Folate status of women in Toronto: Implications of folate fortification and supplementation

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ABSTRACT

OBJECTIVES: To assess the percentage of women of childbearing age with suboptimal levels of folate for protecting against neural tube defects (<906 nM), and assess folate status among the elderly.

METHODS: A total of 1,035 anonymous blood samples from a centralized clinical laboratory, with a catchment area across the Greater Toronto Area, were assessed for red blood cell (RBC) folate concentrations using a chemiluminescent immunoassay. Folate analysis was requested by physicians as part of clinical care. Available data included age, sex, and RBC folate concentration. Descriptive statistics were used to characterize the percent of women who had suboptimal blood folate concentrations, and a multiple regression was used to analyze determinants of folate status.

RESULTS: Our data from 2013 show that 7% of women of childbearing age (15–45 years) had RBC folate concentrations below 906 nM, a substantially lower percentage than in our 2006 study (40%). Results from the multiple regression showed that age is a significant positive predictor of higher RBC folate status ($p < 0.001$).

CONCLUSION: Compared to our earlier data, we report a significant decrease in the suboptimal folate status among women of childbearing age. We also show that age is a predictor of higher RBC folate levels. Our data are limited due to a lack of information regarding patient or physician characteristics, and to the nature of our sample, yet our results are consistent with the continued increase in folate status observed among several population-level studies in the US and Canada post-fortification. Further research is needed to determine the reasons for and future implications of this continued increase in the elderly.

KEY WORDS: Folic acid; prenatal supplementation; folic acid fortification; population-level data; RBC folate; public health initiatives; population health

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Folic acid supplementation during the periconceptional period is associated with a reduction in the risk of neural tube defects (NTDs). NTDs are major malformations of the central nervous system occurring at about 4 weeks of pregnancy, when the neural tube completes its closure.¹ Given that 50% of pregnancies are unplanned, inadequate maternal blood folate concentrations pose a significant risk to this population as a NTD may occur before a woman even knows that she is pregnant. Hence, public health efforts such as mandatory fortification and awareness of periconceptional supplementation are important in ensuring that women of childbearing age (WCBA) achieve protective blood folate concentrations.

Beyond NTDs, low folate intake during pregnancy is associated with an increased risk of oral clefts, congenital cardiovascular defects, certain pediatric cancers, as well as overall adverse pregnancy outcomes.²⁻⁴ Although folate generally has low potential for toxicity, studies based on animal data suggest that folate overexposure is associated with the potential masking of vitamin B12 deficiency, cognitive decline in the elderly and colorectal cancer.^{5,6}

Canada introduced mandatory fortification of white wheat flour, cornmeal and enriched pasta in 1998, with the goal of increasing the average intake of folic acid among WCBA by 30–70%.⁷ Overall, folate-fortified products provide an additional 100–200 µg of folic acid daily.⁷ The incidence of NTDs has decreased by 46% across Canada after the implementation of

folate fortification strategies (1.58 per 1,000 births pre-fortification to 0.86 per 1,000 births post-fortification).⁸

A case-control study by Daly et al. was the first to describe the red blood cell (RBC) folate concentration of 906 nM or above as being optimally protective against the risk of NTDs.⁹ This has been generally accepted as an optimal RBC folate concentration to reduce the incidence of NTDs.¹⁰

Recent data from the Canadian Health Measures Survey (CHMS) showed that only 28% of women routinely take folic acid supplements,¹¹ and data from the Canadian Maternity Experiences Survey documented that 58% of women began taking folic acid supplements at least three months prior to pregnancy,¹² suggesting that there are still segments of the population that

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may benefit from a more targeted approach to supplementation awareness.

Data derived from the nationally-representative CHMS in 2007–2009 showed that 22% of WCBA had blood folate concentrations considered suboptimal for protecting against the risk of NTDs (<906 nM).¹³ Similarly, a previous study from our group analyzed the folate status of women within the Greater Toronto Area, Ontario (GTA)*, and based on data from 2006, the authors found suboptimal blood folate levels in 40% of WCBA and 36% of pregnant women.¹⁰ Finally, given the recent change in clinical climate, where blood folate testing is not routinely warranted,¹⁴ this study aimed to assess population folate levels in Toronto through a convenience sample to identify subpopulations that may benefit from folate screening and continued follow-up.

