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Low vitamin C status in umbilical cords is associated with increased risk of neonatal complications in type 1 diabetic pregnancy: A cross-sectional Study

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Abstract

Vitamin C (vit C) is essential for normal fetal development. Vit C of the umbilical cords of the neonates has been related to the maternal vit C status in late pregnancy in a cohort of type 1 diabetic women. Hereby the common view of the fetus as a "parasite" on the mother´s vit C pool is questioned, thus the preferential fetal transport may be overridden by increased needs of the mother during situations of deficiency, thereby potentially influencing the health of the offspring.

Here, we perform analysis based on data of thirty-four blood samples from the umbilical cords (UC) of the fetus in relation to the fetal outcome in the same cohort.

Neonates with complications had a significantly lower UC vit C level than neonates without (mean (SD)): 85.7 μmol/L (26.45) vs 108.7 μmol/L (15.0), p=0.02, n=25 and 9 respectively and also in case of neonatal hypoglycemia (p=0.02), malformations (p=0.01), respiratory distress (p=0.0415), Apgar score (p=0.0025) and LGA (p=0.02) respectively compared to the neonates without complication. The UC vit C was found a predictor of neonatal complications (p=0.032; n=34) as also the Apgar score at one minute tended to, however not significantly (p=0.073). The UC vit C correlated per se positively with the Apgar score (p= 0.009, n=34). No relations of UC vit C were observed in relation to the gestation age, sex of the neonate, diabetic microangiopathy or smoking habits of the women. No relations between maternal vit C or HbA1c in late pregnancy and fetal outcome were found.

In this study low vit C level of the fetus indicate an increased risk of neonatal complications.

Introduction

We have earlier reported data of maternal vitamin C (vit C) from a cohort of pregnant type 1 diabetic women; in comparison with healthy pregnancies [1]; in relation to the glycemic control during pregnancy [2].), and in relation to obstetrical complications of pregnancy [3]. In the latter study we observed, that the newborns of mothers with low maternal vit C at terme seem not to be able to obtain the same level of vit C in the UC as newborns of mothers with a higher vit C level despite the observed higher UC vit C/maternal vit C ratio; thus, a preferential supply seems not fully to be able to compensate for poor maternal vit C status.

This relation between poor maternal vit C status and the obtained vit C status in the fetus of the pregnant type 1 diabetic women was an unexpected finding in relation to the prevailing view [4], although in line with experimental data from guinea pigs showing that the preferential fetal transport as normally found [5,6] may be overridden by increased needs of the mother during situations of deficiency, thereby potentially influencing the health of the offspring [7,8].

In the present study, we report from the UC vit C levels in relation to neonatal outcome based on data from the thirty-four women with umbilical blood samples taken from their newborns obtained as a measure of the level of vit C of the fetus from the same cohort [1].

Materials and methods

All T1DM women from June 1992 to August 1994 attending the Department of Obstetrics, Aarhus University Hospital (Aarhus, Denmark), were screened for participation in the prospective study on vit C during pregnancy and compared to controls as described previously [1]. The inclusion criteria were pregestational T1DM, age >18 years, no other systemic disease than diabetes, and singleton pregnancy. Blood samples for vit C were taken when the diabetic women attended the maternity ward and were taken in a non-fasting state to avoid hypoglycemic episodes. In total, 76 women with T1DM consented to participate in the prospective study from whom thirty-four blood samples from the umbilical cords also were obtained as a surrogate measure of the level of vit C of the fetus [1]. In the present study, we studied the umbilical cords vit C levels in relation to neonatal outcome. Blood samples for vit C measurements were stabilized in sodium EDTA-anticoagulated vacutainer tubes containing dithiothreitol. Tubes were centrifuged and plasma was removed and deproteinized by the addition of 6% perchloric acid. The samples were kept at minus 80 degrees Celsius until analysis and assayed by HPLC using 3,4-dihydroxybenzylamine hydrobromide as internal standard [9]. A plot of the ratio of vit C to internal standard versus the concentration of six aqueous standards resulted in a linear curve to at least 86 μmol/L (y=0.16x – 0.028, R2=0.99). The within-day and between-day coefficient of variation was 2.6% and 3.9%, respectively,

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of a mean concentration of 19 µmol/L. Limit of detection and limit of quantification were 0.525 µmol/L and 1.75 µmol/L. The analytical recoveries were 111%, 104%, 102%, and 101% at vit C concentrations of 5.75, 28.75, 43.125 and 57.5 µmol/L respectively.

