Maternal–cord blood vitamin C status and its relation to fetal growth and placental apoptosis

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Abstract Background: Although the antioxidant property of vitamin C as well as its endothelial function promotion are well documented, its role in fetal growth during pregnancy is still not conclusive in previous studies.

Objectives: This study aimed to estimate maternal and cord blood vitamin C level and to detect its influence on neonatal growth as well as placental weight and placental apoptosis.

Methods: The study was conducted on 60 healthy singleton pregnant women and their full term neonates at Ain Shams University Hospital in Egypt. Maternal and cord blood vitamin C plasma level estimation as well as quantitative analysis of placental apoptotic index were done in addition to full anthropometric assessment for delivered neonates.

Results: There was a positive significant correlation between maternal and neonatal vitamin C levels ($r = 0.838$, $P < 0.001$). Positive significant correlations between maternal vitamin C levels and neonatal weight ($r = 0.448$, $P < 0.001$), length ($r = 0.67$, $P < 0.001$), BMI ($r = 0.52$, $P = 0.003$), OFC ($r = 0.60$, $P < 0.001$) and placental weight ($r = 0.373$, $P < 0.001$) while a significant negative correlation with placental apoptotic index ($r = -0.817$, $P < 0.001$) were detected. Multiple regression analysis showed that placental weight was the most sensitive predictor of neonatal weight ($t = 4.132$, $P < 0.001$) followed by maternal vitamin C ($t = 3.034$, $P = 0.006$).

Conclusion: Maternal vitamin C level has a significant positive impact on neonatal anthropometry and placental weight while negatively correlating with placental apoptosis. This denotes an important role of vitamin C in fetal and placental growth during pregnancy.

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fetal genome. In late pregnancy; environmental, nutritional, and hormonal influences become increasingly important. Low birth weight significantly contributes to mortality and morbidity in babies both immediately after birth and during the early childhood years. Poor maternal nutrition has been implicated as one of the key “adverse environmental influences in utero,” which could lead to compromised placental and fetal growth and adverse long-term consequences.

In addition, pregnancy is a state of metabolic challenge. Even a normal, healthy pregnancy is a state of oxidative stress compared with non-pregnancy, and is a challenge to be met by the mother and her developing baby. Due to its high metabolic rate and level of mitochondrial activity, the placenta is a key source of this oxidative stress.

Oxygen free radicals have been implicated in the etiology of premature delivery, fetal growth restriction, eclampsia, maternal infections and maternal malnutrition. Risk may, however, depend on the mother’s antioxidant status which potentially protects the maternal–fetal unit, thus increasing intrauterine growth and infant weight at birth. Accordingly, poor dietary intake of antioxidant nutrients or low serum level may particularly play an important role in the pathophysiology of low birth weight. Therefore, antioxidant vitamins, such as vitamins C and E, may play a role in fetal growth.

Vitamin C or ascorbic acid is a hydro-soluble lactone (synthesized from glucose) that is essential to the human body for several functions. Unlike many other animal species, humans and primates lack the terminal enzyme in the biosynthetic pathway for ascorbic acid synthesis, so diet is crucial for its availability in these organisms. Vitamin C counteracts several hydroxyl radicals and may contribute in protecting the fetus from oxygen free radical damage. It is worth noting that vitamin C is involved in the regeneration of oxidized vitamin E. Also vitamin C promotes endothelial cell proliferation during the inflammatory condition, inhibits endothelial cell apoptosis and lipid peroxide formation, and improves endothelial function.

The aim of this study was to estimate maternal plasma vitamin C level & to find its relation with cord blood level of vitamin C in their off-springs and to detect its influence on placental growth, apoptosis as well as neonatal anthropometry; in normal full term uncomplicated deliveries.

Materials and methods

This cross sectional study was conducted on 60 pregnant women admitted for delivery and their newborns at Maternity Hospital, Ain Shams University, Cairo, Egypt in the period between July 2012 and June 2013.

