Original Communication

Perinatal Vitamin A Status in Relation to Neurodevelopmental Outcome at two Years of Age

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\textbf{Abstract}: Information about the effect of antioxidant vitamins nutrition during pregnancy on offspring’s intellectual development is extremely limited. \textit{Objective}: To investigate the correlation of antioxidant vitamins (Vitamin A, E and C) at delivery and the neurodevelopment of early childhood. \textit{Method}: A total of 158 paired maternal-neonatal subjects were recruited. The serum concentrations of vitamin A, E and C in maternal and cord blood after delivery were determined and intellectual development was evaluated by Gesell Development Schedule (GDS) at two years old. \textit{Result}: After adjusting for potential confounders, vitamin A placental transport ratio (VA-PTR) was positively associated with motor area development quotients (DQ) and average DQ ($p<0.01$). Cord VA level was positively related with language area and social area DQ ($p<0.05$). Nevertheless, there was no significant association between cord VE, VC levels, VE PTR or VC PTR and GDS. The adaptive area and average DQ in high cord VA group was higher than those in low VA group ($p<0.05$). Cord VA level and VA-PTR were positively associated with birth head circumference and birth weight, respectively. \textit{Conclusion}: Our data suggested that adequate vitamin A at delivery had beneficial influence on neonatal birth outcomes and children’s neurodevelopment in later childhood.

\textbf{Key words}: vitamin A, children, neurodevelopment, cord blood, Gesell Development Schedule
Introduction

The 'fetal/developmental origins of adult health and diseases' hypothesis firstly put forward by Barker et al.[1] states that environmental factors, particularly maternal undernutrition during pregnant periods of neonatal critical development can have a long-term programming effect on offspring's health in adult life[2, 3]. In recent decades there has been a large growing body of reports suggesting that pre- or postnatal nutritional manipulations may programme adult size and diseases, such as cardiovascular disease, obesity, the metabolic syndrome, cancer, learning and behavior abnormality, etc [4-10]. This is partially supported by evidence from epidemiological studies, animal models and clinical intervention trials[11-13].

The effects of impaired nutritional status during infancy and childhood may have long-term consequences for the cognitive function and mental performance during their later life. Supplementation with micronutrients has been suggested to improve intelligent scores in malnourished children[14]. Extensive animal data, largely on rats, show that nutrition at a sensitive period of brain development may have permanent effects on brain size, the number of brain cells, behavior, learning and memory [15].

Brain development is a dynamical process modulated by some neuroprotective pre- and perinatal nutritional factors such as protein-energetic intake, long-chain polyunsaturated fatty acid, vitamins, minerals, choline and the maternal pseudo-nutritive agent intake[5]. There are well established associations between poor cognition and behavioral development and iron, zinc and iodine deficiency, while few studies has been conducted on vitamin deficiencies [16] Oxidative stress is involved in the development of several neuronal degenerative diseases, and the brain is especially vulnerable owing to its high oxygen use[17]. Antioxidant vitamins, such as vitamin A, E and C, may play an important role in normal neurodevelopment by reducing oxidative stress [18]. For example, prolonged alpha-tocopherol deficiency in infancy is associated with lower subsequent cognitive performance[19]. Vitamin A has a critical role in the learning and memory processes as vitamin A deficiency (VAD) produces impairment of spatial learning, memory and somatostatinergic system in rats[20, 21]. And postnatal VAD may induce a selective memory impairment in rats[22].

Data from animal models have importance in suggesting human interventions. However, public health policy for early nutrition in humans must ultimately depend on human studies, on which information is extremely limited. The extent to which these animal data has relevance to human neurodevelopment however, is uncertain and needs further exploration.

To our knowledge, most researches about the relationship between antioxidant vitamins and individual intellectual level have focused on aged people and children[19, 23], and no report about the association of antioxidant vitamin levels at delivery with neurodevelopment in childhood has been shown. The present study evaluated the association between levels of cord blood antioxidant vitamins A, E and C, and neurodevelopment at two years of age as measured by the Gesell Development Schedules (GDS). Our data suggested that adequate vitamin A at delivery had beneficial influence on neonatal birth outcomes and children’s neurodevelopment in later childhood.

