Assessment of Vitamin B12 status during pregnancy

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Introduction
B12 (cobalamin (Cbl)) deficiency during pregnancy leads to anaemia and neuropsychopathies both in mother and child. Low total B12 (B12t) is common in pregnancy due to haemodilution and changes in vitamin B12–binding proteins making diagnosis of deficiency challenging. Other markers include: holo transcobalamin (holoTC) – the biologically active fraction of B12, methylmalonic acid (MMA) - functional test of mitochondrial B12 (Ado-cbl) utilization, total homocysteine (tHcy) - functional test of cytoplasmic B12 (Me-Cbl) utilization (Figure 1) and combined indicator of B12 status (cB12), which may offer support for diagnosis, but they are also affected by haemodilution and hormonal changes.

Study design
Serum B12t, holoTC, tHcy, folate, MMA and cB12 were measured at: 12 (trimester 1 (T1)), 20 (T2), and 35 (T3) weeks of gestation for pregnant women attending the clinic in Oxford (N=114). Anthropometrics, thyroid-stimulating hormone (TSH), pregnancy history and selected baby outcomes were also available. Ethics reference 08/H0603/46.

Methods
B12t, holoTC, tHcy and folate were measured by Immunoassays (Abbott Architect) and MMA by LCM-MS/MS, cB12 was calculated as in Fedosov et al. Clin Chem Lab Med (2015). cB12 are a set of formulae which combine the results of vitamin B12 markers of an individual with reference combinations at the stipulated age e.g. 

cB12=\log_{10}(\text{holoTC} \times \text{B12t}/\text{MMA} \times \text{tHcy})_{\text{test}} - \log_{10}(\text{holoTC} \times \text{B12t}/\text{MMA} \times \text{tHcy})_{\text{ref}} = \text{test} - 3.79/1+(\text{age}/230)^{-2.6}

The reference combinations were derived from a large database, N=5000, following mathematical modelling. cB12 has been suggested as a more reliable indicator of vitamin B12 status than individual tests. Depending on the cB12 value obtained, B12 status is classified as: elevated (>1.5), adequate (0.5 to 1.5), low (<1.5 to -0.5), possible B12 deficiency (-2.5 to -1.5) and probable B12 deficiency (<-2.5).

Results
The mean (SD) age was 31 (31.7) yrs. The median (interquartile range) for BMI was 23.7 (21.6-26.3) kg/m². The results for markers of vitamins B status are shown in Table 1 and Figure 2. There was a 25% decline in B12 compared to 11% for holoTC between trimester 1 and 3. Using non-pregnancy cut-offs (140 pmol/L, 25 pmol/L, 280 nmol/L, 15 μmol/L, -0.5), the prevalence of B12 deficiency was: (7%,12%,33%), (1%,2%,1%), (8%,13%,14%), (0%,0%,0%), (3.9%,1.8%,5.7%) (according to B12t, holoTC, MMA, tHcy and cB12 in T1, T2, T3 respectively. In multiple regression, age (T1) and TSH (T3) contributed to variation in B12 and MMA. After excluding the final gestation age from the model, 29% of variation in baby’s weight was explained by maternal weight in all trimesters, TSH (T1), MMA and cB12 (T3). MMA in T1 correlated (Spearman’s) with TSH across all trimesters (0.243-0.253, p<0.05).

Conclusions
A significant decline in B12 status was present as pregnancy progressed. Non-pregnancy cut-offs are not suitable for B12 assessment in pregnancy. Other factors e.g. age, thyroid function need to be taken into account when diagnosing B12 deficiency in pregnancy. B12 status influences the baby’s weight.

Feature directions
• Application of pregnancy related reference ranges for B12 status assessment
• Further evaluation of the utility of cB12 during pregnancy

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