Inclusion criteria:

- All cases were healthy, booked pregnant women with regular antenatal care.
- Singleton gestation.
- Absence of significant adverse obstetric history including history of previous abortion, intrauterine fetal death, unexplained neonatal deaths or previous off-spring with chromosomal or congenital anomalies.
- Absence of maternal medications except for iron and folic acid intake, routinely given as per antenatal management protocol.
- Maternal weight, height and calculated body mass index (BMI), falling between 5th and 95th centiles, on growth charts.
- Normal antenatal investigations including complete blood count (Hb% > 12 gm/dl), kidney, liver function tests and negative serology for Rubella, Toxoplasma and Cytomegalovirus.
- All tested babies had antenatal normal biophysical profile and ultrasound serially performed, using MEDI-SON SONAGE 8800 with trans abdominal 3.5 MHz probe, indicating fetal well-being and absence of congenital anomalies.

Exclusion criteria:

- Any maternal disease that could affect either vitamin C level or feto-placental growth like preeclampsia, diabetes, kidney, liver, heart disease, sepsis, sickle cell disease or genital infections.
- Maternal smoking, alcohol or drug abuse.
- Premature deliveries.

Informed consents were obtained from all subjects of the study. The research followed the tenets of the declaration of Helsinki and was approved by hospital ethics committee.

All pregnant women were subjected to

- Full history taking laying stress on their antenatal history, vitamin and mineral supplementation, dietetic history with 24 h recall of feeding, socio-economic status using Park and Park score and drug history.
- Maternal weight and height measurements with calculation of their BMI (kg/m²).
- Neonatal examination including Ballard scoring for gestational age assessment. Apgar scoring as well as full anthropometric measures including weight, length, occipitofrontal circumference (OFC), left mid arm circumference (MAC) and abdominal girth. Anthropometric measures were performed on the same day of delivery using standardized equipments and were plotted on growth charts. Further, calculation of neonatal BMI was done. Babies were classified according to percentiles of weight for GA into appropriate for gestational age (AGA); lies between the 10th and 90th percentiles, small for gestational age (SGA); < 10th percentile and large for gestational age (LGA); > 90th percentile.
- Weighing the placenta and assay of placental apoptosis assay.
- Plasma vitamin C assay for both the mother and their babies.

Sampling procedure

Blood samples were collected from the mothers just before delivery and from cord blood immediately after delivery in lithium heparin collecting tubes and centrifuged for 30 min for plasma separation waiting for vitamin C quantitative analysis within 24 h of sample collection, using the colorimetric technique.
Principle of vitamin C assay

In serum and plasma, vitamin C is found as ascorbic acid as well as its oxidized form, dehydro-ascorbate. Both forms are biologically active. In vitamin C assay, an oxidation is induced prior to analysis so that both forms are measured. A dose response curve of the absorbance unit (optical density, OD at 492 nm) vs. concentration is generated, using the values obtained from the standard. The concentration of the patient sample is determined directly from the linear standard curve. Normal range: 4–15 mg/l.13

Placental apoptosis assay

Placental samples, 2 cm × 2 cm, were collected from the basal plate of the immediately delivered placentas and samples were stored in formalin 10% for pathological examination and quantitative analysis of apoptotic index by determining the ratio of apoptotic nuclei to the total number of nuclei in ten high power fields for assessment of each specimen.14

Statistical analysis

Data were analyzed using Statistical Program for Social Science (SPSS) version 18.0. Quantitative data were expressed as mean ± standard deviation (SD). Qualitative data were expressed as frequency and percentage (%). One-way analysis of variance (ANOVA) when comparing between more than two means. One-Way ANOVA Post Hoc test (Bonferroni) to determine which means differ. Pearson’s correlation coefficient (r) test was used for correlating data. Multiple Linear Regression Analysis by creating a statistical model that shows the relationship between the dependent variable and the explanatory factors. P-value <0.05 was considered significant.

Results

Descriptive data of the enrolled mothers and their offspring are shown in Tables 1 and 2. Forty-two (70%) of babies were AGA, 8 (13.3%) were LGA while 10 (16.6%) were SGA.