The objective of the current study was to follow up on previous population-based studies to capture the current folate status of women within the GTA, in order to determine the percentage of women of childbearing age who are still inadequately protected. Since there is controversy in the literature regarding the impact of high folate levels in the elderly, we also assessed the impact of age on folate status as a secondary objective.

METHODS

Sample collection

In 2013, random, anonymous convenience samples were retrieved from clinical requisitions for folate analysis submitted by health care practitioners in the GTA. The patients’ health care provider, as part of clinical care, requested the tests. Only age, sex, and RBC folate concentration were available to us; the reason for testing and/or diagnosis was not. These samples had been collected at various “blood collection sites” spread across the GTA, and were submitted to a central clinical laboratory for RBC folate analysis. The population cohort in this study, based on the sample collection area, the assay method and the testing clinical laboratory, are the same as in our previous study.¹⁰ Blood samples for RBC folate were collected as per standard laboratory procedures using lavender Vacutainer® tubes containing EDTA. Test analysis included both RBC folate and assessment of hematocrit.

Sample preparation and analysis

Whole blood samples were analyzed to measure RBC folate concentrations as per instrument manufacturer’s assay protocol using an automated chemiluminescent immunoassay (Access Folate, Beckman Coulter Inc., Fullerton, CA). The whole blood samples were first analyzed for hematocrit and then haemolyzed by adding to each sample a mixture of Access RBC folate Lysing Agent, which consisted of a 0.15% ascorbic acid solution. The mixture was left to equilibrate at room temperature for 90 minutes, and the hemolysate was assayed within 1.5 hours. All samples were analyzed within 24 hours of receipt of the sample. RBC folate concentrations were calculated using the formula

below, and were converted to nmol/L using the conversion factor of 1 ng/mL = 2.266 nmol/L.

RBC folate(ng/mL) = hemolysate folate × 21 ÷ (hematocrit/100)

Quality control testing, including stability and dilution testing for this assay, was part of standard assay setup protocol. The linear range of the assay had an upper limit of 2623 nM. Samples that were beyond the linear range of the assay were diluted to bring them within the linear range, and their values were reported after adjusting for dilution. Samples that could not be diluted and had readings as “>2623” were imputed as 2623 nM for data analysis.

Statistical analysis

Descriptive statistics (frequencies, medians, percentiles) were used to characterize the population, based on blood folate concentrations, age and sex. 906 nM was used as the cut-off for optimal RBC folate concentrations. All of the descriptive analyses were conducted using GraphPad Prism (version 5; GraphPad Software, San Diego, CA).

A multiple regression analysis was conducted to analyze whether age or sex were significant predictors of RBC folate concentrations. The model was tested for basic assumptions, including independence of residuals, normality and homoscedasticity. These analyses were conducted using IBM SPSS (version 18; PASW Statistics, Armonk, NY).

RESULTS

Within our cohort of 1,035 individuals, about 40% of the individuals were male (n = 407) and 60% were female (n = 628). There were 235 women of childbearing age (WCBA), between the ages of 15–45 years. The descriptive statistics for the population cohort are presented in Table 1. Among the 235 WCBA, about 7% had blood folate concentrations less than 906 nM. The frequency distribution of RBC folate concentrations among WCBA is presented in Figure 1.

As part of the multiple regression, the assumptions for normality, linearity, independence of residuals and homoscedasticity were met (p > 0.05). We found a statistically significant predictive effect for age (p < 0.001): for every unit increase in RBC folate, a 3.3 unit increase in age was predicted by the model. Regression coefficients and their standard errors are presented in Table 2.

