High rate of maternal vitamin B12 deficiency nearly a decade after Canadian folic acid flour fortification

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Summary

Vitamin B12 deficiency may be an independent risk factor for neural tube defects (NTD). We determined the prevalence of biochemical B12 deficiency (<125 pmol/l) among 10 622 Ontarian women aged 15–46 years who underwent concomitant testing of serum bhCG and B12 9 years after the implementation of Canadian folic acid flour fortification. The overall prevalence of biochemical B12 deficiency was 7.4%. Relative to non-pregnant women, the adjusted odds ratio (95% confidence interval) of biochemical B12 deficiency was 0.78 (0.60–1.0) among women pregnant 28 days gestation or less and was 1.4 (1.1–1.8) after 28 days gestation. About 1 in 20 women may be deficient in B12 in early pregnancy. The impact on maternal and fetal well-being, including preventable NTD, should be considered.

Background

Folic acid flour fortification, universally introduced in Canada by early 1998, was associated with a 50% reduction in fetal neural tube defects (NTD).1,2 This initiative was paralleled by a 45% relative increase in red cell folate levels among women of reproductive age, and a near disappearance of folate deficiency.3

Beyond folate deficiency, we and others have shown that maternal vitamin B12 (B12) deficiency may be an independent, additional risk factor for NTD, marked by a near tripling in the risk of NTD.4,5 About 35% of NTD cases in the province of Ontario may now be attributable to low maternal B12 status.5 Hence, even in the presence of sufficient intake of folic acid around the time of conception, B12 deficiency may remain an ongoing risk factor for NTD.4,5 Since the embryonic neural tube closes by 28 days post conception, determining the prevalence of B12 deficiency in this crucial period may inform future programs designed to lower the risk of NTD.

The focused goal of this study was to determine the prevalence of B12 deficiency among women of reproductive age who underwent pregnancy testing.

Methods

We completed a retrospective cross-sectional study of Ontarian women aged 15–46 years who underwent concomitant testing of highly sensitive serum human beta-gonadotropin (bhCG) and serum
B12 between January and July 2007. Red cell folate test results were also recorded, when available. Testing was performed through Gamma-Dynacare Medical Laboratories, which handles one-third of private laboratory specimens in Ontario. These tests are ordered by physicians, and paid for under the province’s universal health care system; hence, only limited information is available about the study participants, all of which is presented herein. Personal identifiers were removed to ensure patient confidentiality, and study approval was obtained from St Michael’s Hospital’s Research Ethics Board.

Biochemical B12 deficiency was defined at a concentration below 125 pmol/l, the fifth centile value among Ontarian women of reproductive age in the era before folic acid flour fortification. The prevalence of B12 deficiency was further categorized by a gestational age of 28 days or less (the time of neural tube closure), and more than 28 days. Mean measures were compared by an unpaired t-test or ANOVA, and proportions by a chi-square test. Crude odds ratios (OR) and 95% confidence intervals (CI) were calculated using logistic regression analysis (Table 1). The OR were adjusted for age (years) and bhCG concentration (IU/L). SAS, Version 9.1 (SAS Institute, Cary, NC) was used; statistical significance was at a two-sided P < 0.05.

Results
A total of 10,622 women underwent both serum bhCG and B12 testing at a mean (SD) age of 29.9 (7.1) years (Table 1). There were 2891 women (27.2%) who also underwent red cell folate testing. The mean (95% CI) red cell folate concentration was 1401 nmol/l (1385–1418) among non-pregnant women, 1642 nmol/l (1598–1687) in those ≤28 days gestation and 1600 nmol/l (1571–1628) among women >28 days gestation (P < 0.001). The frequency distribution of serum B12 testing among all 10,622 women is shown in Figure 1. There was a significant difference (P < 0.001) in mean serum B12 levels associated with pregnancy (Table 1). Biochemical B12 deficiency was observed among 6.9% of non-pregnant women, 5.2% of those pregnant ≤28 days and 10.1% after 28 days gestation (P < 0.001). The frequency distribution of serum B12 testing among all 10,622 women is shown in Figure 1.

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Table 1: Mean serum vitamin B12 concentrations and the risk of biochemical B12 deficiency among 10,622 women who underwent concomitant pregnancy and B12 testing about 9 years after mandatory Canadian folic acid flour fortification

<table>
<thead>
<tr>
<th>Participant category</th>
<th>Serum B12 concentration (pmol/l)</th>
<th>Fifth centile (95% CI)</th>
<th>Crude Adjusted for age Adjusted for age and serum bhCG concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>All women (n = 10,622)</td>
<td>264 (261–266)</td>
<td>113 (111–116)</td>
<td>791 (7.4)</td>
</tr>
<tr>
<td>By pregnancy status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not pregnant (n = 6888)</td>
<td>266 (262–269)</td>
<td>116 (113–118)</td>
<td>473 (6.9)</td>
</tr>
<tr>
<td>Pregnant ≤28 days (n = 1244)</td>
<td>283 (277–289)</td>
<td>123 (120–126)</td>
<td>65 (5.2)</td>
</tr>
<tr>
<td>Pregnant &gt;28 days (n = 2498)</td>
<td>249 (244–255)</td>
<td>103 (96–106)</td>
<td>252 (10.1)</td>
</tr>
</tbody>
</table>

B12: vitamin B12; bhCG: human beta-gonadotropin; CI: confidence interval.

*Defined as a serum B12 concentration ≤fifth centile (i.e. <125 pmol/l) among women of reproductive age, prior to the era of folic acid flour fortification. Adjusted for age as a continuous variable, in years. Adjusted for serum bhCG concentration as a continuous variable, in IU/L. Based on a serum bhCG concentration <25 IU/L and 5000 IU/L.
Discussion

In this study, vitamin supplement use was not documented, nor was the indication for B12 testing; accordingly, the prevalence of B12 deficiency may have been overestimated. Conversely, we had used a conventional B12 threshold of 150 pmol/l to define abnormal7, or used a more sensitive marker of early B12 deficiency, such as holotranscobalamin5, the prevalence would have been higher. The rate of biochemical B12 deficiency in pregnancy nearly doubled after 28 days gestation, which might be partly attributed to the known hemodilutional effect of pregnancy on serum B12.7 Since serum bhCG provides a rough estimate of pregnancy dating,8 misclassification of gestational age likely attenuated the risk estimates described herein. Non-pregnant participants had a higher risk of biochemical B12 deficiency than women pregnant <28 days, perhaps because B12 deficiency is associated with hypofertility,9 or because non-pregnant women were less prepared for pregnancy, including taking fewer B12-containing prenatal multivitamins.6

B12 deficiency may be associated with an increased risk of NTD,4 especially in the era after folic acid flour fortification.5 The current data suggest that as many as 1 in 20 women in Ontario may be B12 deficient in the critical period of closure of the embryonic neural tube. Together, this may justify completing a multicenter randomized controlled trial comparing periconceptional B12 and folic acid with folic acid alone for the prevention of NTD, both in countries with and without folic acid food fortification. Such a trial might be optimally carried out in an area with a high prevalence of B12 deficiency, such as India, where vegetarianism is a major cause of low B12 status10 and where the incidence of NTD may be among the highest worldwide.11

Conflict of interest: None declared.

References
