Serum and breast milk vitamin A levels of nursing women in three selected districts of Chiang Mai Province, Thailand

Duangruthai Phongchiewboon MPH¹, Jearawan Wannaweke MPH¹, Prasong Tienboon MD, PhD², Pannee Siriwanapa MD³, Kannika Vitsupakorn MPH⁴, Somdet Sricharatanakool PhD⁵, Aram Phongchiewboon MD⁶

1 Chiang Mai Public Health Office, Chiang Mai, Thailand.
2 Department of Pediatrics, Faculty of Medicine, Chiang Mai University, Thailand.
3 Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University, Thailand.
4 Department of Community Medicine, Faculty of Medicine, Chiang Mai University, Thailand.
5 Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Thailand.
6 Department of Anesthesiology, Faculty of Medicine, Chiang Mai University, Thailand.

Address for correspondence and request of reprints: Aram Phongchiewboon, Department of Anesthesiology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand. Email: arammd@gmail.com

Abstract
Vitamin A functions to maintain cell growth, eye development, healthy skin and mucous membranes. Vitamin A deficiency remains a significant health risk in developing countries, particularly affecting infants and children. When maternal vitamin A is deficient, subclinical deficiency can appear in fetus and young children. This study aims to measure the levels of serum and breast-milk vitamin A in nursing mothers in Amphur Omkoi, Amphur Maejam and Amphur Muang of Chiang Mai Province. Lactating Omkoi mothers (n = 86), Maejam mothers (n = 90) and Muang mothers (n = 46) were recruited in this study. Serum and breast-milk were kept frozen and light protected at -80 °C until analysis of vitamin A was done. The samples were extracted with n-hexane and level of vitamin A concentrations were measured using a reversed-phase HPLC. Breast-milk vitamin A level of Omkoi mothers (58.4 ± 11.5 Ug/dl) was not significantly different from that of Muang mothers (68.7 ± 12.5 Ug/dl) and of Maejam mothers (67.5 ± 18.4 Ug/dl). Serum vitamin A level of Omkoi mothers (39.1 ± 19.9 Ug/dl) was not significantly different from that of Muang mothers (49.3 ± 10.6 Ug/dl) and of Maejam mothers (44.5 ± 13.0 Ug/dl). All lactating mothers (except 1 Maejam mother) had normal vitamin A concentrations in breast milk (>30 Ug/dl) and also in serum (>20 Ug/dl).

Keywords: maternal, lactating, vitamin A, retinol, breast-milk

Introduction
Vitamin A is an essential nutrient needed in small amounts by humans to maintain normal functions including vision, growth, development and vital for maintenance of epithelial cellular integrity, immunity function and reproduction. Particularly, it is important for expectant mother¹³ and the developing children.⁴ The richest sources of preformed vitamin A as retinyl ester are liver and fish liver oil, milk, butter and eggs. While beta-carotene, pro-vitamin A, is mainly distributed in yellow and dark-green vegetables and fruits, the World Health Organization (WHO) suggests a conversion of beta-carotene to vitamin A in human to be a ratio of 6:1.⁵ Vitamin A deficiency is a major public health problem in many developing countries and afflicts all children under the ages of five years. It can cause blindness, decrease resistance to infectious diseases and increase mortality.⁶⁻⁸ Most newborns have a marginal vitamin A status when their mothers have inadequate vitamin A intake. Their vitamin A reserve can maintain optimum levels in a few weeks. The baby will receive the proper amounts of vitamin A from breastfeeding and increase its vitamin A store. If not properly nourished, infants and young children can develop the signs of vitamin A deficiency rapidly.⁹⁻¹³ The World Health Organization (WHO) defines that the proper amount of vitamin A (retinol) should be >30 Ug/dl in breast milk and >20 Ug/dl in serum.¹⁴ Serum retinol reflects total body vitamin A stored in the liver. Normally, the levels of vitamin A and status, prenatal care in the first trimester, in breast-milk and serum are 40-50 Ug/dl and 28-94 Ug/dl, respectively.
The prevalence of vitamin A deficiency in lactating mothers can be influenced by maternal education previous experience of breastfeeding, duration of breastfeeding, malnutrition, and probably habits of eating. Previous studies show that breast-milk vitamin A concentrations were 14.1-31.1 μg/dl in Ethiopian mothers, 40-50 μg/dl in Swedish mothers, 26.7-30.1 μg/dl and 29.8-61.3 μg/dl in Bangladesh mothers, 27.4-41.6 μg/dl in Indonesian mothers.\textsuperscript{13-17} The studies performed in Thailand report that the breast-milk vitamin A levels of lactating mothers in Ubon Rajathani and in Chiang Mai were 30-50 μg/dl and 22.0±7.5 μg/dl respectively.\textsuperscript{20-22} The numbers of blind children are increased in many countries in South and East Asia,\textsuperscript{12, 23-25} also in Africa,\textsuperscript{11, 26, 27} which poor dietary intake could be the principal factor. It is postulated that their mothers may nourish vitamin A deficient milk to the infants. This study was purposed to measure the levels of serum and breast-milk vitamin A in the lactating women in Amphur Omkoi (OK) compared with vitamin A level in lactating women in Amphur Maejam (MJ) and Amphur Muang (MU) Chiang Mai.