Table 1. Descriptive characteristics of the population cohort

	Males (n = 407)	Females (n = 628)
Mean age ± SD	56.67 ± 19.79	52.98 ± 21.79
Mean RBC folate (nM)	1695 ± 510.9	1710 ± 561.7
Median RBC folate (nM)	1641	1627
Minimum RBC folate (nM)	256	370
Maximum RBC folate (nM)	3072	3468

Mean age, mean RBC folate, and range of RBC folate concentrations within the sample population. There were 60% females, and 40% males. The minimum RBC folate concentration in males was below the 305 nM cutoff¹³ for folate deficiency, whereas in females, it was above the minimum cutoff. The mean RBC folate concentrations in both males and females were above the 906 nM cutoff⁹ indicative of suboptimal folate status in women of childbearing age.

* Based on the Ontario provincial definition, the GTA consists of the city of Toronto and the regions of Halton, Peel, York and Durham.

Figure 1. Frequency distribution of women of childbearing age, 15–45 years, with suboptimal folate status

DISCUSSION

Following up on population-level studies presenting folate status through biochemical assessment,^{10,13} our study presents a convenience sample from the GTA to evaluate the current folate status among women of childbearing age. Our data from 2006, from the same population and catchment area, showed that 40% of WCBA had suboptimal blood folate concentrations.¹⁰ The Canadian Health Measures Survey 2007–2009 showed that 22% of WCBA were still inadequately protected.¹³ Our data from 2013 show further improvement in this vulnerable population, as only 7% of women had RBC folate concentrations below 906 nM. Clearly there has been a reduction in WCBA with suboptimal folate levels. In our previous study, the RBC folate data had been corrected for both hemoglobin and mean corpuscular volume (MCV), and we had reported 40% of WCBA as having RBC folate levels below the optimal threshold level. In the current study, we were unable to correct for these parameters as they were not available to us. Thus, our current findings of only 7% of women of childbearing age being below the optimal threshold represent a significant and notable change. This reduction in the proportion of population at risk demonstrates an increasing trend in population-wide folate levels post-

fortification, and suggests the success of public health strategies surrounding folic acid supplementation among WCBA.

Our data are especially comparable with Bar-Oz et al.'s study¹⁰ since our population cohort was obtained from the same catchment area in the GTA and the assays were done at the same clinical laboratory, utilizing the same method. In the Bar-Oz et al. study, the median RBC folate concentration was 972 nM among 1,537 participants. Colapinto et al.'s nationally-representative study sample showed median RBC concentrations of 1248 nM among 5,248 participants, although they used a different analyzer (Immulin 2000) to analyze folate concentrations.¹³ Our current study shows much higher median blood folate concentrations of 1688 nM among 1,035 participants, suggesting a gradual increase in population levels. While there has been no change in folate supplementation policy since its inception in 1998, population RBC folate levels have continued to increase both in the US and Canada.^{15,16} Perhaps at a population level, blood folate levels are gradually approaching a plateau due to increased daily folate intake through diet and supplementation.

After the introduction of mandatory folic acid fortification of cereal products in Canada, several studies have shown a decrease in the incidence of NTDs across Canada.^{15,17–19} Traditionally, the incidence of NTDs was higher in the eastern provinces compared to the western ones,¹⁵ with prevalence (per 10,000 births) in the pre-folic acid fortification period being 43.6 in Newfoundland and Labrador²⁰ and 25.5 in Nova Scotia,¹⁸ compared to 11.3 in Ontario.^{15,21} However, over time, the previous geographical differences have been eliminated as a result of the overall effect of folic acid fortification on NTDs.⁸

While rising folate levels have been successful in reducing the proportion of women who may be at risk of a pregnancy with an NTD,⁸ it is unclear what impact increasing folate levels are having as they become more prevalent in the elderly. Our multiple regression analyses (Table 2) showed that age was a statistically significant predictor of increased RBC folate concentrations.