Materials and methods

Subjects and ethical approval

The present research was a prospective cohort study and the subjects were 158 children born to non-smoking women who gave birth at the Tongliang County Hospital, Tongliang Traditional Chinese Medicine Hospital, Tongliang Maternal Children Health Hospital or Baichuan Hospital in Tongliang, Chongqing, China, between March 2, 2005 and May 24, 2005. Only healthy mothers and their infants were recruited into the protocol and the exclusion criteria were pregnancy toxemia, hypertension, diabetes mellitus, thyroid disease, bronchial asthma, active hepatitis, chronic renal failure, heart failure and hereditary neurological illnesses. The number of subjects was sufficient enough to allow the detection of a difference of two developmental quotient points among the children at two years of age with 95% power at the two-tailed 5% level. Potential participants received information about the study and a written informed consent was presented to and signed by the volunteer mothers, the written consent stated that participation was voluntary, their confidentiality was assured and that participants could withdraw from the study at any time. At two years of age, 122 children were tested with the GDS. The research protocol had been reviewed and approved by the institutional Ethics Committee of the child-
ren's hospital at Chongqing Medical University in Chongqing, China.

Personal interview

An about 45-minute questionnaire was administered by a trained interviewer after delivery. The questionnaire elicited demographic information, lifetime residential history (location of birth and duration of residence), history of passive smoking (including number of household smokers), occupational exposure, medication information, and alcohol use during each trimester of pregnancy. Socioeconomic information related to maternal age, educational level, height, weight before pregnancy and income was also collected. Data including the date of delivery, gestational age, neonatal gender, malformations; maternal height, head circumference, pre-pregnancy weight, complications of pregnancy and delivery, and medications used during pregnancy were obtained by the research workers based on the mothers' and neonates' medical records after delivery. Gestational age was estimated based on the maternal report for the last menstrual period and on ultrasound measurement by obstetricians.

Biological sample collection and analysis

Maternal blood (10 ml) was collected within 1 day postpartum, and umbilical cord blood (40–60 ml) was collected at delivery. Samples were transported to the field laboratory at the Tongliang County Hospital immediately after collection. For blood samples, the buffy coat, packed red blood cells, and plasma were separated and stored at -70°C. The serum sample prepared for retinol measurement was protected from light.

Serum retinol and α-tocopherol concentrations were determined using high-performance liquid chromatography (HPLC) following the method with slight modification. Briefly, retinol was extracted with hexane after deproteinization with ethanol containing retinyl acetate as the external standard, and evaporated to dryness with nitrogen gas. The residue was dissolved in 0.1 ml methanol. A portion (20µl) of the sample was injected into the column (Symmetry Shield RP, 3.9×150 mm) installed with the HPLC apparatus (Waters 1525 Binary HPLC Pump, Waters Breeze, USA). The mobile phase was a methanol–DH2O mixture (95:5) for α-tocopherol: 98:2). Concentration of retinol was determined by spectrophotometry (Waters 2487 Dual λ Absorbance Dector, USA) at 315 nm (for α-tocopherol: 280 nm).

Serum ascorbic acid concentration was measured by HPLC following Zhanguo C and Esteve MJ's method using 100 mmol/L potassium dihydrogen phosphate as mobile phase at pH 3.5 by phosphoric acid and the detected absorbance λ was 254 nm. All procedures were performed in dark room to protect the serum from light.

Duplicate analyses for serum retinol were performed on one tenth of the samples and the estimated variability was 0.02µmol/l. Three control serum samples with low (0.70µmol/l), medium (1.40µmol/l) and high (2.79µmol/l) concentrations of serum retinol were provided by the retinol standard solution (Sigma, USA) with pooled serum. The between-day CVs for low, medium and high concentration for serum retinol were 5.68%, 3.16% and 1.85% respectively. Similarly, the between-day CVs for serum α-tocopherol were 4.6%, 2.2% and 2.0% at 10 µmol/l, 20 µmol/l and 40 µmol/l respectively, and for serum ascorbic acid were 3.2%, 2.8% and 1.1% at 30 µmol/l, 60 µmol/l and 120 µmol/l respectively.