In this work, vitamin C levels in both maternal sera and cord blood of involved neonates were within normal levels (The mean value of maternal vitamin C was 7.29 ± 2.56 mg/l while in cord blood it was 13.05 ± 3.70 mg/l).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Descriptive data of enrolled mothers.</th>
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<tbody>
<tr>
<td>Parameters</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Maternal age (years)</td>
<td>28.44(4.81)</td>
</tr>
<tr>
<td>Maternal weight (kg)</td>
<td>90.66(13.31)</td>
</tr>
<tr>
<td>Maternal height (cm)</td>
<td>161.43(2.25)</td>
</tr>
<tr>
<td>Maternal BMI (kg/m²)</td>
<td>34.67(5.01)</td>
</tr>
<tr>
<td>Weeks of gestation (wks)</td>
<td>39.23(1.33)</td>
</tr>
<tr>
<td>Parity</td>
<td>1.43(1.38)</td>
</tr>
<tr>
<td>Gravidity</td>
<td>2.73(1.76)</td>
</tr>
<tr>
<td>Social status (Park &amp; Park score)</td>
<td>14.03(3.91)</td>
</tr>
<tr>
<td>Plasma vitamin C (mg/l)</td>
<td>7.29(2.56)</td>
</tr>
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<td>BMI: body mass index.</td>
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</table>

A positive significant correlation between vitamin C levels of mothers and cord blood vitamin C levels was detected (Fig. 1). In multiple regression analysis, vitamin C level of mothers was the most sensitive predictor of vitamin C level of the neonate (t = 2.866, P = 0.008). Other independent factors, including placental apoptotic index (t = 0.273, p = 0.787), placental weight (t = 0.329, p = 0.745), gestational age (t = −0.091, p = 0.929) and neonatal weight (t = 1.322, p = 0.198) were not significant predictors.

Also, positive significant correlations between maternal vitamin C levels and neonatal weight (r = 0.448 & p < 0.001) (Fig. 2), length (r = 0.67, P < 0.001), BMI (r = 0.52, P = 0.003) and OFC (r = 0.60, P < 0.001) as well as placental weight were detected (r = 0.373 & p < 0.001) (Fig. 3). Moreover, a negative significant correlation was found between maternal vitamin C level and placental apoptotic index (r = −0.817 & p < 0.001) (Fig. 4). Further, placental apoptotic index had a negative significant correlation with neonatal weight (r = −0.460, P = 0.011), BMI (r = −0.528, P = 0.003) as well as with placental weight (r = −0.577, P = 0.001).

On comparing between the three groups of neonates; AGA, SGA and LGA, we found that maternal weight and BMI were significantly higher among the LGA group compared to AGA and SGA groups. Also, the LGA group had significantly higher placental weight and lower apoptotic index compared to both AGA and SGA. Significantly higher values of both maternal and neonatal plasma vitamin C were shown in the LGA group compared to SGA and in the AGA group compared to SGA (Table 3).

Further, maternal BMI had significant positive correlations with neonatal weight (r = 0.458, P = 0.011) and neonatal BMI (r = 0.484, P = 0.007) while, maternal age had no significant correlations with neonatal anthropometry, placental weight, apoptotic index or vitamin C levels of mothers and babies.

Multiple regression analysis showed that placental weight was the most sensitive predictor of neonatal weight (t = 4.132, P < 0.000) followed by maternal vitamin C (t = 3.034, P = 0.006). Maternal BMI (t = 2.005, P = 0.056), placental apoptotic index (t = −0.052, p = 0.959) and gestational age (t = −1.801, P = 0.084) were not significant predictors of neonatal weight.