The following data were recorded: Age, duration of diabetes, presence of diabetic microangiopathy, glycemic control, diurnal blood pressure, albumin excretion rate, creatinine, creatinine clearance, pregnancy and labor data, and the neonate's Apgar score at one minute, birth weight, and presence of malformations and the neonatal complications. Neonatal complications were defined as respiratory distress, hypoglycemia, hyperbilirubinaemia, neonatal septicemia, indications of prenatal growth rate; large for gestational age (LGA) (macrosomia) and small for gestational age (SGA) respectively than normal for the gestational age defined as a weight above the 90th or below the 10th percentile for the gestational age. Prematurity were considered an obstetrical complication and not per se regarded as a complication of the neonates. The study was part of an evaluation of morbidity in diabetic pregnancy with respect to nephropathy and retinopathy approved by the local Ethics Committee (jr.nr.1992/2523, 1998/4147, and 2026-99). It was performed in concordance with the Helsinki II declaration and all women had given their informed consent. The collection of samples for vit C was approved by the local Ethics Committee (jr.nr. 1992/2328). Furthermore, and approved by the local Ethical Committee, the pregnant women were not informed about the focus of the study on their vit C levels during pregnancy to avoid the women to start vit C supplementation by themselves that could biaz the results. They were informed that we wanted to measure tracers during pregnancy and in their babies. Hypovitaminosis C was defined as a plasma vit C <23 µmol/L.

Statistics was performed with SigmaPlot 12; a Systat software. Difference between two means was tested with Student's t-test if data followed Gaussian distribution; otherwise, Mann-Whitney's test was used. Values are given as mean ± SD if not otherwise stated. Median (25%–75% interval) indicates variable of non-Gaussian distribution and values were subjected to non-parametric testing. Multiple linear and logistic regressions were used as predictive analysis of qualitative and quantitative data respectively. A two-sided $p<0.05$ was chosen as level of significance.

Results

Clinical data from the pregnant diabetic women from whom thirty-four blood samples from the umbilical cords were also obtained as a measure of the level of vit C of the fetus are shown in Table 1. The range (0%–100%) of umbilical cords vit C in the cohort was 19.7 µmol/L–130.4 µmol/L.

Results regarding fetal related features and the type and distribution of complications in the neonates are shown in Tables 2 and 3 respectively.

Multiple regression analysis showed the level of UC vit C as a predictor of Apgar score (r= 0.45, p=0.009, n=34) and neonatal complications (p=0.032; n=34). The level of UC vit C in the group of neonates without neonatal complication was found significantly higher compared to the UC vit C levels in the group of neonates with neonatal complications (p=0.02) and to the subgroups of neonates characterized of hypoglycemia (p=0.02), LGA (p=0.04), malformations (p=0.01), respiratory distress(p=0.042) and Apgar score ≤ 7 (p=0.0025) respectively; see Table 3.

The obtained Apgar score of the newborn at one minute tended to be a predictor of neonatal complications, however not significantly (p=0.073).

Table 1. Maternal data and characteristics of the study population. Values are reported as mean (SD) for normally distributed quantitative data, as median (5%-95%) indicating non-Gaussian distribution or as frequencies for qualitative data.

