FOLIC ACID AND FOLATE BINDING PROTEIN IN PREGNANCY

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Summary Folic acid and folate binding protein (FABP) concentrations were determined in 208 pregnant women as well as in 50 non-pregnant women. Serum folate levels in pregnant women were significantly lower than those of the non-pregnant group. Anaemic pregnant women also had lower serum folate concentrations than those of the non-anaemic pregnant women. Ninety-eight of 208 (47.1%) pregnant women had serum folate levels of less than 4 ng/ml. No significant difference in red cell folate concentration between the pregnant and non-pregnant groups was demonstrated, and only 5 of 206 (2.4%) of pregnant women had red cell folate levels of less than 200 ng/ml. Serum FABP levels in pregnant women were also found to be significantly higher ($p<0.001$) than those of the non-pregnant women. These levels were also found to be higher in the anaemic pregnant group and in pregnant women with serum folate concentration less than 4 ng/ml than those of the non-anaemic pregnant women and pregnant women with serum folate concentration higher than 4 ng/ml, respectively.

There was a direct relationship between the serum FABP levels and the gestation period. As the period of pregnancy advanced higher serum FABP levels were demonstrated. No correlation between the gravida and the serum FABP levels was found in this study.

It is well established that the homeostasis of serum folic acid level changes considerably in pregnancy. The serum folate levels usually fall with the advance of pregnancy (1–3). This is probably due primarily to an increased requirement with a relative lack of nutrients.

The folate in serum is bound by proteins and this binding is highly specific. This serum folic acid binding protein (FABP) is barely measurable in normal serum but is significantly increased in folate-deficient serum and in the serum of pregnant women (4–7). Few studies on folate and FABP in the serum of pregnant women...
have been reported, and therefore the present study was undertaken to determine the effect of pregnancy on the serum and red cell folic acid levels and FABP.

MATERIALS AND METHODS

The study was carried out on 208 pregnant women selected randomly from outpatients during their first attendance at the antenatal clinic of the Department of Obstetrics and Gynaecology, Faculty of Medicine, Siriraj Hospital. Their ages ranged from 16 to 42 years. Venous blood samples were taken from the antecubital vein of non-fasting subjects for the determination of haemoglobin, haematocrit, serum and red cell folate levels and serum folate binding protein. The history of gestation and parity of these subjects were also recorded. The studies were also performed on non-fasting 50 non-pregnant women with no history of taking oral contraceptives.

Red cell and serum folate levels were determined by the methods of HOFFBRAND et al. and WATERS and MOLLIN (8-9), respectively. Serum FABP was determined by using $^3$H-folic acid ($^3$H-PGA) as described by WAXMAN and SCHREIBER (10). Haemoglobin concentration was determined using the cyanmethaemoglobin method and haematocrit values were obtained after centrifuging the blood for 5 minutes at 10,000 g.

RESULTS

The mean values ± one standard deviation (S. D) and ranges of serum and red cell folate levels determined in 208 pregnant and in 50 non-pregnant women are shown in Table 1. A significant difference ($p<0.001$) in the mean values of serum folic acid level was found between these two groups of subjects. Pregnant women with haemoglobin concentration of less than 10 g/100 ml were taken in the present study as the anaemic subjects. A significant difference ($p<0.05$) in the mean serum folate level was demonstrated between the anaemic and non-anaemic groups. Thirty of 44 (68.2%) anaemic pregnant women and 68 of 164 (41.5%) non-anaemic pregnant women were found to have serum folic acid levels less than 4 ng/ml. Only 2 (4.5%) and 3 (1.9%) pregnant women of the anaemic and non-anaemic groups had red cell folate concentration less than 200 ng/ml.

The prevalence of serum folic acid deficiency in pregnant women is shown in Table 2. There were 29.8% and 47.1% of pregnant women with serum folic acid levels below 3 ng/ml and 4 ng/ml respectively. However, all cases had red cell folate levels higher than 100 ng/ml.

The mean serum FABP determined in 134 pregnant women was statistically ($p<0.001$) higher than that of the non-pregnant women as shown in Table 3. In comparing the results between the anaemic and non-anaemic pregnant groups, the former had also higher serum FABP than that of the latter. Table 4 also illustrates the higher serum FABP values in pregnant women with serum folate less than 4 ng/
Table 1. Comparison of serum and red cell folate values in anaemic and non-anaemic pregnant women and non-pregnant women.

