Serum Folate by Two Methods in Pregnant Women Carrying Fetuses with Neural Tube Defects, Gizele Thame,1,2* Elvira Maria Guerra-Shinohara,3 and Antonio Fernandes Moron1,4 (1 Departamento de Saúde Materno-Infantil, Faculdade de Saúde Pública, Universidade de São Paulo, São Paulo-SP, Brazil CEP 05054-010; 2 Seção de Hematologia, Instituto Adolfo Lutz, São Paulo-SP, Brazil; 3 Departamento de Análises Clínicas e Toxicológicas, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo-SP, Brazil CEP 05508-900; 4 Setor de Medicina Fetal da Disciplina de Obstetrícia, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo-SP, Brazil CEP 0437-002; address correspondence to this author at: Rua Aíbi, 146, São Paulo-SP, Brazil CEP 05054-010; fax 55-11-5083-5409, e-mail gizelethame@hotmail.com)

Neural tube defects (NTDs) are the most common severe congenital malformations that occur as a result of failure of neural tube closure at the beginning of pregnancy (24–28 days after conception). Genetic (1–3), environmental (4), and nutritional factors (5–9) can be involved in the etiology of NTDs.

Among the nutritional deficiencies, maternal folate status during pregnancy is considered one of the most important risk factors for NTDs, leading to a recommended dietary folate allowance for pregnant women (600 μg/day) from supplements and fortified food (10).

Folate is a cofactor in several enzymatic reactions in the formation of new tissues. Low maternal red cell folate (RCF) and serum folate concentrations have been strongly associated with the occurrence of NTDs (8, 9), and maternal RCF concentrations in particular have been shown to correlate with the occurrence of NTDs in a concentration-dependent fashion (5). However, some case-control studies did not show lower mean folate concentrations in pregnant women carrying fetuses with NTDs (6, 7, 9).

The differences among the cited studies may have been attributable to intrinsic differences in the methods used for folate determination. Currently, the major analytical approaches used for folate determination are microbiological assays, radioassays, chemiluminescence assays, HPLC, and ion-capture assays.

The aim of the present study was to compare serum folate results obtained by two automated folate tests in 55 pregnant women carrying unaffected fetuses or fetuses with NTDs.

Seventeen pregnant women carrying fetuses with NTDs, detected by ultrasound, were seen between February and October 1997 at the prenatal service of the Fetal Medicine Department of Sao Paulo Hospital, UNIFESP (Sao Paulo, Brazil). Mean (SD) maternal age and gestational age were 24.5 (6.1) years and 30.6 (5.5) weeks, respectively. None of the pregnant women had a history of children with anomalies. The NTDs were anencephaly (4), encephalocele (5), and meningomyelocele (8).

Controls were 38 pregnant women from the prenatal services of the University Hospital of the University of Sao Paulo. Mean (SD) maternal age and gestational age were 26.2 (7.0) years and 29.8 (4.1) weeks, respectively. The absence of NTDs was confirmed after the birth of the fetus.

The study was approved by the Ethics Committee of the institution, and written consent was obtained from each pregnant woman.

Venous blood (7 mL) was collected into evacuated tubes without anticoagulant for determination of serum folate. The serum samples were immediately divided into two aliquots, protected from light, and stored at −40 °C for a maximum period of 2 weeks before being analyzed by the IMx and ACS:180 methods.

Serum folate concentrations were measured by two laboratories, one using the ion-capture assay (IMx System®; Abbott Laboratories) and the other a chemiluminescence method (ACS:180; Chiron Diagnostics Corporation/Bayer). Both methods are based on binding by a specific folate-binding protein. Samples from the NTDs and control groups were analyzed at the same time. Neither laboratory received any previous information about the case–control samples, and the operator could not identify the samples.

The results were compared by Mann–Whitney and Wilcoxon tests. Correlation coefficients were calculated by the Spearman method. A linear regression model (Deming regression) was also adjusted, and scatter plots and difference plots were constructed.

The measured folate concentrations were not necessarily those directly associated with risk of NTDs at the time of closure of the neural tube because the samples were obtained later, at approximately the 30th week of pregnancy.