High folate concentrations among the elderly have been associated with a potential for masking vitamin B12 deficiency and cognitive decline,²² however, the direction of effect is not only dependent upon folate status but also on B12 status. Data from NHANES show that among the elderly, high folate with low B12 status was associated with anemia and cognitive impairment; however, high folate status with normal B12 levels was actually protective against cognitive impairment.⁵ A recent study showed that higher folate intake in the elderly is protective against Alzheimer's disease.²³ In contrast, other studies bring attention to the high risk of folate deficiency among the elderly, especially because many have concurrent medical conditions or are on medications that may impair folate absorption. Given the lack of consensus in the literature regarding the potential risk of folate overexposure in the elderly, further research is necessary for a better understanding of long-term consequences of folate overexposure in this subpopulation.

Women from lower socio-economic status (SES) often have a breadth of concomitant issues compared to women of higher SES. They are at higher risk of inadequate folate intake through, among others: poor diet, poor compliance with medications or supplement use, possible teratogenic substance use (e.g., alcohol), and high BMI.²⁴ These women may currently be inadequately

Table 2. Summary of multiple regression analysis to determine whether age or sex are significant predictors of RBC folate concentrations

Variable	B	SE _B	β
Intercept	1477.889	78.451	
Age	3.308	0.834	0.129*
Sex	32.960	36.232	0.030

Parameter estimates of the multiple regression model.

* Indicates $p < 0.001$. B indicates the unstandardized beta coefficient, SE_B indicates the standard error associated with the coefficients, and β indicates the standardized beta coefficient. For every unit increase in RBC folate, a 3.3 unit increase in age is predicted by the model.

protected and may require higher doses of folic acid (>1 mg) under the guidance of a health care professional.²⁴ Since we did not have access to demographic data in our study population, this group of at-risk women may potentially be under-represented in our sample. Thus, we suggest that future efforts should focus on the need for higher folate doses among women who may fall into these high-risk groups for NTDs. Specific ethnic communities that have higher incidence for NTDs, such as Sikh, Celtic and women from Northern China,²⁴ could be targeted through intensive community outreach and awareness programs. Similarly, women with a previous child with NTD, those treated with antifolate medications, obese women, and smokers should also be targeted.²⁴

Limitations

While we had a large sample size and a broad catchment area across the GTA, our study was limited by the lack of information about patient characteristics associated with our convenience sample. To determine the comparability of our sample with the Toronto population, we compared our population demographics with the 2011 Toronto census. Data from the 2011 Census indicate that Toronto's population is 48% male and 52% female.²⁵ Within our sample, we found 40% males and 60% females. The 2011 Census data for Toronto indicate a population distribution of 12.7% aged 65 years or over, 69.9% aged 15–64, and 17.5% aged 0–14.²⁵ Our sample has a distribution of 34.3% aged 65 or over, 63.4% aged between 15 and 64, and 2.3% between 0 and 14. While our population age distribution is not exactly comparable to the census distribution, this is perhaps because of the clinical representation of hematological abnormalities in certain age groups (more common in adults or older adults, less common in children). RBC folate testing in Canada, within the clinical setting, is becoming largely uncommon, and based on guidelines for laboratory investigation,¹⁴ would not be requested during routine bloodwork, but as a diagnostic investigation if a physician suspects anemia or folate deficiency. With that consideration in mind, our sample population is more “clinically representative” of the age distribution among which RBC folate testing would be clinically warranted – patients with perceived health issues.

Our data also came from the same population base as Bar-Oz et al., thus serving as an appropriate before–after comparator when analyzing the changes in folate status over time. In our study, RBC folate concentrations served as an apt biomarker of folate status because they are representative of long-term, tissue stores of folate. In contrast, plasma folate is indicative of more recent folate intake, and may be susceptible to fluctuation. We also utilized the same assay reagents and instruments as Bar-Oz et al., yet some changes may have occurred in the formulation of the reagents between 2006 and 2013, which may have subtle effects on our results. Nevertheless, this does not change our interpretations or conclusions.

