Vitamin D and Epstein-Barr virus antibody levels in a prospective cohort of MS patients


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Background
Low serum 25(OH)-vitamin D (25(OH-2D) and Epstein-Barr virus (EBV) levels are known risk factors for multiple sclerosis (MS). EBV infection is associated with an increased risk of developing MS, and MS disease activity is associated with anti-EBV nuclear antigen 1 (EBNA-1) antibody levels. It has been shown that intrathecal production of anti-EBNA-1 by B-lymphocytes can be attenuated by stimulation with vitamin D [1]. We hypothesize that there is a seasonal variation in EBNA-1 antibody production, negatively associated with 25(OH)-2D levels.

Objective
To study the seasonal variation in EBNA-1 IgG antibody levels and association between anti-EBNA-1 antibody levels and 25(OH)-2D in a prospective cohort of patients with relapsing-remitting MS.

Methods
Participants
The study comprised 90 patients with active relapsing-remitting MS, all participants in a randomized clinical trial of ω-3 fatty acids (the OFAMS study). Repeated, paired measurements of 25(OH)-D3 and anti-EBNA-1 levels were obtained at baseline, and from trial-months 6, 12, 18 and 24. None of the patients had used immunomodulatory treatment the last six months prior to enrolment. At trial-month six, all patients were initiated on interferon β-1a (IFN-β, Rebif®, Merck-Serono, Geneva, Switzerland) 44 μg three times weekly.

Measurements
25(OH)D was measured with a radioimmunoassay kit (ImmunoDiagnostics Systems, Boldon, UK). Immunoglobulin (g) G antibodies to Epstein-Barr virus nuclear antigen 1 (EBNA-1) was measured with the Liaison® quantitative chemiluminescent assay.

Results
Seasonal variation in 25(OH)-D and EBNA-1 IgG antibody levels
There was a statistically significant variation in EBNA-1 IgG antibody levels between sampling months (F 1,8854, p =0.043, one-way ANOVA) (Figure 1). Cosinor analysis of seasonality (Figure 2) revealed a significant seasonality in 25(OH)D levels, with a mean change of 20.5 +/- SE 1.6 mmol/L (p<0.0005), but not in EBNA-1 IgG levels (mean change 0.068 +/- SD 0.374 U/mL, p>0.35).

Association between 25(OH)D and EBNA-1 IgG
There was a significant association between 25(OH)D and EBNA-1 IgG level. In the hierarchical linear model of the association between 25(OH)D and EBNA-1 IgG level (Table 1), there was a weak, but significant effect of IFN-β treatment, age, gender, HLA DRB1*15 status, BMI and vitamin A levels as covariates.

Conclusion
Our study demonstrate monthly differences in EBNA-1 IgG, and an association between 25(OH)D and EBNA-1 IgG. Further studies should expand on by investigating the effect of vitamin D on EBV viral antigen production in B cells, and also investigate potential immunomodulatory effects of solar radiation other than vitamin D synthesis.

Table 1. Association between EBNA-1 IgG levels and vitamin D during the whole study period – results from the stepwise analysis.

<table>
<thead>
<tr>
<th>25(OH)D</th>
<th>B</th>
<th>95% CI</th>
<th>p</th>
<th>25(OH)D</th>
<th>B</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.230</td>
<td>-0.44</td>
<td>-0.023</td>
<td>0.030</td>
<td>-0.246</td>
<td>-0.471</td>
<td>-0.022</td>
<td>0.030</td>
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<tr>
<td>Interferon β-1a</td>
<td>-0.162</td>
<td>[-14.20, 14.00]</td>
<td>0.982</td>
<td>-0.591</td>
<td>[-15.51, 14.33]</td>
<td>0.938</td>
<td>-0.0214</td>
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<tr>
<td>Age</td>
<td>0.425</td>
<td>[-2.721, 3.065]</td>
<td>0.791</td>
<td>-0.002</td>
<td>[-3.189, 3.185]</td>
<td>0.999</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>-36.97</td>
<td>[102.8, 28.9]</td>
<td>0.271</td>
<td>-36.06</td>
<td>[-103.2, 29.0]</td>
<td>0.728</td>
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<tr>
<td>HLA DRB1*15</td>
<td>86.98</td>
<td>[-3.363, 177.3]</td>
<td>0.059</td>
<td>94.68</td>
<td>[2.423, 186.9]</td>
<td>0.044</td>
<td></td>
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<tr>
<td>Body mass index</td>
<td>2.165</td>
<td>[-7.893, 12.22]</td>
<td>0.673</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Retinol</td>
<td>11.81</td>
<td>[-13.37, 37.29]</td>
<td>0.364</td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Vitamin D: 25(OH) – vitamin D; AIC: Akaike’s Information Criteria; CI: confidence interval; B: Regression coefficient; p: p-value. * n: Number of patients contributing to the analysis. Significant associations (p<0.05) are marked in bold font.

References
2. Barnett AG, Dobson AJ. SpringerLink (Online service). Analysing Seasonal Health Data.

Disclosures