Variable	N	Value
Age (yr)	34	27.8 (4.2)
Diabetes duration (yr)	34	11.0 (1.0-34.5)
Parity (n)	34	2.0 (1.0-3.3)
Systolic blood pressure at entry (mmHg)	28	119.5 (10.1)
Diastolic blood pressure at entry (mmHg)	28	70.8 (7.2)
Retinopathy (n=Non/Simplex/Proliferative)	34	19/12/3
BMI at delivery (kg/m²)	31	28.6 (4.3)
Normo-/Micro- or Macroalbuminuri (n/n)	34	29/5
HbA1c at delivery (%)	34	6.9 (1.0)
Creatinine clearance at delivery (ml/min)	21	109.0 (36.5)
Vit C in late pregnancy (µmol/L)	32	30.1 (13.1)
Obstetrical complications of pregnancy (n=yes/no) [3]	28	15/13
Smoker (n=yes/no/unknown)	34	13/30/1

Table 2. Results regarding foetal related features

Variable	N	Value
Gestations age (weeks)*	34	38.0 (33.3-38.0)
Neonatal complication (n=yes/no)	34	25/9
Delivery (n=induced, section (elective/ acute)	34	0/14/20 (15/5)
Birth weight of newborn (gram)	34	3734.6 (806.5)
Birth weight ratio (ratio)	34	1.2 (0.25)
Apgar score at one minute (between 1-10)	34	9.0 (3.1-10)
Sex of neonate (n=male/female)	34	17/17
Vit C of UC (µmol/L)	34	91.6 (25.7)
Umbilical cord/maternal vit C ratio	32	3.0 (1.3-11.0)

• *Six neonates were born premature born < 37 gestation week (UC level 91.3 (24.7) µmol/L. Prematurity were considered an obstetrical complication. Data are listed as mean (SD), median (5%–95%) or n-values.

Table 3. The type and distribution of complications in the neonates of T1DM women (*n*=34). Recorded neonatal complications[2] were small and large for gestation age; SGA and LGA respectively, hyperbilirubinamia, hypoglycemia, respiratory distress, malformation, Apgar score at one minute ≤ 7, acute section, septicemia and others

Complications	N	UC Vit C µmol/L
Foetus with and without neonatal complications	34	91.43 (25.9)
Foetus with neonatal complications	25	85.7 (26.5)*
Foetus without neonatal complication	9	108.7 (14.95)
[1]Foetal malformation	4	69.6 (33.9)*
Foetal hypoglycemia	14	86.3 (24.2)*
Foetal hyperbilirubinamia	14	98.3 (22.4)
Acute section	5	96.0 (19.95)
Large for gestation age LGA	16	86.3 (28.2)*
Apgar score ≤ 7 at one minute	6	69.0 (25.9)**
Respiratory distress	7	89.2 (19.6)*

[1] The fetal malformations consisted of two neonates with cardiac malformations with transposition and atrium septum defect and two others were related to skeletal abnormalities; [2] Neonates may have more than one complication; thus we also recorded one neonate with clavicle fracture (UC level 81.7 µmol/L) one neonate with sepsis (UC level 124.2 µmol/L 7), 2 neonates with hypocalcamia (UC level 100.1 µmol/L), one neonate with anemia (66.3 µmol/L), 1 neonate small for gestation age SGA (UC level 75.6) and one neonate with polycytamia (UC level 92.0 µmol/L) among the 25 neonates with neonatal complications.

No relationship between the way of delivery (acute cesarean section, elective cesarean and induced delivery; 5/15/14) and umbilical cord vit C status was observed. Nor were any relations of UC vit C in relation to the gestation age, sex of the neonate or the presence or not of diabetic microangiopathy or smoking habits of the women observed. We did not find any relation between maternal vit C or HbA1c in late pregnancy and fetal outcome.

