Maternal Plasma and Breast Milk Vitamin D level from the USM Birth Cohort Study

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Overview

• The Monsoon Study
• The USM Birth Cohort Study
The Monsoon Study

Objectives

1. To compare levels of vitamin D between indoor and outdoor workers in Kelantan

2. To compare levels of vitamin D during monsoon and non-monsoon season among indoor and outdoor workers in Kelantan
Outdoor & Indoor Workers Definition

Outdoor Workers ➤ Fishermen

• Group that receives regular and significant solar ultraviolet rays (UVR) exposure [Gies et al., 1995]

• Those reporting occupational sun exposures for more than 2h/day between 0800 and 1400 h [Azizi et al., 2009]

Indoor Workers ➤ Office staff

• Persons who are employed in nonindustrial, nonagricultural indoor settings (indoor work environments) [Mendell et al., 2002]

• Stayed indoors more than half of their working time [Chao and Wang, 1994]

Study design & setting

Funding: Fundamental Research Grant Scheme (MOHE)

Setting:

- Wisma Persekutuan Kota Bharu
- Kelantan Fishermen Villages (Kota Bharu, Bachok & Semerak)
Flowchart

1st Phase

Non-Northeast Monsoon Season (May – June 2012)

Indoor Workers (n=143)
Outdoor Workers (n=138)

Data Collection
Analysis of Outcome

2nd Phase

Northeast Monsoon Season (Jan – Feb 2013)

Indoor Workers (n=121)
Outdoor Workers (n=118)

Data Collection
Analysis of Outcome

Same subjects were followed up

Comparative Point Prevalence Study

Vitamin D Concentrations between Non Monsoon and Monsoon Season (WORKPLACE)

![Graph showing vitamin D concentrations](image)

- Non-Northeast Monsoon
  - Outdoor: 124.7 nmol/l, P<0.001
  - Indoor: 115.1 nmol/l, P<0.001

- Northeast Monsoon
  - Outdoor: 48.1 nmol/l
  - Indoor: 49.2 nmol/l

NS
## Vitamin D Concentrations between Non Monsoon and Monsoon Season (SEX)

<table>
<thead>
<tr>
<th>Workplace</th>
<th>Vitamin D Concentration (nmol/l)</th>
<th>P-value</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Outdoor Workers</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non Monsoon</td>
<td>80.4 (n=10)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Monsoon</td>
<td>76.1 (n=8)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td><em>Indoor Workers</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non Monsoon</td>
<td>39.8 (n=101)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Monsoon</td>
<td>68.3 (n=42)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

### Conclusion

1. Indoor workers have lower vitamin D level as compared to outdoor workers
2. Females have lower vitamin D level as compared to males
The USM Birth Cohort Study

Objectives

General objective

• To examine maternal plasma and breast milk vitamin D concentrations.

Specific objectives

• To determine levels of maternal plasma vitamin D in the second and third trimester of pregnancy.
• To determine levels of breast milk vitamin D at birth, 2, 6 and 12 months of postnatal age.
• To examine the associations between maternal plasma and breast milk vitamin D concentrations.
Study design:
Longitudinal study

Setting:
Two health clinics in Kubang Kerian

Data collected:
Apr 2010 – Dis 2012

Study sample & Sampling method

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study sample (pregnant women)</td>
<td>Inclusion criteria:</td>
</tr>
<tr>
<td></td>
<td>§ Malay ethnic</td>
</tr>
<tr>
<td></td>
<td>§ Aged 19 – 40 years old</td>
</tr>
<tr>
<td></td>
<td>§ Singleton pregnancy</td>
</tr>
<tr>
<td></td>
<td>Exclusion criteria:</td>
</tr>
<tr>
<td></td>
<td>§ With chronic diseases</td>
</tr>
<tr>
<td></td>
<td>§ Preterm labour</td>
</tr>
<tr>
<td>Sample size</td>
<td>103 pregnant women</td>
</tr>
<tr>
<td>Sampling method</td>
<td>Non-probability Convenience Sampling</td>
</tr>
<tr>
<td>Ethical approval</td>
<td>Human Research Ethics Committee (USM)</td>
</tr>
<tr>
<td></td>
<td>Medical Research Ethics Committee (MOH Malaysia)</td>
</tr>
</tbody>
</table>
Flow Chart

Pregnant women (15-22 weeks of gestation) aged 19-40 years old.