<table>
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<th>Table 2</th>
<th>Descriptive data of delivered neonates and placentas.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Neonatal sex</td>
<td>Male 26(43.3) Female 34(56.6)</td>
</tr>
<tr>
<td>BALLARD score (wks.)</td>
<td>38.86(0.86)</td>
</tr>
<tr>
<td>Neonatal weight (kg)</td>
<td>3.25(0.68)</td>
</tr>
<tr>
<td>Neonatal length (cm)</td>
<td>49.83(2.36)</td>
</tr>
<tr>
<td>Neonatal head circumference (cm)</td>
<td>34.88(1.28)</td>
</tr>
<tr>
<td>Neonatal BMI (kg/m²)</td>
<td>12.94(1.85)</td>
</tr>
<tr>
<td>Neonatal left MAC (cm)</td>
<td>11.71(1.19)</td>
</tr>
<tr>
<td>Neonatal Abdominal girth (cm)</td>
<td>31.50(2.98)</td>
</tr>
<tr>
<td>Placental weight (gm.)</td>
<td>628.33(77.32)</td>
</tr>
<tr>
<td>Cord blood vitamin C (mg/l)</td>
<td>13.05(3.70)</td>
</tr>
<tr>
<td>BMI: body mass index, MAC: mid arm circumference. #: number(%).</td>
<td></td>
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</table>
Discussion

In the current work, vitamin C level of cord blood was higher than its level in maternal plasma. This result corroborates with previous studies that found similar findings, they explained their results by that placenta is permeable to dehydroascorbic acid but not to ascorbic acid then the fetus converts dehydroascorbic acid to ascorbic acid and accumulates it.\textsuperscript{15,16} Also increased vitamin C levels in cord blood could be due to fetal synthesis of vitamin as dehydroascorbic acid, the oxidized form of ascorbic acid, that is reduced to L-ascorbic acid by fetal erythrocyes that gets trapped in the fetal circulation.\textsuperscript{17}

Figure 1  A scattered graph showing a positive significant correlation between neonatal plasma vitamin C levels and maternal plasma vitamin C levels ($r = 0.838$ & $p < 0.001$).

Figure 2  A scattered graph showing a positive significant correlation between maternal vitamin C levels and neonatal weight. ($r = 0.448$ & $p < 0.001$).
In our study a positive significant correlation between vitamin C levels of mothers and cord blood vitamin C levels was detected. In addition, vitamin C level of mothers was the most sensitive predictor of vitamin C level of the neonate by multiple regression analysis. These results were consistent with a previous study that suggested that maternal vitamin C intake can influence fetal vitamin C levels.\(^1\)

In this work, forty-two (70%) of babies were AGA, 8 (13.3%) were LGA while 10 (16.6%) were SGA. Though the normal population distribution is 10% SGA, 80% AGA and 10% LGA\(^1\) the higher SGA proportion in our study may be attributed to small sample size and difference in ethnic population also, it may be explained by other studies that reported approximately 10 percent of term infants in developed

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**Figure 3** A scattered graph showing a positive significant correlation between maternal vitamin C levels and placental weight. \((r = 0.373 & p < 0.001)\).

**Figure 4** A scattered graph showing a negative significant correlation between maternal vitamin C levels and placental apoptotic index \((r = -0.817 & p < 0.001)\).
countries are SGA, compared with 23 percent of term infants in developing countries. Moreover, a recent study showed that 26.8% of males and 22.4% of females were born term but SGA in low- and middle-income countries.

Comparison between AGA, LGA and SGA neonates of our study revealed significant higher values of both maternal and neonatal vitamin C in LGA compared to SGA and in AGA compared to SGA. The effect of vitamin C on neonatal anthropology was further highlighted by finding positive significant correlations between maternal vitamin C level and all neonatal anthropology as well as placental weight and negative correlation with placental apoptotic index. Similarly, Jain et al. found a significant positive relationship between newborn birth weight and maternal plasma vitamin C levels in addition to significant positive relationship between newborn birth weights and newborn vitamin C levels. Another study found that maternal serum vitamin C levels during the second trimester were positively correlated with birth weight and length in full-term babies. They thought that there was probably not a large change in vitamin C levels between the second trimester and the term, because it might not be reasonable to believe that lifestyle, such as dietary pattern, changes profusely during pregnancy. In contrast, Wang et al. studied the effect of maternal antioxidant vitamins on birth weight and found a positive significant effect of vitamin A but not E and C.