Although the present results showed a strong positive correlation between the IMx and ACS:180 methods (r = 0.83; P < 0.01 and r = 0.81; P < 0.01 for the NTDs and control groups, respectively; Fig. 1), the mean folate values obtained with the ACS:180 method were ~20% higher than those obtained with the IMx method (Table 1) in both the NTDs and control groups (P < 0.01).

As shown in Table 1, folate concentrations by the IMx method were lower in the NTDs group than in controls. In contrast, this difference was not detected (P = 0.38) when the ACS:180 method was used. The area under the ROC curve for the ACS:180 method was only 0.58, vs 0.75 for the IMx, indicating its virtual lack of diagnostic accuracy. The 95% confidence intervals for the areas under the curves were 0.42–0.73 and 0.62–0.88 for the ACS:180 and IMx, respectively.

Our results are similar to those reported by Gunter et al. (11), who pointed out the wide variability of the folate values obtained with different methods (microbiological assay, radioassay, chemiluminescence, HPLC, and ion-capture assay), with the serum folate concentrations measured by the ACS:180 method being, on average, 61% higher than those obtained with the ion-capture assay (IMx).

The differences between the results obtained with each method may have been attributable to the different characteristics of the methods: the ACS:180 folate method is a competitive protein-binding assay, whereas the IMx
method is an ion-capture assay that uses a protein-binding assay based on saturation analysis. The ACS:180 method uses a purified folate-binding protein (bovine), but no information about the characteristics of the folate-binding protein is provided with the IMx method. The characteristics of folate binders can be the main determinants of specificity in these assays, perhaps explaining the differences in the results obtained in the present study.

Although the ACS:180 folate assay used in this study is no longer available, our study emphasizes the critical importance of considering the analytical performance when selecting the method for analysis, which can indeed affect the results.

In addition to folate values, homocysteine values and polymorphisms of the methylenetetrahydrofolate reductase (MTHFR) gene have been indicated as risk factors for NTDs (1–3). Moreover, the T allele of the MTHFR gene has been associated with an accumulation of formylated tetrahydrofolate in red blood cells, which is not the normal form of RCF (12). This form of folate can give falsely high RCF values in at least one radioassay (13). Thus, further studies using the two methodologies presented here are needed to elucidate the effect of these folate analogs in individuals homozygous for the C677T mutation in the MTHFR gene to determine the specificity of the protein binders of each method.

The presence of conflicting reports in the literature may be attributable to the different methods used. The present study shows the importance of specifying the method used by each clinical laboratory and of establishing reference values for the nutrient studied for each population and method used.

References


Table 1. Comparison of folate values obtained by the IMx and ACS:180 methods in pregnant women in the NTDs and control groups.

<table>
<thead>
<tr>
<th></th>
<th>NTDs group (n = 17)</th>
<th>Control group (n = 38)</th>
<th>Mann–Whitney test</th>
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<tbody>
<tr>
<td>IMx</td>
<td></td>
<td></td>
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<tr>
<td>Mean (SD)</td>
<td>12.6 (4.4)</td>
<td>19.6 (8.7)</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Median</td>
<td>11.3</td>
<td>20.6</td>
<td></td>
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<tr>
<td>ACS:180</td>
<td></td>
<td></td>
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<tr>
<td>Mean (SD)</td>
<td>21.0 (9.0)</td>
<td>23.6 (12.6)</td>
<td>P = 0.38</td>
</tr>
<tr>
<td>Median</td>
<td>18.1</td>
<td>22.9</td>
<td></td>
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<tr>
<td>Wilcoxon test</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
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</table>

Fig. 1. Regression analysis scatter plot (A) and difference plot (B) for the ACS:180 and IMx methods in NTDs and control groups.

(A), solid line indicates the regression line for the NTDs group (●); dotted line indicates the regression line for the control group (○). For the NTDs group: ACS:180 = −2.0 + 1.8(IMx) (S.D. = 4.0, R² = 0.82). For the control group: ACS:180 = −0.3 + 1.2(IMx) (S.D. = 7.0, R² = 0.70). (●), for the NTDs group (○), the mean (SD) difference is 0.01 (3.9), and the 95% confidence interval is −7.7 to 7.7. For the control group (○), the mean (SD) difference is 0.01 (6.9), and the 95% confidence interval is −13.8 to 13.9.