32-39 weeks of gestation.

At birth
Maternal-infant anthropometry and breast milk samples taken

Mother and child followed-up 2 month, 6 month and 1 year.

Parameters

Prenatal period

- Maternal anthropometry
- Glucose & lipids profiles
- Oxidative stress markers
- Plasma 25(OH) D

Postpartum period

- Infant feeding
- Infant anthropology
- Maternal dietary intake
- Maternal physical activity
- Nicotine exposure
- Breast milk 25(OH) D

USM Birth Cohort Study
25(OH) D Analysis

- Analysis performed by the Canterbury Health Laboratories, New Zealand.
- HPLC–tandem mass spectrometry (Lewis & Elder, 2008).
- Definition of vitamin D status (WHO, 2003):
  - deficiency (<25nmol/L)
  - insufficiency (25-50nmol/L)
  - normal (>50nmol/L)
- Breast milk 25(OH)D: Conversions to antirachitic activity in IU/L were based on the following: 5 pg/mL of 25-hydroxyvitamins D-3 and D-2 = 1 IU/L (Dawodu et al., 2012).


Statistical analyses

Performed with IBM SPSS (PASW) Statistic 19. Significant level set at $p<0.05$.

Repeated measures ANOVA
- to assess the levels of maternal plasma and breast milk 25(OH) D concentrations.

Pearson’s correlation test
- to examine the association between maternal plasma and breast milk 25(OH) D concentrations.

Outcome measures
- Breast milk 25(OH) D concentrations at birth, 2, 6 and 12 months of postnatal age.

Independent variables
- Maternal plasma 25(OH) D concentrations in the 2nd and 3rd trimesters of pregnancy.
### Results

Maternal anthropometric measurements

<table>
<thead>
<tr>
<th>Variable</th>
<th>mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.71 (4.61)</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>18.21 (2.73)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.55 (0.06)</td>
</tr>
<tr>
<td>Pre-pregnancy BMI (kg/m²)</td>
<td>23.09 (4.13)</td>
</tr>
</tbody>
</table>
Distributions of Maternal Socio-demographic Characteristics

**Distribution of Maternal Parity**
- Multipara: 40.6%
- Nullipara: 31.9%
- Primipara: 27.5%

**Distribution of Maternal Employment Status**
- Employed: 77.4%
- Unemployed: 22.6%

**Distribution of Household Income**
- High: 32.3%
- Low: 49.4%
- Middle: 27.5%

**Distribution of Maternal Parity**
- Multipara
- Nullipara
- Primipara

**Distribution of Maternal Employment Status**
- Employed
- Unemployed

**Distribution of Educational Level**
- University
- Primary & Secondary
- Pre-University

**Maternal vitamin D status during pregnancy**

<table>
<thead>
<tr>
<th>25 (OH) Vitamin D (nmol/L)</th>
<th>2nd trimester</th>
<th>3rd trimester</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deficient (&lt;25)</td>
<td>3.9</td>
<td>0</td>
</tr>
<tr>
<td>Insufficient (25-50)</td>
<td>57.3</td>
<td>39.8</td>
</tr>
<tr>
<td>Normal (&gt;50)</td>
<td>38.8</td>
<td>60.2</td>
</tr>
</tbody>
</table>
Maternal plasma 25(OH) D concentrations were significantly increased (p<0.001) from the 2nd to 3rd trimesters of pregnancy.

No difference in the prevalence of multivitamin intake between 2nd and 3rd trimesters of pregnancy.
Plasma 25(OH)D concentration between women with and without multivitamin intake during pregnancy

Maternal plasma 25(OH)D concentrations were significantly associated with multivitamin intake. The physiological change may also contribute to the significant increase.

Breast milk 25(OH)D antirachitic activity

Low levels of 25(OH)D in the breast milk samples
Trend of breast milk 25(OH)D antirachitic activity during the first year of postnatal life

Decreasing trend in breast milk 25(OH)D concentrations were observed during the first year of postnatal life, though the differences were not significant (p=0.145).