All the biochemical indexes were measured by expert examiners in the Paediatric Laboratory of Chongqing Medical University, China.

Measurements relevant to birth outcomes and child neurodevelopment

Neonatal birth weight was measured to the nearest 50 g using a digital scale, and crown–heel length and head circumference were recorded to the nearest 0.1 cm with a board and a nonstretch plastic tape measure. All scales were checked daily against standard metallic weights and were calibrated if necessary.

The GDS was selected to assess neurodevelopment. It was developed by Gesell and Amatruda in 1940, and it included physical, emotional, and behavioral development other than intellectual or academic skill assessment. The GDS was selected for comparability to other studies in the Chinese population and in other countries. Children at two years of age in the present study were administered the version of the GDS for 0–3 year-old children revised by the Chinese Pediatric Association and Beijing Mental Development Cooperative Group in order to adapt to the Chinese population. The results were shown as development quotients (DQs) in motor area, adaptive area, language
area, social area, and average of the these four domains. The standardized mean±standard deviation (S.D.) of the developmental quotients (DQs) is 100±15. A child with a DQ lower than 85 is considered to have a high probability of some organic impairment. We used the score of 84 as the cutoff point for determining normal and developmental delay[29,39].

The test was conducted by trained professionals, who took a one-year course at Shanghai Jiaotong University and passed standardized exams to become certified, to maximize reliable assessment and valid interpretation. The examiners on the GDS were blind to the vitamin A status of both mothers and children. Furthermore, the two examiners split the testing by domains, not by subjects. As a result, for any one domain, all subjects were tested by the same examiner.

Statistical analysis

Using Shapiro-Wilk test, the distribution of each set of data was tested for normality before analysis. When necessary, data were normalized using natural-log transformations. Data were presented as mean and standard deviation (SD) for data with normal distribution and median (P_{25}, P_{75}) for non-normal distribution data. Tests of significance were two-tailed and p<0.05 was considered statistically significant.

Simple linear regression analysis was used to identify the independent variables that were statistically significantly associated with GDS. Multiple linear regression was used to analyze the association between vitamin levels and GDS with adjustment for potential confounders, including gestational age, gender, educational level, ETS and neonatal head circumference. Variance inflation factors (VIF) calculation values indicated the multicollinearity between these factors, and factors with VIF>10 were eliminated from the multiple linear regression model to avoid biases of parameter estimate. Gender, ETS and gestational age were associated with one or more domains of DQs (p < 0.1) and were considered to be potential confounders. Maternal education level and maternal age were significantly correlated (r = -0.274, p = 0.0005), so adjusting for them together would lead to instability. Therefore, maternal education level was selected as a covariate because it contributed more than maternal age to neurodevelopment outcome. In addition, birth head circumference is reported to be related to neurodevelopment in some studies[40].

We calculated the age-adjusted DQs of the four domains and the average GDS. The following covariates were examined: gender of neonate (1=female, 2=male), gestational age (days), educational level of mother (1=high school, 2=high school), parity (1=no parity, 2=1 parity, 3=2 parities, 4=3 parities and 5=4 parities before), environmental tobacco smoking (ETS) during pregnancy (1=yes, 2=no), maternal age at first hospital visit (years), reported height before pregnancy (cm), maternal height (cm), maternal head circumference (cm), body mass index (BMI; in kg/m²) before conception, qualitative variables were treated as dummy variables in the multivariate statistical analysis. To avoid instability in the analysis models, only maternal-neonate paired cases with valid data for all of the variables (no missing values) were included.

Cord vitamin A variable was dichotomized into “high” and “low” level, with the median value as the cutoff point. The dichotomization was performed so that subjects with levels for either of those variables that are outliers compared with other subjects in this study, but are reasonable compared with what has been observed in previous studies, could be incorporated in our analysis without introducing significant biases. Two-sample t-tests were used to compare the DQ of the four domains and average DQ of “high” and “low” cord vitamin A group with normal distribution and homogeneous variance while Wilcoxon sign-rank test was used for data that was not normally distributed.

