
<td>89</td>
<td>3.8 / 2.2</td>
<td>80</td>
<td>587</td>
<td>2.1</td>
<td>6.9592</td>
<td>CHF</td>
</tr>
<tr>
<td>14</td>
<td>156</td>
<td>30,800</td>
<td>136§</td>
<td>12</td>
<td>675</td>
<td>417</td>
<td>4.7 / 3.5</td>
<td>133</td>
<td>946</td>
<td>4.4</td>
<td>5.2052</td>
<td>ACH</td>
</tr>
<tr>
<td>15</td>
<td>36</td>
<td>15,160</td>
<td>85§</td>
<td>3</td>
<td>131</td>
<td>115</td>
<td>1.6 / 0.8</td>
<td>482</td>
<td>996</td>
<td>3.8</td>
<td>13.631</td>
<td>EHBA</td>
</tr>
<tr>
<td>16</td>
<td>4</td>
<td>6,100</td>
<td>65</td>
<td>4</td>
<td>477</td>
<td>365</td>
<td>17.9 / 9.3</td>
<td>733</td>
<td>1935</td>
<td>3.5</td>
<td>4.7355</td>
<td>EHBA</td>
</tr>
<tr>
<td>17</td>
<td>156</td>
<td>55,700</td>
<td>156</td>
<td>5</td>
<td>134</td>
<td>144</td>
<td>2.9 / 1.0</td>
<td>43</td>
<td>698</td>
<td>3</td>
<td>9.7474</td>
<td>ACH</td>
</tr>
<tr>
<td>18</td>
<td>192</td>
<td>77,300</td>
<td>176</td>
<td>192</td>
<td>96</td>
<td>119</td>
<td>3.2 / 1.3</td>
<td>316</td>
<td>737</td>
<td>3.6</td>
<td>18.601</td>
<td>EHBA</td>
</tr>
<tr>
<td>19</td>
<td>7</td>
<td>7,720</td>
<td>68</td>
<td>7</td>
<td>95</td>
<td>72</td>
<td>7.4 / 4.4</td>
<td>322</td>
<td>1236</td>
<td>2.7</td>
<td>32.662</td>
<td>EHBA</td>
</tr>
<tr>
<td>20</td>
<td>47</td>
<td>11,800</td>
<td>91§</td>
<td>47</td>
<td>212</td>
<td>149</td>
<td>14.3 / 7.3</td>
<td>100</td>
<td>1500</td>
<td>4.5</td>
<td>3.1203</td>
<td>Al. syn.</td>
</tr>
<tr>
<td>21</td>
<td>137</td>
<td>30,200</td>
<td>151</td>
<td>137</td>
<td>125</td>
<td>115</td>
<td>5.0 / 2.5</td>
<td>94</td>
<td>745</td>
<td>2.5</td>
<td>7.3323</td>
<td>EHBA</td>
</tr>
<tr>
<td>22</td>
<td>33</td>
<td>12,450</td>
<td>87.5§</td>
<td>12</td>
<td>150</td>
<td>208</td>
<td>4.6 / 2.5</td>
<td>178</td>
<td>3562</td>
<td>4</td>
<td>10.757</td>
<td>CHF</td>
</tr>
</tbody>
</table>

AST= aspartate aminotransferase, ALT= alanine aminotransferase, BT/BD= total bilirubin/direct bilirubin, GGT= gamma glutamyltransferase, AP= alkaline phosphatase, Alb= albumin, Vit D= vitamin D, EHBA= extrahepatic biliary atresia, PTCH= post-transplant chronic hepatitis, N/D= no diagnosis, Neo Hep= neonatal hepatitis, Al. syn.= Alagille syndrome, CHF= congenital hepatic fibrosis, ACH= autoimmune chronic hepatitis.

* Weight with more than 2 average standard deviations (z score) regarding age.
† Height with more than 2 standard deviations below the average (z score) regarding age.
‡ Weight with percentile lower than 5m regarding age (NCHS).
§ Height with percentile lower than 5 regarding age (NCHS).