Likewise, the current study showed a significant positive influence of maternal vitamin C level on placental weight and a significant negative influence on placental apoptotic index. Similarly, a previous study showed a significant lower trophoblast expression for the endothelial scavenger receptor low-density lipoprotein receptor-1 (LOX-1) and the apoptotic index in normal full-term pregnancy in women with higher levels of prenatal vitamin C, also, Hung et al. concluded that concomitant administration of 50 mM of vitamins C and E was observed to reduce apoptotic and autophagic changes in the trophoblast layer of placenta at normoxia.

Placental apoptosis is likely to be the underlying pathological mechanism affecting fetal growth. Our study showed negative significant correlations between placental apoptotic index and neonatal weight, BMI as well as placental weight. Moreover, placental weight was the most sensitive predictor of neonatal weight by multiple regression analysis followed by maternal vitamin C. Similarly, a recent study reported that placental weight is an important determinant of both birth weight and fetal growth. Another study reported that apoptosis is an essential feature of normal

<table>
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<tr>
<th>Table 3</th>
<th>Comparison between AGA, SGA and LGA neonates regarding maternal BMI, placental weight and apoptosis and vitamin C levels.</th>
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<tbody>
<tr>
<td></td>
<td>SGA (n = 10)</td>
</tr>
<tr>
<td>Maternal BMI (kg/m²)</td>
<td>32.96 ± 5.0</td>
</tr>
<tr>
<td>Placental weight (gm)</td>
<td>541.61 ± 37.60</td>
</tr>
<tr>
<td>Placental apoptotic index</td>
<td>0.38 ± 0.13</td>
</tr>
<tr>
<td>Maternal plasma vitamin C (mg/l)</td>
<td>4.85 ± 0.50</td>
</tr>
<tr>
<td>Neonatal plasma vitamin C (mg/l)</td>
<td>8.90 ± 0.71</td>
</tr>
</tbody>
</table>

SGA = small for gestational age. AGA = Appropriate for gestational age. LGA = Large for gestational age. GA = Gestational age. BMI: body mass index.

* Bonferroni.
placental development but is exaggerated in association with placental disease, they stated that widespread apoptosis of the syncytiotrophoblast may impair trophoblast function leading to the reduction in nutrient transport seen in IUGR.\(^2\)\(^3\)

In the current study, maternal BMI positively influenced neonatal weight and BMI. This corroborates with other studies that found a progressive increase in the mean infant birth weight with increased maternal BMI. They stated that increased BMI predisposes to development of gestational hypertension and infant macrosomia. Also, the tendency for an individual to give birth to a macrosomic infant may suggest a genetic element; while mothers with low BMI had babies with the lowest birth weight in their population.\(^2\)\(^4\)\(^5\)\(^1\)

In this work, maternal age had no significant correlation with neonatal anthropometry, placental weight and apoptosis or vitamin C levels of mothers and babies. This was in agreement with Acharya et al. who concluded that maternal age had no significant association with IUGR.\(^2\)\(^6\) Also Hayward et al. found no differences in placental weight between adult and teenage pregnancies.\(^2\)\(^7\) Other studies showed conflicting results with a positive association between increasing maternal age and increasing risk for IUGR\(^2\)\(^8\) while, another study revealed that placentas from older mothers had a lower incidence of apoptosis compared with those from younger mothers.\(^2\)\(^9\) Regarding the relation between maternal age and vitamin C levels, similar to our results Kiondo et al. found no association between maternal age and vitamin C levels.\(^3\)\(^0\)

**Conclusion**

Maternal vitamin C level has a significant positive impact on neonatal anthropometry, placental weight and apoptosis correlating with placental apoptosis. This denotes an important role of vitamin C during pregnancy. Further longitudinal studies, preferably randomized controlled trials to evaluate different effects of vitamin C supplementation during pregnancy are warranted.

**Conflict of interest**

We have no conflict of interest to declare.

**Acknowledgement**

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**References**


