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Folate and Vitamin B12 Status among Non-Pregnant, Non-Lactating Women of Reproductive Age and Predicted Risk for Neural Tube Defects, Morogoro Region, Tanzania

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Abstract

Introduction: Population risk for neural tube defects (NTDs) can be determined using red blood cell (RBC) folate. However, a paucity of biomarker and surveillance data among non-lactating, non-pregnant women of reproductive age (NPWRA) from Africa limits accurate assessment. Our study assessed folate and vitamin B12 status among non-lactating NPWRA and predicted population risk of NTDs in Tanzania.

Methods: A cross-sectional biomarker survey of non-lactating NPWRA (15–49 years) in the Morogoro region, Tanzania was conducted during June–October 2019. Questionnaire interview

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Ethical Approval This study received ethical approval from the Tanzania National Institute for Medical Research (NIMR).

Disclaimer The findings and conclusions in this paper are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Consent to Participate All participants provided written informed consent in Swahili prior to data collection. An additional informed consent was obtained from women of reproductive age who participated in the survey and provided blood samples.

Conflicts of interest/Competing Interests The authors declare no conflicts of interest.

responses and non-fasting blood samples were collected. Folate was assessed using the CDC microbiologic assay kit and vitamin B12 was measured using an electrochemiluminescence immunoassay. Complex survey design analyses were conducted using SAS-callable SUDAAN (v11.0.1).

Results: Of the 761 participating non-lactating NPWRA, 294 (39.8%) had RBC folate insufficiency (<748 mol/L). The prevalence of RBC folate insufficiency was lower among non-lactating NPWRA living in urban than rural areas (PR: 0.72, 95% CI: 0.52–0.99) but did not differ by age or household wealth index. Vitamin B12 insufficiency was uncommon (< 221 pmol/L, 2.7%). The estimated NTD risk was 10.5 (95% uncertainty interval: 8.1–13.3) per 10,000 births.

Discussion: Elevated NTD risk was predicted in the Morogoro region of Tanzania, where ~ 40% of non-lactating NPWRA had RBC folate insufficiency and $< 3\%$ had vitamin B12 insufficiency. The NTD risk is consistent with surveillance data for the area, limited folic acid fortification of staple foods, and low vitamin B12 insufficiency. Further studies are needed to better understand the context of these findings, especially the impact of micronutrient fortification in Tanzania.

Keywords

Folate; Vitamin B12; Biomarker; Neural tube defects; Tanzania

Introduction

Folate and vitamin B12 deficiencies are associated with an increased risk of adverse maternal and infant health outcomes (Rogne et al., 2017). Folate is a water-soluble B9 vitamin needed for cell growth as well as DNA synthesis, repair, and methylation (Bailey et al., 2015). Folate deficiency due to inadequate folic acid intake during the periconceptional period increases the risk for neural tube defects (NTDs) and anemia, particularly megaloblastic anemia (Bailey et al., 2015; CDC, 1992; Garrett & Bailey, 2018). Vitamin B12, another water-soluble B vitamin, functions as a required co-factor in one-carbon cycle metabolism and plays a critical role in the biosynthesis of hemoglobin and erythropoiesis (Allen et al., 2018). Evidence suggests that vitamin B12 deficiency is associated with an increased risk of NTDs through its involvement in folate metabolism or as an independent risk factor (Bailey et al., 2015; Molloy, 2018).

To address micronutrient deficiencies, Tanzania mandated fortification in 2011 of large-scale industrially produced wheat and maize flour with several key micronutrients, including folic acid and vitamin B12 (Tanzania Ministry of Health, 2011; Tanzania NBS, 2013). However, systematic monitoring of fortification practices has been limited. Also, most maize flour production is fragmented (i.e., not produced industrially in large-scale mills), creating an implementation barrier for fortification (Bymolt & d'Anjou, 2017).

Assessing folate and vitamin B12 status among non-pregnant women of childbearing age is important to understand their risk of having a pregnancy affected by NTDs given the critical period for neural tube development during the early weeks of pregnancy, often before most women know they are pregnant. However, a paucity of biomarker and surveillance data among non-lactating non-pregnant women of reproductive age (NPWRA) from Africa

limits accurate assessment of the population risk for NTDs. This is especially challenging for folate status given the dearth of RBC folate data available using the World Health Organization (WHO) recommended microbiologic assay (Cordero et al., 2015). Our study assessed folate and vitamin B12 status among non-lactating NPWRA in the Morogoro region of Tanzania and predicted the population risk of NTDs.