### Discussion

The existence of adequate levels of plasma vitamin D is dependant upon the ingestion of vitamins D2 and D3 and the cutaneous biosynthesis of vitamin D3 after exposure to ultra-violet light. Absorption depends upon sufficient bile flow and occurs at the level of the jejunum and the ileum, where it is transported as chylomicrons to the liver. In this organ it undergoes 25-hydroxylation transforming it into 25-OHD. Next the 25-OHD returns to the circulation and is transported to the kidneys where it undergoes 1 hydroxylation to form 1.25 dihydroxyvitamin D [1.25 (OH2) D] or 24.25 dihydroxyvitamin D [24.25 (OH2) D] depending upon the state of vitamin D sufficiency. The secretion of parathyroid hormone (PTH) stimulates the synthesis of 1.25 (OH2) D3.
in the kidney, which promotes the mobilization of bone and intestinal calcium and regulates the synthesis of the PTH itself by negative retroaction. The form of the vitamin which is to be found circulating in greatest quantities is 25(OH)D while 1.25 (OH)2D is the active form of the vitamin.7

Studies evaluating plasma vitamin D levels in children and adolescents with chronic hepatopathy are shown in Table 2. Heubi et al.10 tested plasma vitamin D levels after a test dose of the vitamin in children with cholestasis and compared them to children without cholestasis observing significant malabsorption in the first group. Bucuvalas et al.,15 evaluating calcium absorption and plasma vitamin D levels in children with chronic hepatopathy, concluded that other factors must contribute to the reduction in bone mass in these patients, since vitamin D and calcium levels were found to be normal in the majority of the cholestatic patients studied. We would remind that a sample of only nine individuals was studied. A Thai16 study into bone density and 25-OHD in patients with biliary atresia found that 25-OHD levels were significantly lower among the group with cholestasis than among the group without cholestasis. In the current study we observed vitamin D values that were significantly lower among the cholestatic patients when compared with normal children (Figure 1).

Chin et al.,17 investigating numerous aspects of malnutrition in patients with chronic hepatopathy, found a prevalence of 25% of hypovitaminosis D in their group of patients with chronic hepatopathy without distinguishing cholestasis occurrences. Argao et al.18 however, found a prevalence of 29% of hypovitaminosis D among children with chronic cholestasis. This study found vitamin D deficiency in 36% of the patients with cholestasis.

The cut-off point used in our study to define hypovitaminosis D (9 ng/ml) was based on the values provided by the suppliers of the 25-hydroxyvitamin D - Nichols Institute Diagnostics - Paris kit. The same was

![Figure 2](image-url)  
**Figure 2** - Blood levels of vitamin D (ng/ml) between cholestatic patients, according to the nutritional status (Mann-Whitney)

![Figure 3](image-url)  
**Figure 3** - Comparison of the medians of vitamin D blood levels between patients using oral supplement or not (Mann-Whitney U)
Table 2 - Studies that assess blood levels of vitamin D in children and adolescents with chronic hepatic diseases

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Design</th>
<th>Sample</th>
<th>Age group</th>
<th>Technique</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heubi et al. (1989)</td>
<td>Controlled cross-sectional with interference</td>
<td>Chronic hepatic diseases patients with cholestasis n = 6</td>
<td>1 to 19 years</td>
<td>CBPA†</td>
<td>Measures of 25(OH)D after test dose of vitamin D: malabsorption in cholestatic patients</td>
</tr>
<tr>
<td>Bucuvalas et al. (1990)</td>
<td>Controlled cross-sectional</td>
<td>Chronic hepatic diseases patients with cholestasis n = 9</td>
<td>4m to 16 years</td>
<td>CBPA†</td>
<td>Low levels of vitamin D in 1 among 9 cholestatic patients</td>
</tr>
<tr>
<td>Chin et al. (1992)</td>
<td>Cross-sectional</td>
<td>Chronic hepatic diseases patients n = 27</td>
<td>6m to 6 years</td>
<td>HPLC ‡</td>
<td>Hypovitaminosis D: 25% of the patients</td>
</tr>
<tr>
<td>Argao et al. (1993)</td>
<td>Cross-sectional</td>
<td>Chronic hepatic diseases patients n = 56</td>
<td>2m to 20 years</td>
<td>HPLC ‡</td>
<td>Hypovitaminosis D: 29% of the patients</td>
</tr>
<tr>
<td>Chongsrisawat et al. (2001)</td>
<td>Cross-sectional</td>
<td>EHBA patients n = 42</td>
<td>6m to 10 years</td>
<td>RIA §</td>
<td>Vitamin D significantly lower in the cholestatic patients</td>
</tr>
<tr>
<td>This study (2002)</td>
<td>Controlled cross-sectional</td>
<td>Chronic hepatic diseases patients with cholestasis n = 22</td>
<td>4m to 18 years</td>
<td>RIA §</td>
<td>Vitamin D significantly lower in the cholestatic patients</td>
</tr>
</tbody>
</table>

* number of individuals; † Competitive Protein Binding Assay; ‡ High Pressure Liquid Chromatography; § Radioimmune assay.

done in the study by Pugliesi et al., who also used the radioimmunoassay technique to evaluate children with no hepatopathy. Chin et al. and Argao et al., cited above, used the HPLC technique and considered values less than 15 ng/ml to be low (Table 2).