Multiple linear regression models were used to analyze the relationship between cord antioxidant vitamin levels and birth outcomes (birth weight, birth length and birth head circumference) after adjusting for the variables namely gestational age, gender, educational level and ETS, which have been reported to be correlated with birth outcomes in some studies[41–43]. Some researchers suggested that maternal pre-gestational weight, and body height correlated significantly with neonatal birth weight and birth length, respectively[44–45]. Thus maternal weight before pregnancy, maternal height and maternal head circumference were included in the analysis of birth weight, length and head circumference, respectively.
Table 1: Demographic Characteristics of the study sample (n=157)

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>Mean±SD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maternal (n=157)</strong></td>
<td></td>
</tr>
<tr>
<td>Maternal age (years) *</td>
<td>26.9 (23.9,31.4)</td>
</tr>
<tr>
<td>Height(cm) †</td>
<td>158.14 ± 3.9 (150–168)</td>
</tr>
<tr>
<td>Weight before pregnancy (kg) †</td>
<td>51.64 ± 6.2 (37.5–70)</td>
</tr>
<tr>
<td>BMI before pregnancy (kg/m²) †</td>
<td>20.65 ± 2.4 (15.99–27.69)</td>
</tr>
<tr>
<td><strong>Maternal education [n (%)]</strong></td>
<td></td>
</tr>
<tr>
<td>&lt; High school</td>
<td>91 (58.0)</td>
</tr>
<tr>
<td>≥ High school</td>
<td>66 (42.0)</td>
</tr>
<tr>
<td>Prenatal ETS exposure (hr/day) [n (%)]*</td>
<td>0.08 (0.0,0.33)</td>
</tr>
<tr>
<td>Maternal vitamin A (µmol/L) (n=148) †</td>
<td>1.05 ±0.37 (0.18–2.56)</td>
</tr>
<tr>
<td>Maternal vitamin E (µmol/L) (n=149) *</td>
<td>38.3 (26.0,54.9)</td>
</tr>
<tr>
<td>Maternal vitamin C (µmol/L) (n=149) *</td>
<td>50.1 (31.2,82.8)</td>
</tr>
<tr>
<td><strong>Neonatal (n=157)</strong></td>
<td></td>
</tr>
<tr>
<td>Gender of newborn (% female)</td>
<td>44.9</td>
</tr>
<tr>
<td>Gestational age (days) †</td>
<td>277.3 ± 9.2 (250–308)</td>
</tr>
<tr>
<td>Birth weight (kg) †</td>
<td>3.4 ± 0.4 (2.55–4.35)</td>
</tr>
<tr>
<td>Birth length (cm) (n=154) †</td>
<td>50.3 ± 1.5 (44–55)</td>
</tr>
<tr>
<td>Birth head circumference (cm) (n=156) *</td>
<td>34 (33.5,33.5)</td>
</tr>
<tr>
<td>Cord vitamin A (µmol/L) (n=148) †</td>
<td>0.68 ±0.19 (0.3–1.4)</td>
</tr>
<tr>
<td>Cord vitamin E (µmol/L) (n=153) *</td>
<td>19.7 (13.3,33.0)</td>
</tr>
<tr>
<td>Cord vitamin C (µmol/L) (n=154) *</td>
<td>47.4 (31.9,77.5)</td>
</tr>
<tr>
<td>Vitamin A placental transport ratio (VA-PTT) †</td>
<td>0.72±0.33</td>
</tr>
</tbody>
</table>

* Data was shown as median (P₂₅, P₇₅); † Data was shown as mean ± standard deviation.

Results

A total of 158 maternal–neonate pairs were recruited between March 2, 2005 and May 24, 2005. Complete epidemiological and clinical data were obtained from 157 pairs. The demographic, clinical characteristics and biochemical indexes from this prospective cohort study are presented in Table I. Eighty-nine out of 148 neonates (60.1%) had serum retinol <0.7 µmol/ L status (vitamin A deficiency, VAD), and 52 out of 148 neonates (35.1%) had serum retinol (0.7–1.05) µmol/ L (marginal VAD, MVAD). The prevalence of maternal VAD and MVAD were 12.8% (19/148) and 43.9% (65/148), respectively.