Methods

The Morogoro Regional Micronutrient Household and Biomarker Survey (MRMHBS), jointly conducted by the Tanzania Ministry of Health and U.S. Centers for Disease Control and Prevention (CDC), was a cross-sectional biomarker survey of NPWRA in the Morogoro region of Tanzania, covering both urban and rural areas. It used a probabilistic population-based survey with multi-stage and stratified sampling from eligible households with NPWRA between the ages of 15–49 years, selected from six of the seven districts in the Morogoro region. A household was eligible if its current resident(s) lived in the current dwelling for at least 3 months, were present at the time of the interview, consented to participate in the survey, prepared or purchased food for the house, and had at least one NPWRA in the household. Household and NPWRA interviews were conducted from June to October 2019. In eligible households with more than one NPWRA, a simple randomization was performed to select one person to participate in the NPWRA questionnaire that included questions about her medical and pregnancy status and history, as well as supplementation and medication intake.

Consenting NPWRA provided non-fasting blood samples, taken by trained phlebotomists, in purple-top EDTA vacutainers for whole blood and red-top vacutainers for serum. The blood samples were maintained at 4–8 °C and sent to a regional laboratory for sample processing within 6 h of collection. After processing, serum and whole blood lysate samples were stored in a –70 °C freezer until laboratory analysis. Serum folate and whole blood folate (WBF) were assessed at the Tanzania Food and Nutrition Centre using the CDC folate microbiologic assay kit (WHO, 2015; Zhang et al., 2018). RBC folate was calculated using imputed serum folate (SFOLi), whole blood folate (WBF), and hematocrit (Hct), based on the formula: $[WBF - SFOLi(1 - Hct/100)] / (Hct/100)$ (Zhang et al., 2020). SFOLi was calculated by taking the average of 567 serum folate values from the survey population. Serum levels of vitamin B12 were measured using an electrochemiluminescence immunoassay on the Roche™ clinical analyzer (Cobas c501). RBC folate deficiency and insufficiency were defined as < 305 nmol/L and < 748 nmol/L, respectively (Bailey et al., 2015; WHO, 2017). Serum vitamin B12 deficiency was defined as < 148 pmol/L and insufficiency as < 221 pmol/L (Allen et al., 2018).

Proportions and prevalence ratios with 95% confidence intervals (CI) were calculated with sample weights to account for complex survey design. Confidence intervals were used to examine differences between groups. Calculations were performed to examine the distribution of RBC folate and vitamin B12 as well as the geometric means for the overall NPWRA population and by selected subgroups. Adjusted models used variables selected a priori. The wealth index, a composite measure of a household's cumulative living standard using indicators of their assets and amenities, was calculated using the

methodology described by Rutstein and Staveteig (2014). NTD prevalence (per 10,000 births) with 95% uncertainty intervals (UI) was modeled using an established Bayesian method based on population RBC folate concentrations for predicting NTD risk among the overall population as well as among subpopulations (Crider et al., 2014). Analyses were conducted using SAS-callable SUDAAN (version 11.0.1).

The study received ethical approval from the Tanzania National Institute for Medical Research (NIMR). All participants provided written informed consent in Swahili. Additional informed consent was obtained from NPWRA for their participation in the questionnaire and blood assessments.

Results

A total of 997 (98.8%) households with at least one NPWRA participated in the study, and 960 (96.3%) NPWRA participated in both the survey questionnaire and provided blood samples.

After excluding those who were lactating during the data collection period, an overall total of 761 NPWRA was included in the final analysis. Their characteristics are shown in Table 1. Approximately 40% of the non-lactating NPWRA were 35 years or older, more than half were married (57.3%), and about two-thirds were multiparous (68.2%). About half of the women had malaria in the past six months, and the percentage who had any supplement use was low. Households were evenly divided across low, medium, and high wealth indexes.