Our study was limited by the information available to us (age, gender and RBC folate concentration), limiting our ability to analyze any associations between important clinical covariates. Similarly, because we did not have the diagnostic reason for requisition of folate testing, we were unable to make any predictions about whether a test was requested to determine

folate deficiency, overexposure or general health bio-monitoring. Given the lack of information available to us regarding patient characteristics, we cannot rule out the possibility that the demographics of our sample may have changed between 2006 and 2013, despite being retrieved from the same catchment area, which could affect the accuracy of the rates presented in our study – yet our results still have meaningful implications for the general population.

In fact, our sample's limitations of uncorrected RBC folate concentrations and a population cohort where hematological abnormalities may be clinically suspected may actually be a strength in terms of its implications for the general population. Our findings of 7% of WCBA being suboptimally protected could be even lower compared to the percentage in the general population, given that our data were not adjusted for normocytic, non-anemic individuals. In the 2006 study by Bar-Oz et al., 40% of RBC folate levels were below 900 nM among a sample where anemia was ruled out. The fact that there is now a major improvement in RBC concentrations in a sample where anemia and other conditions cannot be ruled out, suggests that this is a genuine and clinically significant improvement, despite the sample limitations.

CONCLUSION

Though our data are limited by not knowing the reason for folate testing or the lack of hemoglobin and MCV correction, extrapolation of our data to *real* findings among the normal population is quite meaningful precisely because of the significant reduction of suboptimal status within a population where clinical deficiency may be suspected. These results demonstrate a decrease in the percentage of women of childbearing age who are suboptimally protected against NTDs, in comparison to most recent data.^{10,13} However, they also show a significant association with increasing age and higher folate concentrations, a cause for potential concern because the elderly are a vulnerable population with respect to folate overexposure. In combination with data on prevalence of supplement use among Canadians, continued monitoring of folate status among vulnerable populations and targeted public health initiatives for folate supplementation are recommended.

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RÉSUMÉ

OBJECTIFS : Évaluer le pourcentage de femmes en âge de procréer ayant des niveaux sous-optimaux de folates protégeant contre les anomalies du tube neural (<906 nM) et évaluer le bilan en folates chez les personnes âgées.

MÉTHODE : Nous avons évalué les concentrations en folates érythrocytaires dans 1 035 échantillons de sang anonymes provenant d'un laboratoire clinique centralisé servant la région du Grand Toronto, à l'aide d'un immunodosage en chimioluminescence. Les analyses de folates avaient été demandées par des médecins dans le cadre de soins cliniques. Les données disponibles étaient l'âge, le sexe et la concentration en folates érythrocytaires. Nous avons eu recours à la statistique descriptive pour caractériser le pourcentage de femmes ayant des concentrations sous-optimales de folates sanguins, et à la régression multiple pour analyser les déterminants du bilan en folates.

RÉSULTATS : Nos données de 2013 montrent que 7 % des femmes en âge de procréer (15–45 ans) avaient des concentrations en folates érythrocytaires inférieures à 906 nM, un pourcentage considérablement plus bas que dans notre étude de 2006 (40%). Les résultats de la régression multiple montrent que l'âge est un prédicteur positif significatif des bilans élevés en folates érythrocytaires ($p < 0,001$).

CONCLUSION : Comparativement à nos données antérieures, nous constatons une diminution significative des bilans en folates sous-optimaux chez les femmes en âge de procréer. Nous montrons aussi que l'âge est un prédicteur des niveaux de folates érythrocytaires élevés. Nos données sont limitées par le manque d'information sur les caractéristiques des patients ou des médecins et par la nature de notre échantillon, mais nos résultats sont conformes à la hausse continue du bilan en folates observée dans plusieurs études populationnelles menées aux États-Unis et au Canada après l'enrichissement des aliments. Il faudrait pousser la recherche pour déterminer les raisons et les conséquences futures de cette hausse continue chez les personnes âgées.

MOTS CLÉS : acide folique; supplémentation prénatale; enrichissement en acide folique; données populationnelles; folates érythrocytaires; initiatives de santé publique; santé des populations