At two years of age, 122 children were tested with the GDS. Children lost to follow-up were due to changes of address or telephone number, parents going out to work, etc. The retention rate for the full cohort was 77.2% at the 2-year follow-up. There was no significant difference in maternal age (p= 0.843), education (p = 0.669), gestational age (p = 0.353), birth weight (p = 0.407), birth length (p = 0.732), birth head circumference (p = 0.482), cord VA (p< 0.656), cord VE (p=0.280), cord VC (p=0.265) of the children between women or their children retained in the study and those who were lost to follow-up.

Table II shows the distribution of DOs. All DO domains were significantly inter-correlated (p < 0.001), with r-values ranging from 0.34 to 0.82. The frequency of developmental delay as shown in Table 2 ranged from 2.5% (social) to 9.8% (language), with 1.6% for the average score.

The results of multiple regression analysis are shown in Table III. Variance inflation factor (VIF) of every independent variable was calculated and the variable with >10 VIF was excluded from models. All independent variables together (adjusted R²) ex-
Table II: Distribution of Gesell Development Schedule scores (n=122)

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD (range)</th>
<th>Normal [n (%)]</th>
<th>Developmental Delay [n (%)]*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motor area</td>
<td>97.36 ± 7.60 (74–116)</td>
<td>117 (95.9)</td>
<td>5 (4.1)</td>
</tr>
<tr>
<td>Adaptive area</td>
<td>100.86 ± 10.75 (76–129)</td>
<td>111 (91.0)</td>
<td>11 (9.0)</td>
</tr>
<tr>
<td>Language area</td>
<td>99.84 ± 9.68 (74–127)</td>
<td>110 (90.2)</td>
<td>12 (9.8)</td>
</tr>
<tr>
<td>Social area</td>
<td>101.43 ± 6.62 (76–117)</td>
<td>119 (97.5)</td>
<td>3 (2.5)</td>
</tr>
<tr>
<td>Average</td>
<td>99.90 ± 7.00 (76–117)</td>
<td>120 (98.4)</td>
<td>2 (1.6)</td>
</tr>
</tbody>
</table>

* Normal. >84; Developmental delay, <=84

Table III: Results of multiple regression analyses of Gesell scores at age two (n=122)

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variable</th>
<th>β (95% CI)</th>
<th>p value</th>
<th>R²/R² adj</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motor area</td>
<td>Vitamin A placental transport ratio (VA-PTR)</td>
<td>7.2064 (1.9093,12.5036)</td>
<td>0.0082</td>
<td>0.0713/0.0615</td>
</tr>
<tr>
<td>Adaptive area</td>
<td>Cord vitamin A</td>
<td>10.1617 (-0.8873,21.2107)</td>
<td>0.0710</td>
<td>0.1194/0.0811</td>
</tr>
<tr>
<td></td>
<td>VE-PTR</td>
<td>-0.8688 (-1.8019,0.0643)</td>
<td>0.0676</td>
<td></td>
</tr>
<tr>
<td>Language area</td>
<td>Cord vitamin A</td>
<td>10.5896 (1.2605,19.9188)</td>
<td>0.0265</td>
<td>0.1554/0.1186</td>
</tr>
<tr>
<td></td>
<td>Maternal education</td>
<td>2.3355 (0.4661,4.2049)</td>
<td>0.0149</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prenatal ETS exposure</td>
<td>6.1704 (0.5346,11.8062)</td>
<td>0.0322</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neonatal birth head circumference</td>
<td>-1.8745 (-3.3822,-0.3668)</td>
<td>0.0154</td>
<td></td>
</tr>
<tr>
<td>Social area</td>
<td>Cord vitamin A</td>
<td>8.0576 (1.3515,14.7637)</td>
<td>0.0191</td>
<td>0.0867/0.0673</td>
</tr>
<tr>
<td></td>
<td>Neonatal birth head circumference</td>
<td>-1.1785 (-2.2562,-0.1007)</td>
<td>0.0324</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>VA-PTR</td>
<td>7.0190 (2.1355,11.9026)</td>
<td>0.0053</td>
<td>0.1435/0.1062</td>
</tr>
<tr>
<td></td>
<td>Maternal education</td>
<td>1.9834 (0.5564,3.4104)</td>
<td>0.0070</td>
<td></td>
</tr>
</tbody>
</table>