Malnutrition levels, verified by both NCHS percentiles and by the Z Score were around 30 - 40%, and as such were similar to those observed in studies performed in Porto Alegre. Notwithstanding, there are reports in the literature in which the prevalence of malnutrition in hepatopathies can reach 70%. In a healthy population less than 1% severe deficits and around 2.3% moderate deficits are found.

In order to assess the relationship between nutritional status and other variables the Z score height/age was used. The relationship between well-nourished and malnourished patients and the period of cholestasis and plasma vitamin D levels did not reveal any statistically significant differences. The fact that the median of the plasma vitamin D levels presented lower among well-nourished cholestatic patients (Figure 2) cannot be valued since it has no statistical significance and also because other studies which have made the same comparison have not observed any significant relationship between nutritional status and plasma vitamin D levels.17,18

We observed that hypovitaminosis D occurred irrespective of the use of the prescribed oral supplement. On comparing the median of the plasma vitamin D levels of patients who used the oral supplement with those who did not we did not find any differences, as Figure 3 demonstrates. In the studies listed in Table 2, only Heubi et al. and Bucuvalas et al. referred to the use of oral vitamin D supplements with doses of 2,500 to 50,000 IU/day. In our locale the commercial offering Aderogyl D3 12 drops twice a day is used, which equates to approximately 26,000 UI/day of cholecalciferol and 66,000 UI/day of retinol acetate. The most recent recommendation is for periodic monitoring of plasma 25-OHD levels associated with adequate solar exposure and a diet with normal quantities of calcium and phosphorous. If vitamin D deficiency occurs,
oral vitamin D3 should be used at levels 3 to 10 times those recommended for the age of the patient, 25-OHD (Calderol™) 3 to 5 mg/kg/day or 1.25(OH)2D (Rocaltrol™) 0.05 to 0.2 µg/kg/day. While these vitamins are being used, there should be careful monitoring of their plasma levels.

The use of higher doses of vitamin D3 or the use of 1.25(OH)2D (Rocaltrol™) is viable, although there are risks of intoxication, for which reason careful monitoring is recommended. This fact, associated with the prevalence of hypovitaminosis found among the patients evaluated in this study, reinforce the importance of introducing plasma vitamin D assays to the investigation and observation protocols of cholestatic patients in our environment.

The prevalence of osteopenia was not included in the objectives of our study, but we know that this is related to vitamin D deficiency. Vieira11 performed bone densitometry on 20 patients with chronic cholestasis cared for at the HCPA aged between 3 and 18 and observed that all of them presented decreased total bone mass. Although osteopenia is related with chronic cholestasis in children, its pathogenesis is not clearly defined. Some authors have not managed to relate plasma vitamin D levels with bone density.14,18 Chongrisawat et al.,16 however, compared bone mineral density with plasma 25-OHD levels in cholestatic children and found that patients with osteoporosis presented lower vitamin D values. Nevertheless, vitamin D deficiency is not the only determinant of metabolic bone disease in cholestatic children and adults, but this deficiency can be corrected and thus one of the factors which contribute to this morbidity be prevented.

We conclude that the average plasma vitamin D level in the cholestatic children and adolescents evaluated was significantly lower than that of normal children and adolescents. We found a prevalence of hypovitaminosis D of 36% among the cholestatic patients studied. We did not observe a relationship between plasma vitamin D levels and nutritional status, period of cholestasis or regular use of vitamin supplements. Nevertheless we recommend the monitoring of plasma vitamin D levels in cholestatic patients in order to detect such deficiencies and to be able to monitor more effective supplementation.

Acknowledgements

To the head of the Radioimmunoassay laboratory of the Hospital de Clínicas de Porto Alegre, Dr José Romildo de Jesus and the Biochemist at the same laboratory, Dr. Ligia Crossetti for their technical support in the performance of the plasma vitamin D assays.

The nutritionist Carla Rosane Silveira for her collaboration in the performance of the anthropometric evaluation of the patients in this research.

References