RBC folate status, including deficiency and insufficiency by demographic characteristics are reported in Table 2. Few participants ($n = 7$, 1.1%) had RBC folate deficiency (< 305 nmol/L), while nearly 40% ($n = 294$, 39.8%) had RBC folate insufficiency (< 748 nmol/L). The prevalence of RBC folate insufficiency was higher among non-lactating NPWRA living in rural (46.5%, 95% CI: 37.7–55.4) than urban areas (33.5%, 95% CI: 25.7–42.3; PR: 0.72, 95% CI: 0.52–0.99) but did not differ by age or wealth index. Results were similar when prevalence ratios were adjusted for residence, age, and wealth index (data not shown).

Twenty (2.7%) non-lactating NPWRA were found to have vitamin B12 insufficiency (< 221 pmol/L), and one participant had vitamin B12 deficiency (< 148 pmol/L). Vitamin B12 insufficiency by demographic characteristics are shown in Table 3. Prevalence of vitamin B12 insufficiency did not differ statistically by residence, age, or household wealth index.

The geometric means of RBC folate were lowest among non-lactating NPWRA residing in rural areas, aged 35–49 years, and from high wealth index households (Supplemental Table 1). Lower geometric means of serum vitamin B12 also were seen among those residing in rural areas and from high wealth index households, but among younger (15–24 years) instead of older (35–49 years) non-lactating NPWRA.

Overall, the predicted NTD prevalence per 10,000 live births based on RBC folate concentrations was 10.5 [95% uncertainty interval (UI): 8.1–13.3]. Predicted NTD prevalence was similar by residence (rural: 10.6, 95% UI: 8.2–13.4 vs. urban: 9.9, 95% UI: 7.6–12.6), age (15–24 years: 10.3, 95% UI: 7.9–13.0 vs. 25–34 years: 9.6, 95% UI: 7.4–12.2

vs. 35–49 years: 11.7, 95% UI: 9.1–14.7) and wealth index (low: 9.8, 95% UI: 7.5–12.4 vs. medium: 9.8, 95% UI: 7.5–12.4 vs. high: 11.1, 95% UI: 8.6–13.9) (Table 4). Selected percentiles of RBC folate among non-lactating NPWRA are shown in Supplemental Table 2.

Discussion

Nearly 40% of the non-lactating NPWRA in the Morogoro region of Tanzania had RBC folate insufficiency while only about 1% had RBC folate deficiency. Maternal RBC folate, a measure of average folate status over the previous 120 days, provides an important indicator for the risk of NTDs in infants (Bailey et al., 2015). Higher RBC folate concentrations have been associated with a lower risk of NTDs in observational studies (Daly et al., 1995; Tinker et al., 2015) and Bayesian modeling analyses (Crider et al., 2018). The RBC folate insufficiency and deficiency in this study are lower than reported RBC folate status using data from the microbiologic assay (MBA) from other surveys in Africa, i.e., the 2015 Ethiopian National Micronutrient Survey (32% with RBC folate deficiency) and the 2015–2016 Malawi Micronutrient Survey (81.4% with RBC folate insufficiency) (Ethiopian Public Health Institute, 2016; Malawi NSO, 2017). However, these countries had not implemented folic acid fortification during the survey periods and other factors, such as heterogeneity of population characteristics (e.g., variations in diet, supplement use), differences in inclusion criteria (e.g., lactating vs. non-lactating women), or methodological differences (e.g., reagents prepared in-house vs. using CDC folate MBA kits) could drive some of the differences observed. Additionally, Morogoro was one of three regions in Tanzania that implemented the USAID-supported “Tuboreshe Chakula Project” from 2011 to 2015 that facilitated maize flour fortification to small scale maize flour processors, which may have contributed to folic acid intake in the population (Abt Associates, 2015).

Examining our findings on RBC folate status among women of reproductive age in the context of other studies from Africa presents several challenges, primarily the different types of folate assays used and whether the study population excluded pregnant and/or lactating women of reproductive age. Few studies are available on RBC folate status among non-pregnant, non-lactating women that used the WHO recommended microbiologic assay (Rogers et al., 2018). Other assays used to assess RBC folate concentrations, such as protein-binding assay and high-performance liquid chromatography along with tandem mass spectrometry, have been shown to vary greatly which makes direct comparisons difficult. Even assay-specific cutoff values have been shown to be problematic and could be misinterpreted when applied to data not matched for the specific assay (Rogers et al., 2018). More studies have looked at folate status using serum/plasma folate (Rogers et al., 2018), and a study among WRA in two regions of Dar es Salaam, Tanzania found that plasma folate concentrations have improved after fortification (Noor et al., 2017). However, unlike RBC folate which reflects folate stores and has been associated with NTD risk, serum folate reflects recent intake (Bailey et al., 2015). Additionally, comparison to other folate status studies is challenging when they do not exclude pregnant and/or lactating women since these women have higher folate intake requirements.