Model included gestational age, gender, maternal education level, neonatal birth head circumference, cord vitamin A level, vitamin A placental transport ratio (VA-PTR), cord vitamin F level, VE-PTR, cord vitamin C level, VC-PTR and prenatal ETS exposure as covariates.

plained 6.15%, 8.11%, 11.86%, 6.73% and 10.62% of the variability in motor, adaptive, language, social area DQs and average DQ at two years of age, respectively. Increased vitamin A placental transport ratio (VA-PTR), which was calculated as the ratio of VA concentration in cord serum to that in maternal, was positively associated with an increase in motor area DQ (β=7.21; 95% CI, 1.91 to 12.50; p = 0.0082), and average DQ (β= 7.02; 95% CI, 2.14 to 11.90; p = 0.0053) after adjusting for gestational age, gender, maternal education level, neonatal birth head circumference and prenatal ETS exposure. In the same model, cord VA level was significantly positively associated with language area DQ (β=10.59; 95% CI, 1.26 to 19.92; p = 0.0265), and social area DQ (β= 8.06; 95% CI,1.35 to 14.76; p = 0.0191). An increase of 1% in VA-PTR was associated with an increase of 7.2 and 7.0 points in motor and average DQs, respectively. Similarly, an increase of 1μmol/L in cord vitamin A was associated with an increase of 10.6 and 8.1 points in language and social DQs, respectively. There was no significant association among cord VE, VC levels, VE placental transport ratio (VE-PTR) or VC placental transport ratio (VC-PTR) and GDS.

Table IV shows the comparison of GDS DQ scores by dividing cord vitamin A into high/low groups using median of cord VA (0.65μmol/L) as the dividing point. The adaptive area DQ (p<0.05) and average DQ (p<0.05) in high cord blood VA group was significantly higher than those in low VA group, and language area DQ in high VA group was higher than that in low VA group but without significance (p = 0.0658). Results of multiple regression analyses of birth weight, length and head circumference are presented in Table V. Cord VA level was significantly positively associated with birth head circumference (β=1.20; 95% CI,1.08 to 2.21; p = 0.0218) after adjusting for gestational age, gender, maternal education level, maternal head circumference, cord VA

Table IV: Comparison of GDS DQ scores at 2 years of age by dividing cord vitamin A into high/low groups †

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean± SD</th>
<th>p</th>
<th>Mean± SD</th>
<th>p</th>
<th>Mean± SD</th>
<th>p</th>
<th>Mean± SD</th>
<th>p</th>
<th>Mean± SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>High (n=61)</td>
<td>98.32±6.83</td>
<td>0.7221</td>
<td>102.89±10.24</td>
<td>0.0476</td>
<td>102.05±8.36</td>
<td>0.0658</td>
<td>102.74±4.85</td>
<td>0.3960</td>
<td>101.59±5.31</td>
<td></td>
</tr>
<tr>
<td>Low (n=53)</td>
<td>96.96±8.47</td>
<td>98.89±11.55</td>
<td>98.06±11.02</td>
<td>0.0289</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Using median of cord vitamin A level (0.65μmol/L) as dividing point
‡ Wilcoxon non-parametric tests were used for the different comparison of DQs domain scores between high and low cord serum vitamin A level; else by two-sample t-tests.

Table V: Results of multiple regression analyses of birth weight, length and head circumference