Materials and methods

Chemicals and reagents: All solvents were HPLC grade and were employed as supplied by manufacturers. High purity water obtained through a Milli Q water purification system (Millipore, Bedford, MA, USA) was used in all procedures. All-trans retinol and alpha-tocopherol were of analytical-reagent grade and purchased from Sigma Chemical Company (St. Louis, MO, USA).

Study subjects: The study complied with the Declaration of Helsinki and with the approval by the Faculty of Medicine Ethics Committee for Human Research in Chiang Mai University for collection of maternal blood and breast-milk protocol (Reference number 0515(05)/240/2003). Geographic distribution of the three selected areas is described briefly. The OK and the MJ are mostly mountainous, and locate in the Southwest and the West of Chiang Mai Province respectively. The MU is a city of Chiang Mai and located on plain land. Written informed consent were obtained from all mothers before enrollment. Inclusion criteria were healthy; living in the OK, MJ and MU District in Chiang Mai; and being in lactation period. A total of 222 healthy mothers (86 in OK, 90 in MJ and 46 in MU) 14 day postpartum were enrolled in the study. Exclusion criteria were chronic diarrhea, breast abscess and incompliance for the study. Local public-health volunteers were well trained to interview subjects' history and to collect their blood and breast-milk samples. Socio-economic information including maternal age, race, income, frequency of pregnancy and food intake were recorded in the study.

Anthropometric measurement: The subjects had their height and weight measured. Nutritional status was assessed by using body mass index (BMI) and calculated as the ratio of weight (kg) to height squared (m²). The BMI less than 18.5 kg/m² were considered indicative of chronic energy deficiency while the BMI greater than 25 kg/m² were considered obese.\textsuperscript{28}

Breast-milk and blood samples: Because the infants were breast-fed on demand, breast-milk samples (5-10 ml) were collected independently of the time (usually between 10.00-12.00 a.m.) in a sterile 50-ml Falcon tube from either one of the breasts using a breast pump. Venous blood samples were drawn into a glass tube covered with aluminum foil. The blood was allowed to clot for 20 minutes and serum was separated immediately. Sample tubes were wrapped with aluminum foil to protect from light, transported in the ice box to the Department of Biochemistry, Faculty of Medicine, Chiang Mai University and stored at -80°C until analysis.

Creameatocrit determination: Creameatocrit, which was used to estimate the fat content of breast milk, was measured as described by Lucas and coworkers.\textsuperscript{29} Briefly, breast milk was filled in three capillary tubes and spun at 10,000 rpm, 25°C for 10 minutes in a Hematocrit Centrifuge.

Sample extraction: Breast milk and serum samples were thawed and homogenized for 3-5 strokes. The samples (200 UL) were spiked with internal standard (300 μg/dl alpha tocopherol) (5 UL), then vigorously extracted twice with an equal volume of n-hexane (200 UL). The combined layers of hexane were pooled in an amber colored glass tube and evaporated under nitrogen gas stream. The extracted vitamin A was redissolved in a mobile-phase solvent and further analyzed by using the reversed-phase HPLC\textsuperscript{30} with a slight modification.

HPLC measurement of retinol concentration: The method was validated with respect to reproducibility, linearity, accuracy and standard ization. A 50 UL sample was usually injected into the high performance liquid
Statistical differences were analyzed by the Student’s t test, and P values <0.05 were considered statistically significant. Regression analysis was used to describe the relationship between breast milk and serum vitamin A.