<table>
<thead>
<tr>
<th></th>
<th>Serum folate (ng/ml)</th>
<th>Red cell folate (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-pregnant</td>
<td>Pregnant women</td>
</tr>
<tr>
<td></td>
<td>Anaemic group</td>
<td>Non-anaemic group</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>44</td>
</tr>
<tr>
<td>Mean ± S. D.</td>
<td>7.9 ± 3.7</td>
<td>3.8 ± 2.4</td>
</tr>
<tr>
<td>Range</td>
<td>4.0–20.0</td>
<td>0.8–11.2</td>
</tr>
<tr>
<td>&lt;4 ng/ml</td>
<td>0</td>
<td>30 (68.2%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Number examined</th>
<th>Mean ± S. D.</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red cell folate (ng/ml)</td>
<td>18</td>
<td>498.0±170.1</td>
<td>598.9±253.5</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>656.2±362.9</td>
<td>181.6–1723.9</td>
</tr>
<tr>
<td></td>
<td>162</td>
<td>581.6±212.2</td>
<td>181.6–1723.9</td>
</tr>
<tr>
<td>&lt;200 ng/ml</td>
<td>0</td>
<td>304.7–734.5</td>
<td>181.6–1723.9</td>
</tr>
</tbody>
</table>

There was a direct relationship between the serum FABP levels and the gestation period as shown in Table 5. As pregnancy advanced, the serum FABP content increased considerably.

The mean serum FABP levels with different number of gravidity are shown in

ml (76.6±89.9 pg/ml) than pregnant women with the normal serum folate level (61.9±81.9 pg/ml).

There was a direct relationship between the serum FABP levels and the gestation period as shown in Table 5. As pregnancy advanced, the serum FABP content increased considerably.

The mean serum FABP levels with different number of gravidity are shown in
Table 4. Mean values ± one S.D. and ranges of serum FABP (pg/ml) in pregnant women with low and normal serum folate concentration.

<table>
<thead>
<tr>
<th></th>
<th>Number examined</th>
<th>Mean ± S.D.</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant women</td>
<td>60</td>
<td>21.3 ± 12.2</td>
<td>3.1–48.6</td>
</tr>
<tr>
<td>Pregnant women</td>
<td>134</td>
<td>69.1 ± 85.7</td>
<td>3.6–363.1</td>
</tr>
<tr>
<td>Serum folate &lt; 4 ng/ml</td>
<td>66</td>
<td>76.6 ± 89.9</td>
<td>6.0–335.4</td>
</tr>
<tr>
<td>Serum folate &gt; 4 ng/ml</td>
<td>68</td>
<td>61.9 ± 81.9</td>
<td>3.6–363.1</td>
</tr>
</tbody>
</table>

*t-test: Non-pregnant women vs pregnant women, p < 0.001; serum folate < 4 ng/ml vs > 4 ng/ml, p > 0.05.

Table 5. Relationship between serum FABP levels and the stages of pregnancy.

<table>
<thead>
<tr>
<th>Period of Pregnancy</th>
<th>Number examined</th>
<th>Mean ± S.D. (pg/ml)</th>
<th>Range (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First trimester</td>
<td>10</td>
<td>30.8 ± 17.7</td>
<td>3.6–56.0</td>
</tr>
<tr>
<td>Second trimester</td>
<td>52</td>
<td>67.1 ± 82.1</td>
<td>6.0–363.1</td>
</tr>
<tr>
<td>Third trimester</td>
<td>67</td>
<td>72.5 ± 87.7</td>
<td>9.5–332.2</td>
</tr>
</tbody>
</table>

*t-test: First trimester vs second trimester and third trimester, p > 0.05.

Table 6. Relationship between serum FABP levels and the number of gravidity.

<table>
<thead>
<tr>
<th>Gravida</th>
<th>Number examined</th>
<th>Mean ± S.D. (pg/ml)</th>
<th>Range (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47</td>
<td>79.9 ± 98.4</td>
<td>10.6–363.1</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td>76.3 ± 92.3</td>
<td>3.6–340.5</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>37.4 ± 26.8</td>
<td>3.6–111.0</td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>76.3 ± 99.7</td>
<td>10.2–329.1</td>
</tr>
<tr>
<td>5–9</td>
<td>18</td>
<td>54.7 ± 72.3</td>
<td>7.7–332.2</td>
</tr>
</tbody>
</table>

Table 6. The results showed no correlation between these two parameters in this study.