Our study also reported low levels of vitamin B12 insufficiency (2.7%) and deficiency (0.1%) for non-pregnant, non-lactating women of reproductive age in the Morogoro region

of Tanzania. Similarly low vitamin B12 insufficiency (< 150 pmol/L, 0.5–0.7%) has been reported in a study from the South Kivu and Kongo Central Provinces in the Democratic Republic of Congo (DRC) among non-pregnant women of reproductive age (15–49 years; Harvey-Leeson et al., 2016). However, other African countries, including Ethiopia and Malawi, have reported a much higher prevalence of vitamin B12 deficiency (~ 13% of non-pregnant women of reproductive age in both countries; Ethiopian Public Health Institute, 2016; Malawi NSO, 2017). The concurrent deficiencies in vitamin B12 and folate are not surprising given the interplay of these two B vitamins in the one-carbon metabolic pathway. Such wide variation in prevalence should be further explored. This could include examining factors potentially driving some of the observed variations, such as fortification policy and exposure to environmental sources like intake of foods derived from microbial interaction through milk and meat from ruminant animals (i.e., cattle and sheep) as well as fish (Watanabe & Bito, 2018).

The predicted prevalence of NTDs was estimated in this population using the RBC folate distribution, as has been previously performed among populations in the United States, Guatemala, and India (Crider et al., 2018; Rosenthal et al., 2016; Finkelstein et al., 2021). RBC folate concentrations associated with 9–14 NTDs/10,000 live births, 586–747 nmol/L in the CDC microbiologic assay are considered elevated risk (Crider et al., 2018). In this population, the estimated NTD prevalence (10.5 NTDs per 10,000 live births) was comparable to NTD prevalence estimates from surveillance/registry programs reported for Africa (11.7 per 10,000 births; where fortification policies had not yet been implemented), South Africa (9.8 per 10,000 births postfolic acid fortification), and Dar es Salaam, Tanzania (9.9 per 10,000 births) (Zaganjor et al., 2016; Sayed et al., 2008; Kishimba et al., 2015). Additionally, estimates in this study are similar to the pre-fortification NTD prevalence in the U.S. based on population-based surveillance registries of 10.7 per 10,000 live births. The post-fortification prevalence in the U.S. dropped to 7.0 per 10,000 live births (resulting in 1300 averted cases of NTDs each year), which is consistent with the predicted post-fortification NTD estimate using RBC folate concentrations of 7.3 NTDs per 10,000 live births (Crider et al., 2018; Williams et al., 2015). While Tanzania has mandatory folic acid and vitamin B12 fortification, the expected impact might not have reached all populations, especially since the primary staple food (maize) is often produced through small- and medium-scale millers and toll mills that are voluntary or exempt from fortification requirements (Teachout et al., 2023). Identifying centrally processed and widely available staple foods that are included in mandatory fortification requirements is essential for successful implementation of fortification programs and thereby decreasing prevalence of NTDs (Garrett & Bailey, 2018). Expanding the coverage and reach of fortifiable staple food products across the population in Tanzania could offer an opportunity for additional NTD prevention.

The study has several strengths and limitations. Folate status was measured using the WHO recommended microbiologic assay. The study is population-based with high participation rates for both questionnaire and blood sample collection. Exclusion of pregnant and lactating women allows for a more accurate estimate of biomarker status among women of reproductive age. However, the cross-sectional survey design limits the assessment of biomarker status change over time, especially if there are any changes to food fortification

practice. Furthermore, vitamin B12 status was assessed with only one biomarker, serum vitamin B12. For a more accurate assessment of vitamin B12 status, the addition of a second biomarker, such as serum methylmalonic acid, has been recommended (Allen et al., 2018).