Results

Maternal characteristics: As shown in Table 1, there was no significant difference for the ages of lactating mothers in MU, MJ and OK (26.6±3.6, 25.6±6.3 and 23.6±6.1 years, respectively). The average weight of MU mothers (54.0±8.3 kg) was higher than the MJ mothers (51.0±7.5 kg) and the OK mothers (48.9±5.8 kg). The average height of the MU mothers (155.5±5.3 cm) was greater than the MJ mothers (152.4±5.4 cm) and the OK mothers (150.6±5.4 cm). The average BMI indices for the MU, MJ and OK mothers were in the range of 22.3±3.2, 21.9±3.0 and 21.6±3.1 kg/m², respectively. However, many OK mothers appeared to be diet deficient (31 in 86 mothers) and overweight (26 in 86 mothers). These parameters show normal anthropometric values for all studied lactating mothers. In this study for ethic group, 83 in 86 OK mothers (97.7%) and 67 in 90 MJ mothers (74.5%) were hill-tribes (mostly Karen), while 39 in 46 MU mothers (83%) were Thais. The frequency of pregnancy was higher for the OK mothers (1.9±0.8) and MJ mothers (2.2±0.8) than for the MU mothers (1.6±0.7). In socioeconomic view, the OK mothers were much poorer than the MJ and MU mothers.

Validation of HPLC quantification of vitamin A (retinol): An HPLC chromatogram obtained from analysis of the standard retinol solution containing alpha-tocopherol are shown in Figure 1. The retention time was 2.4 min for the retinol and 7.4 min for the alpha-tocopherol, which was almost constant in all measurements. The vitamin A concentrations (51.2±4.2 Ug/dl) measured in our laboratory had a good correlation (R² = 0.932) with the ones (50.0±3.9 Ug/dl) assayed in the ISO 9003 laboratory without any significant difference (mean difference = 0.9 Ug/dl, P value = 0.897) (Figure 2).

Analysis of retinol in maternal breast milk and serum: expressed as individual and mean±SD values (Table 2 and Figure 3). Breast-milk vitamin A levels in the MU mothers and OK mothers were 68.7±21.5 Ug/dl and in breast milk were not significantly different, and none of the subjects had the values below 30 Ug/dl, which is the indication of inadequate vitamin A status. Nearly all of the MJ mothers had normal breast-milk vitamin A levels (67.5±18.4 Ug/dl), except one (24.5 Ug/dl). Alternatively, the amounts of breast-milk vitamin A were 25.9±5.4 Ug/g fat for the MU mothers, 19.6±6.3 Ug/g fat for the MJ mothers, and 21.2±10.7 Ug/g fat for the OK mothers.

Serum vitamin A levels in the MU mothers (49.3±10.6 Ug/dl) were slightly higher than, but insignificantly different from those in the MJ mothers (44.5±13.0 Ug/dl) and the OK mothers (39.1±19.9 Ug/dl). Nevertheless, none of them were below 20 Ug/dl, which is the indication of inadequate vitamin A status. Apparently, the amounts of breast-milk vitamin A were higher than those of serum vitamin A.

Discussion

A previous report demonstrates five social factors (including cultural and social values, economy, household structure, social supports and health) can affect maternal health and nutrition status.11 Our study was conducted on the lactating mothers in OK, MJ and MU. Most of the mothers in OK were hill tribes (specifically Karen)
with low income. Their incomes were averagely three-fold and six-fold lower than the MJ and MU mothers respectively; however, it did not affect the status of maternal vitamin A levels in breast milk and serum. Additionally, low education did not affect the vitamin A status in the lactating OK mothers who lived in rural areas or high lands. Consistently, there was no association between maternal vitamin A deficiency and socioeconomic stratum, family income or level of education. Our record also indicates that these subjects usually consume traditional foods such as green leaf vegetables, ripen fruits and carrots, which are the main sources of vitamin A.

Figure 1. HPLC profile of vitamin A (retinol). Alpha-tocopherol was used as internal standard.

Figure 2. Correlation of vitamin A concentrations assayed in the BCCMU Laboratory and in the NFI Laboratory.
Figure 3. Vitamin A concentrations in breast milk and serum of lactating mothers living in MU, MJ and OK. Data are expressed as scatter plot and mean ± SD values.
Table 1. Characteristics of the studied mothers.