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variable</th>
<th>n</th>
<th>B (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight</td>
<td>Gestational age</td>
<td>125</td>
<td>0.0102 (0.0036, 0.0169)</td>
<td>0.0029</td>
</tr>
<tr>
<td></td>
<td>Gender of newborn</td>
<td>125</td>
<td>0.2146 (0.0866, 0.3427)</td>
<td>0.0012</td>
</tr>
<tr>
<td></td>
<td>Vitamin A placental transport ratio (VA-PTR)</td>
<td>125</td>
<td>0.7721 (0.0779, 0.4663)</td>
<td>0.0064</td>
</tr>
<tr>
<td></td>
<td>Maternal weight before pregnancy</td>
<td>125</td>
<td>0.0184 (0.0104, 0.0265)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Birth length</td>
<td>Gestational age</td>
<td>125</td>
<td>0.0381 (0.0109, 0.0653)</td>
<td>0.0064</td>
</tr>
<tr>
<td></td>
<td>Vitamin C placental transport ratio (VC-PTR)</td>
<td>125</td>
<td>0.1168 (-0.0115, 0.2452)</td>
<td>0.0739</td>
</tr>
<tr>
<td></td>
<td>Maternal height</td>
<td>125</td>
<td>0.1025 (0.0352, 0.1698)</td>
<td>0.0046</td>
</tr>
<tr>
<td>Birth head circumference</td>
<td>gestational age</td>
<td>125</td>
<td>0.0228 (0.0013, 0.0442)</td>
<td>0.0375</td>
</tr>
<tr>
<td></td>
<td>Gender of newborn</td>
<td>125</td>
<td>0.7567 (0.3469, 1.1665)</td>
<td>0.0004</td>
</tr>
<tr>
<td></td>
<td>Cord vitamin A</td>
<td>125</td>
<td>1.1957 (0.1772, 2.2142)</td>
<td>0.0218</td>
</tr>
</tbody>
</table>

Model included gestational age, gender, maternal education level, cord vitamin A level, vitamin A placental transport ratio (VA-PTR), cord vitamin E level, VE-PTR, cord vitamin C level, VC-PTR and prenatal ETS exposure as covariates. Maternal weight before pregnancy, maternal height and maternal head circumference were included in analysis of birth weight, length and head circumference, respectively.

level, VA-PTR, cord VE level, VE-PTR, cord VC level, VC-PTR and prenatal ETS exposure. And VA-PTR was significantly associated with birth weight ($\beta=0.27; 95\% CI, 0.08 to 0.47; p = 0.0064$).

Discussion

In this study, a prospective cohort research was conducted to evaluate the association between levels of cord blood antioxidant vitamins, namely vitamin A, E and C, and neurodevelopment of two years as measured by GDS. We found that after adjusting for the potential confounders, cord VA or VA-PTR were positively significantly related to at least one domain DQ of GDS at twoyear of age of the children. Moreover, the VA level at birth was associated with birth outcomes.

More and more researchers are paying attention to the relationship between early nutritional status and neurodevelopment. But there is limited information on the effects of vitamin A status during pregnancy on neurodevelopment in later childhood or adulthood. In fact, VAD is a public health problem throughout much of the developing world. Approximately 7 million pregnant women are VAD and 6 million mothers suffer from night blindness during pregnancy. Comparing with VAD, MVAD is more prevalent in pregnant women. In the present study, the percentage of pregnant women with MVAD and VAD in suburb Chongqing, China was 43.9% and 12.8%, respectively, which indicated that it was a moderate public health problem of VAD in the locality according to the criterion of WHO[46], and strategies should be devised to conquer this problem. Up to now, the normal value for cord serum retinol concentration has not been clearly established; however, given that concentration < 0.7 μmol/L is the borderline of deficiency as in adults[47], the data in

the present study indicate severe public health problem for neonates in the locality.

Both cord blood VA and the vitamin A placental transport ratio are used to report the vitamin A status. They are different ways in reflecting the VA status between mothers and neonates. Cord blood VA seems to truly reflect the newborns’ vitamin A status. Placental transfer was calculated as the ratio of newborn to maternal serum concentration, which may be more actually reflect the transfer efficiency of vitamin A across the placenta compared with cord serum vitamin A level. In our study, the ratio was about 72% which was higher than that the 50% of another investigation[69]. The placental transport efficiency indicates fetoplacental signaling of fetal nutrient demand[69], which corresponds to the high VAD and MVAD prevalence in the locality. In the same cohort, we found that when the maternal VA levels varied in a large range, the neonatal VA levels maintained in a relatively small scope (data not shown), which indicated that placenta plays an important role in regulating VA transportation from mothers to offspring. To our knowledge, there was no report that compared these two ways in evaluating neonatal vitamin A status. More studies are needed to assess which is the preferred measure for analyzing the relationship between vitamin A status and child development.