Correlations between maternal plasma 25(OH)D in the 2nd trimester and breast milk 25(OH)D concentrations. (Mean plasma vit D = 48.4 nmol/L)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Postnatal age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At birth</td>
</tr>
<tr>
<td>r (p)</td>
<td>0.18 (0.080)</td>
</tr>
</tbody>
</table>

Correlations between maternal plasma 25(OH)D in the 3rd trimester and breast milk 25(OH)D concentrations. (Mean plasma vit D = 59.1 nmol/L)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Postnatal age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At birth</td>
</tr>
<tr>
<td>r (p)</td>
<td>0.22 (0.031)</td>
</tr>
</tbody>
</table>
In general, the mean ARA of the human milk in healthy lactating women, unsupplemented or supplemented with existing recommended vitamin D intake, ranges from 10 to 80 IU/L (Table 1).

These values lead to low vitamin D intake in the breast-fed infant compared with the recommended intake of 400 IU/d of vitamin D if human milk is sole source of vitamin D (Dawodu et al., 2012).
### Comparison of vitamin D status among studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Subject</th>
<th>25(OH)D (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>USM birth cohort</td>
<td>Pregnant women (19-40 years)</td>
<td>48.40</td>
</tr>
<tr>
<td></td>
<td>Malays (2nd trimester): 48.40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Malays (3rd trimester): 59.10</td>
<td></td>
</tr>
<tr>
<td>The Monsoon Study</td>
<td>Non Pregnant women (19-59 years)</td>
<td>39.80</td>
</tr>
<tr>
<td></td>
<td>Malays (Indoor): 39.80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Malays (Outdoor): 80.40</td>
<td></td>
</tr>
<tr>
<td>Rahman et al. (2004)</td>
<td>Postmenopausal women (50-65 years)</td>
<td>44.40</td>
</tr>
<tr>
<td>Green et al. (2008)</td>
<td>Non-pregnant women (18-40 years)</td>
<td>43.00</td>
</tr>
<tr>
<td>Moy &amp; Bulgiba (2011)</td>
<td>Non-pregnant women (≥35 years)</td>
<td>36.20</td>
</tr>
</tbody>
</table>

• Many women had **inadequate levels of vitamin D** during pregnancy, which aligns well with the Monsoon Study and the few other reports on vitamin D status in Malaysian women (Green et al 2008; Rahman et al 2004).

• The poor vitamin D status can be attributed to **avoidance of sunlight** and **traditional dress style** that cover most of the body while outdoors, and **inadequate vitamin D intake**.
Maternal vitamin D status

• Some studies suggest that low 25(OH)D predicts increased risk of gestational diabetes (Clifton-Bligh et al., 2008), bacterial vaginosis (Bodnar et al., 2009), preterm birth and threatened preterm delivery (Shibata et al, 2011), and C-section (Merewood et al., 2009).

• The high prevalence of low vitamin D status in pregnant women indicates that a significant proportion of infants could be born with vitamin D deficiency (Hollis and Wagner, 2004).

Breast milk vitamin D status

• The risk of vitamin D deficiency in infants increase when the mother is vitamin D deficient during pregnancy (Kovacs, 2012).

• The risk increases further due to low vitamin D metabolites level in breast milk of the vitamin D deficient mothers during lactation.

• Nevertheless, breast milk in general is still the best food for the baby.

• Mothers should receive adequate information on strategies to increase vitamin D level during pregnancy and lactation.
Correlations between maternal plasma and breast milk vitamin D concentrations

- Maternal 25 (OH) D level reached the highest level at the 3rd trimester. Perhaps due to increased intake from supplements and gradual storage of fat soluble vitamin D in the body.

- Hence, significant correlation was only detected between maternal plasma 25(OH)D in the 3rd trimester and breast milk 25(OH)D at birth.

- This may explain the beneficial effect of increased intake of vitamin D during pregnancy on the concentration of 25(OH) D in human milk.

- This aspect warrants further research.

Conclusion

1. The high prevalence of inadequate levels of maternal plasma and breast milk vitamin D might impose negative effects on maternal and infants’s health.

2. Appropriate strategies to improve vitamin D status among pregnant and lactating mothers is warranted.
Acknowledgements

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http://www.iaso.org/events/ico/ico-2014/