<table>
<thead>
<tr>
<th>Maternal characteristics</th>
<th>MU (n = 47)</th>
<th>MJ (n = 89)</th>
<th>OK (n = 85)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>26.6±5.6</td>
<td>25.6±6.3</td>
<td>23.6±6.1</td>
</tr>
<tr>
<td>Weight (kg.)</td>
<td>54.0±8.3</td>
<td>51.0±7.5</td>
<td>48.9±5.8</td>
</tr>
<tr>
<td>Height (cm.)</td>
<td>155.5±5.3</td>
<td>152.4±5.4</td>
<td>150.6±6.4</td>
</tr>
<tr>
<td>BMI (kg/m²):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18</td>
<td>2</td>
<td>25</td>
<td>31</td>
</tr>
<tr>
<td>18-25</td>
<td>40</td>
<td>38</td>
<td>32</td>
</tr>
<tr>
<td>&gt;25</td>
<td>5</td>
<td>26</td>
<td>22</td>
</tr>
<tr>
<td>Average</td>
<td>22.3±3.2</td>
<td>21.9±3.0</td>
<td>21.6±3.1</td>
</tr>
<tr>
<td>Race:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thai (persons)</td>
<td>8</td>
<td>22</td>
<td>2</td>
</tr>
<tr>
<td>Hill-tribe (persons)</td>
<td>39</td>
<td>67</td>
<td>83</td>
</tr>
<tr>
<td>Income (baht/month)</td>
<td>11,629±9,847</td>
<td>5,037±4,600</td>
<td>1,726±2,579</td>
</tr>
<tr>
<td>Frequency of pregnancy</td>
<td>1.6±0.7</td>
<td>2.2±0.8</td>
<td>1.9±0.8</td>
</tr>
</tbody>
</table>

Table 2. Vitamin A concentrations in breast milk and serum of the studied mothers.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>MU (n = 47)</th>
<th>MJ (n = 86)</th>
<th>OK (n = 90)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast-milk vitamin A (Ug/dl):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>58.9 - 105.8</td>
<td>24.5 - 116.5</td>
<td>33.3 - 100.2</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>68.7±21.5</td>
<td>67.5±18.4</td>
<td>58.4±11.5</td>
</tr>
<tr>
<td>Breast-milk vitamin A (Ug/g fat):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>15.8 - 41.0</td>
<td>4.8 - 36.2</td>
<td>7.9 - 57.8</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>25.9±5.4</td>
<td>9.6±6.3</td>
<td>21.2±10.7</td>
</tr>
<tr>
<td>Serum vitamin A (Ug/dl):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>30.4 - 76.0</td>
<td>17.2 - 68.1</td>
<td>5.04 - 95.8</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>49.3±10.6</td>
<td>44.5±13.0</td>
<td>39.1±19.9</td>
</tr>
</tbody>
</table>

Vitamin A (particularly retinyl ester) is mostly accumulated in milk fat, and plasma proteins (especially retinol-binding protein and transthyretin), many methods require a saponification process to hydrolyze ester bond. However, due to the process being troublesome and time consuming, we did not include the saponification to liberate free retinol from retinyl ester in clinical samples. A previous study can confirm that the saponification is not necessary and that yield of retinol is better than those obtained using the saponification. In addition, hexane extraction without the saponification can make the analysis more rapidly efficient. The HPLC based measurement of vitamin A concentration is versatile, reproducible and standardized.

According to previous evidences, the amount of vitamin A in milk increases with excessive intake of vitamin A. Serum retinol and BMI of Indonesian mothers were positively associated with milk retinol level. The levels of breast-milk and serum vitamin A of hill-tribe mothers were not different from those of well-nourished Thai mothers and not lower than the cut-off values recommended by the WHO. The frequency of pregnancy was higher for the OK mothers (1.9±0.8) and MJ mothers (2.2±0.8) than for the MU mothers (1.6±0.7). This could be contributed by the availability of traditional food such as meat, milk, eggs, carotenoids, green leave vegetables and ripen fruits in such a rural area as OK and MJ District. Baron et al. found that serum vitamin A deficiency was not present at any time during pregnancy in these adolescents and the increase of vitamin A intake at the end of pregnancy did not change the decline in serum retinol.33 Presumably, all subjects will have an essentially adequate intake of vegetables and fruits as their usual habit. Suggestively, monitoring of breast-milk and serum vitamin A and nutrient intervention is recommended in order to promote a healthy mother and newborn. Education programs which stress on nutrition and health promotion should be given to pregnant women as well as lactating women regularly and designed according to socioeconomic and cultural factors.
Acknowledgements

This work was completed under a research grant supported by Universal Health Care Coverage from Chiang Mai Public Health Office. We thank Dr. Rattawut Sukmee MD, Head of Chiang Mai Public Health, Dr. Surasing Wisrutratana DDS, PhD and Dr. Wantanee Chawapongse PhD for encouraging the project and also to volunteered Public Health Officers in the studied area for their great assistance.

References