DISCUSSION

Since the study was performed on blood samples taken from non-fasting women that is pregnant women and non-pregnant women, the data from these 2 groups of subjects were therefore comparable. The result in the present study showed that serum folate levels in pregnant women were significantly lower (p < 0.001) than those of the non-pregnant women. Sixty-two subjects (29.8%) and 98 subjects (47.1%) had serum folate levels lower than 3.0 and 4.0 ng/ml, respectively. In comparisons of folate levels of the anaemic and non-anaemic pregnant women, those in the former group were found to be significantly lower (p < 0.05) than those of the latter group. However, no pregnant women in the present study had red cell folate levels lower than 100 ng/ml, and only 5 out of 206 (2.4%) pregnant women had red cell folate content lower than 200 ng/ml. It has been pro-
posed that a low serum folate level alone should be regarded as an indication of a negative balance and, if accompanied by a low red cell folate value, as indicating folate deficiency (11). Based on this concept, the prevalence of folic acid deficiency in pregnant women in the present study would be only 2.4%.

In non-pregnant women, the serum binding capacity for exogenous folic acid of 21.3±12.2 pg/ml was in the same order of magnitude as that reported by WAXMAN and SCHREIBER (10). Many previous studies could not measure this small amount of serum FABP because larger amounts of ³H-PGA were used. An optimum serum and ³H-PGA ratio was therefore essential for determining such a small amount of serum FABP in normal subjects as shown in the present study.

Results in the present study showed that serum FABP was significantly increased during pregnancy to a level about three-fold that observed in non-pregnant women. These results were in accordance with findings that the folic acid binding capacity relative to serum proteins in pregnant women was twice that of the normal subjects as determined by chromatography (7). In another study using Sephadex filtration, FABP was also found to be present in sera of a significant number of pregnant women while it could not be detected in the serum of non-pregnant women (6).

There was a reverse relationship between serum FABP and the haemoglobin concentration in pregnant women. Anaemic pregnant women were found to have higher serum FABP than the non-anaemic pregnant group. This finding indicated that there was an increased serum FABP during pregnancy and this increment was even higher in the anaemic pregnant women.

The close reversed correlation between serum FABP in pregnant women and their serum folate concentration was also observed in the present study. Pregnant women with serum folate level less than 4 ng/ml had higher serum FABP than that of pregnant women with normal serum folate concentration. This result was in accordance with the finding of DA COSTA and ROTHENBERG (6) that only 9 of the 24 pregnant women (38%) with leucocyte folate binder had serum folate greater than 5 ng/ml while 17 of 27 pregnant women (63%) without leucocyte folate binder had serum folate greater than 5 ng/ml. All these findings described above indicated that serum FABP levels were increased in pregnant women with low haemoglobin and low serum folate concentrations.

Serum FABP levels were found to be increased progressively as pregnancy advanced. This indicated a direct relationship between serum FABP and the gestation period. This relationship was not shown in previous studies (6–7, 12). There was no correlation between serum FABP and parity in the present study.

The mechanism that causes a rise in serum FABP in pregnant women is not exactly known. It has been suggested that this might be due to hormonally induced synthesis of folate binder as it also occurred in women taking oral contraceptives (6). As mentioned earlier, serum folate decreased during pregnancy and as the urinary folate excretion was higher in pregnant than in non-pregnant women,
it was therefore postulated that urinary loss of folate could be one factor contributing to the fall of serum folate activity (13–15). In order to compensate for the loss of high urinary folate and the greater demand of folate during pregnancy, the body therefore increased synthesis of serum FABP for binding the required folate. This would result in the increased serum FABP during pregnancy. Findings of an increased total folate binding proteins in the serum of pregnant women and a 600-fold concentration of milk FABP over serum FABP also support this explanation (12, 16, 17). The increased FABP in milk was supposed to act as trapping mechanism to accumulate folate from blood into milk and in the infant's gut to facilitate the absorption by preventing folate uptake by intestinal microorganisms (16).

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REFERENCES

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