Conclusion

In a population-based cross-sectional study, nearly 40% of the non-pregnant, non-lactating women in the Tanzania Morogoro region had RBC folate insufficiency, with an estimated NTD prevalence of 10.5 per 10,000 live births. This level is consistent with other surveillance/registry data for Tanzania and some of the surrounding countries where mandatory folic acid fortification has been implemented. Further studies are needed to better understand the context of these findings, especially the effect of micronutrient fortification, among non-pregnant, non-lactating women of reproductive age.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Significance

Inadequate red blood cell (RBC) folate is associated with an increased risk of neural tube defects (NTDs), and NTD prevalence can be predicted from population-level RBC folate status. Additionally, vitamin B12 deficiency may impact NTD risk independently or through folate metabolism. Biomarker data among non-pregnant, non-lactating women of reproductive age from Africa is limited, hindering accurate assessment of population-level NTD risk. Especially challenging is the dearth of RBC folate data available using the WHO recommended microbiologic assay. Our study presents population estimates of RBC folate and vitamin B12 status among non-pregnant, non-lactating women of reproductive age in Tanzania to understand NTD risk.

Table 1

Characteristics of non-pregnant, non-lactating women aged 15–49 years in the Morogoro Region of Tanzania

	<i>N</i> (unweighted)	Weighted % (95% CI)
Total	761	
Residence		
Urban	387	50.8 (39.8, 61.7)
Rural	374	49.2 (38.3, 60.2)
Age (years)		
15–24	188	25.0 (21.3, 29.0)
25–34	271	35.4 (32.0, 39.0)
35–49	302	39.7 (35.4, 44.1)
Marital Status		
Single/never married	224	29.8 (26.6, 33.3)
Married	440	57.3 (53.8, 60.8)
Separated, Divorced, Widowed	79	10.4 (7.9, 13.6)
Other	18	2.5 (1.6, 4.0)
Parity		
Nulliparous	147	19.3 (15.9, 23.2)
Uniparous	93	12.5 (10.7, 14.7)
Multiparous	521	68.2 (64.1, 72.0)
Wealth Index of Household ¹		
Low	268	33.6 (26.7, 41.4)
Medium	249	34.1 (29.4, 39.2)
High	243	32.3 (23.4, 42.7)
Medical Conditions		
Malaria in the past 6 months	391	51.4 (47.5, 55.3)
Diabetes	5	0.6 (0.2, 1.4)
Chronic diarrhea	39	5.1 (3.5, 7.4)
Seizures	9	1.3 (0.8, 2.4)
HIV/AIDS	24	3.0 (2.1, 4.2)
Supplement Use		
Folic acid supplement use (any)	71	9.2 (7.3, 11.6)
Iron supplement use (any)	18	2.3 (1.1, 4.5)
Vitamin B12 supplement use (any)	64	8.0 (6.1, 10.4)

¹Wealth index is missing for one woman's household

Table 2

RBC folate deficiency and insufficiency by demographic characteristics among non-pregnant, non-lactating women aged 15–49 years in the Morogoro Region of Tanzania

	<i>N</i> ¹	Weighted % (95% CI)	Prevalence ratio (95% CI)
RBC folate deficiency (< 305 nmol/L)			
Total	7	1.1 (0.5, 2.2)	
Residence			
Urban	4	1.5 (0.6, 3.6)	2.47 (0.58, 10.44)
Rural	3	0.6 (0.2, 2.0)	Ref
Age (years)			
15–24	1	1.0 (0.1, 6.7)	Ref
25–34	2	0.7 (0.2, 2.9)	0.71 (0.06, 8.82)
35–49	4	1.5 (0.5, 4.2)	1.59 (0.14, 17.54)
Wealth Index			
Low	2	0.6 (0.1, 2.4)	0.32 (0.06, 1.70)
Medium	1	0.3 (0.04, 2.2)	0.16 (0.02, 1.45)
High	3	1.8 (0.7, 4.8)	Ref
RBC folate insufficiency (< 748 nmol/L)			
Total	294	39.8 (34.0, 45.9)	
Residence			
Urban	128	33.5 (25.7, 42.3)	0.72 (0.52, 0.99)
Rural	166	46.5 (37.7, 55.4)	Ref
Age (years)			
15–24	72	40.8 (31.4, 51.2)	Ref
25–34	100	37.1 (29.6, 45.3)	0.91 (0.70, 1.18)
35–49	122	41.5 (35.0, 48.2)	1.02 (0.77, 1.34)
Wealth Index			
Low	103	38.5 (30.9, 46.6)	0.91 (0.67, 1.22)
Medium	95	38.3 (31.0, 46.3)	0.90 (0.72, 1.13)
High	95	42.4 (33.4, 52.0)	Ref