It is well known that vitamin A and its derivatives, the retinoids, play important roles in the brain development and in the adult central nervous system [50–52]. More recently, it has been reported that VA is involved in adult brain function, especially for learning and memory[53–55]. Our previous studies revealed that MVAD beginning from pregnancy impaired learning and memory in offspring rats, and the impairment could not be fully reversible by vitamin A supplementation after birth or even later [56,57]. The data suggest that in rodent models, VAD can be targeted to later gestational windows and documents the need of VA for more advanced stages of development. Even partial VAD affects the sensitive developing CNS, especially during the critical period of hippocampus development.

Epidemiologic studies about the effects of vitamin A status on neuronal function and vitamin A supplementation on improving cognition have mostly focused on adults, the aged or the postnatal[58–60]. Humphrey JH, et al [61] pointed out that neonatal vitamin A supplementation had beneficial effects on all developmental scores evaluated with Bayley Scales of Infant Development (BSID) over the first three years of life. Kumar MV, et al [62] showed that multiple micronutrient fortified salt including vitamin A is effective in improving cognition in children. However, contradictory results were observed in some intervention trials. No impact of weekly vitamin A supplementation during gestation on mental and psychomotor development was observed in Indonesian infants at the age of 6 or 12 months[63].

Despite the above reports, few studies on the influence of vitamin A status at perinatal period and subsequent neurodevelopment in later childhood had been carried out. In the present study, multiple regression analysis showed that increased VA-PTR was associated with higher motor area and average DQs, and cord blood VA level was positively associated with language area and social area DQs. Children with higher cord blood VA showed higher scores of adaptive and average domain compared to children with lower cord VA. It is widely accepted that the brain grows rapidly during the last third of gestation and the early postnatal stage, which makes it vulnerable to an inadequate diet[58]. Our data indicated that VA status was beneficial for the brain development during this critical period. More studies are needed to provide evidence or definitive explanation about the association between cord retinol status and neurodevelopment of children at early life.

In addition to vitamin A, there are other two important antioxidant vitamins, vitamin E and vitamin C. Alpha-tocotrienol may be neuroprotective by antioxidant-independent as well as antioxidant-dependent mechanisms[64]. It is reported that VE and VC are associated with cognitive function[65–67]. The data in the present study showed that there was no significant association between cord VE level, VC level, VE-PTR or VC-PTR and GDS. One possible explanation was the relatively normal cord VE and VC levels compared with VA level, which might cover the influence of the two antioxidant vitamins. Further study is needed to investigate the relationship between perinatal VE and VC levels and neurodevelopment in children.

Moreover, we found that cord VA level was significantly positively associated with birth head circumference, and VA-PTR was significantly associated with birth weight, which was in agreement with the results of other studies[68]. A previous study reported that head circumference measurement and head growth evaluation constitute the simplest, most inexpensive and easily administered tools to assess the development of the central nervous system and to identify neonates at risk of neurodevelopmental disorders[69].
There are other demographic factors reported to be associated with children’s neurodevelopment, such as maternal education level [70]. Our data indicated that maternal education level was associated with children’s language and average DQ.

One of the limitations of the present study is that some unknown confounding factors were not adjusted, such as family income, information about the caregivers, etc. In the personal interview, we collected the income information. However, we did not include the family income variable in our model due to cultural reasons that people in China usually misrepresent their actual income when being asked, as indicated in our previous studies and other studies. Another limitation is the relatively small sample size, which might limit the precision to detect the relationships. If follow-up of the present cohort could be conducted for a few years, there may be more evidence to confirm the present results. Finally, we did not have data on the dietary intake of nutrients by the 24-h recall questionnaires which lead to insufficiently evaluate the effect of maternal and children’s dietary habit on their nutrient status and in turn the children’s cognitive and behavioral development.

In conclusion, the present study was the first to investigate the relationship between antioxidant vitamins, especially VA status, and neurodevelopment in children at two years of age. The data provide evidence that decreased serum VA concentration at birth and the VA-PTR would be associated with adverse birth outcomes and impaired neurodevelopment in later childhood. Continued follow-up of the present cohort could observe the long-term implication on school performance.

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Conflict of interest

The authors do not have any possible conflicts of interest.

References


56. Mao CT, Li TY, Liu YX, Qu P. (2005) Effects of marginal vitamin A deficiency and intervention on


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