¹*N*=717, unweighted

Table 3

Serum vitamin B12 insufficiency by demographic characteristics among non-pregnant, non-lactating women aged 15–49 years in the Morogoro Region of Tanzania

	<i>N</i> ¹	Weighted % (95% CI)	Prevalence ratio (95% CI)
Serum vitamin B12 insufficiency (≤ 221 pmol/L) *			
Total	20	2.7 (1.6, 4.5)	
Residence			
Urban	14	3.7 (2.0, 6.7)	2.24 (0.71, 7.08)
Rural	6	1.6 (0.6, 4.3)	Ref
Age (years)			
15–24	6	2.6 (1.2, 5.7)	Ref
25–34	6	2.6 (1.0, 6.7)	1.00 (0.32, 3.13)
35–49	8	2.8 (1.3, 5.7)	1.07 (0.37, 3.09)
Wealth Index			
Low	5	2.1 (0.9, 4.9)	0.61 (0.19, 2.01)
Medium	8	2.6 (1.0, 6.9)	0.77 (0.22, 2.67)
High	7	3.4 (1.5, 7.2)	Ref

¹*N*=761, unweighted

* One non-pregnant, non-lactating woman had vitamin B12 deficiency (< 148 pmol/L)

Table 4

Predicted median neural tube defect risk per 10,000 live births by demographic characteristics and red blood cell folate distribution among non-pregnant, non-lactating women aged 15–49 years (*N* = 717)

	Predicted median NTD risk per 10,000 (95% UI)	RBC folate distribution (percentile)				
		5	25	50	75	95
Total	10.5 (8.1, 13.3)	29.5 (23.3, 37.2)	12.1 (10.2, 14.1)	7.5 (6.3, 9.0)	4.7 (3.7, 5.9)	2.6 (1.9, 3.5)
Residence						
Urban	9.9 (7.6, 12.6)	30.4 (23.9, 38.5)	11.0 (9.4, 13.0)	7.1 (5.9, 8.5)	4.6 (3.6, 5.8)	2.8 (2.1, 3.8)
Rural	10.6 (8.2, 13.4)	27.5 (22.0, 34.5)	13.3 (11.3, 15.6)	8.4 (7.0, 10.0)	4.8 (3.8, 6.0)	2.4 (1.7, 3.3)
Age (years)						
15–24	10.3 (7.9, 13.0)	26.1 (21.0, 32.4)	11.9 (10.1, 13.9)	7.9 (6.6, 9.4)	5.1 (4.1, 6.4)	1.9 (1.3, 2.7)
25–34	9.6 (7.4, 12.2)	24.5 (19.8, 30.1)	11.1 (9.5, 13.1)	7.3 (6.1, 8.8)	4.7 (3.7, 5.9)	2.5 (1.8, 3.4)
35–49	11.7 (9.1, 14.7)	31.7 (24.8, 40.5)	13.5 (11.4, 15.8)	7.5 (6.3, 9.0)	4.6 (3.6, 5.8)	2.9 (2.1, 3.9)
Wealth Index						
Low	9.8 (7.5, 12.4)	24.5 (19.8, 30.1)	11.9 (10.1, 13.9)	7.6 (6.4, 9.1)	4.6 (3.6, 5.8)	2.6 (1.9, 3.5)
Medium	9.8 (7.5, 12.4)	30.0 (23.7, 38.0)	11.0 (9.4, 12.9)	7.3 (6.1, 8.8)	4.7 (3.8, 6.0)	2.5 (1.8, 3.4)
High	11.1 (8.6, 13.9)	30.8 (24.2, 39.1)	13.3 (11.3, 15.6)	7.6 (6.3, 9.1)	4.7 (3.7, 5.9)	2.9 (2.1, 3.9)
Serum Vitamin B12						
Status						
B12 Insufficient (sample size too small)		-	-	-	-	-
B12 Sufficient	10.2 (7.8, 12.9)	28.2 (22.4, 35.3)	11.9 (10.1, 13.9)	7.5 (6.3, 9.0)	4.7 (3.7, 5.9)	2.6 (1.9, 3.6)

NTD = neural tube defect; RBC = red blood cell