Discussion

For decades it has been the general believe that the foetus is protected from vit C deficiency at the expense of the mother [5-6]; the fetus acts as a "parasite" in normal pregnancies as described by Teel et al already in 1938 [4]. The result of the present study, however, challenge the general believe that the maternal vit C status is an irrelevant consideration in relation to fetal outcome by virtue of the finding of the level UC vit C as a predictor of Apgar score (p=0.009, n=34) and neonatal complications (p=0.032; n=34). We have earlier presented data from the present cohort of T1 diabetic women that indicates that the pregnancies are associated with a significantly higher gradually decline of maternal vit C from 1st to 3rd trimester compared to control pregnancies [1] and nearly the half of the women with obstetrical complications were characterized of having hypovitaminosis C in late pregnancy that may limit the obtainable level of UC vit C in the foetus [3]. The reason for this extra increased draw on the mothers vit C in the diabetic group is not clear but might be on basis of an increased hyperglycemia- induced oxidative stress [10] as also found in the placenta in T1DM [11-13] leading to an increased maternal turnover of vit C. In accordance we have earlier reported the glycemic control during pregnancy as a predictor of vit C in late pregnancy in the present cohort [2]. In case of normal pregnancy, vit C is thought to be actively transported to the foetus by the sodium-dependent vitamin C transporter 2 (SVCT2) [14]. In agreement, in vivo studies in knockout mice devoid of SVCT2 show that offspring die immediately after birth [15-16]. Consequently, this in vivo experimental evidence suggests that vit C transplacental transport is primarly governed by SVCT2 and thus constitutes the primary means of foetal vit C supply (17-18) and on the expense of the maternal vit C status. In accordance, a study in T1DM of 57 placentas from a DAPIT sub-cohort of deliveries in women with T1DM could not demonstrate any difference regarding vit C mean levels in umbilical cords or placenta, regardless if the women had been vit C supplemented or not during pregnancy [19].

However the results from the present study contradict the postulation that a foetus is protected from vit C deficiency by the SVCT2 transporter in case of diabetes and as also found in pregnant smokers and their respective umbilical cords compared to non-smokers [20] and in vivo studies in non-diabetic guinea pigs [7-8] and in the (SVCT1) line knockout mice [21]. Taken together the placental transport of vit C essential for foetal survival still need to be resolved and is probably not solely mediated by the SVCT2 transporter. You could speculate if a GLUT 1 mediated placental transport of vit C as dehydroascorbic acid (the oxidized form of vit C; DHA) by glucose transporters (GLUT 1) yet is a physiologically relevant alternative in the placenta [14] as GLUT 1 transporters also are present in the human placenta [22-25]. Thus transplacental vit C transport could be compromised in diabetic hyperglycaemia through competitive inhibition of DHA transport with consequences for the resultant foetal vit C level; a level of UC vit C that we found a significant predictor of foetal outcome and on the other hand in case of the presence of hypoglycaemia, LGA (macrosomia), Apgar score below 7, respiratory distress and malformation in the neonates we also found the UC vit C levels significantly lower.

As hyperglycemia is well known involved in adverse pregnancy outcome [26], theoretically the presented results could be connected as follows; maternal hyperglycemia leads partly to postnatal neonatal hypoglycaemia because of the hyperglycaemic induced fetal hyperinsulinaemia and to macrosomia according to the Pedersen hypothesis [27], partly to low UC because of low maternal vit C in late pregnancy with possible consequences for normal foetal development [28]; thus vit C supplementation of the maternal diet in diabetic rats reduces the rate of malformation in the offspring [29]. Also, the strong positive correlation between UC vit C and Apgar score and relation to respiratory distress could relate the UC vit C level to the very common need for admission to neonatal care that is very frequent among diabetic neonates [30]. Although we did not find the presence of hyperbilirubinaemia related to the level of UC vit C, interestingly a recent placebo-controlled study found a significant effect of vit C intervention on reducing neonatal bilirubin levels in healthy pregnancies [31].

Limitations of the present study are obvious; the small number of participants and that the registration of neonatal complications of pregnancy was done retrospectively on the case report forms, which in some cases may be imprecise. However, no studies so far have been identified that have investigated UC vit C in relation to foetal outcome in the diabetic pregnancy. Further studies are needed to decide the relevance of the present findings, but if found true of important clinical impact.

Conclusions

In conclusion, the results from this small study of a pregnant T1DM cohort suggest, that the obtainable vit C of the foetus as measured by umbilical values in the newborn is associated to the obtained Apgar score and predictable of an increased risk of neonatal complications. Further investigations must disclose the possible clinical significance of vit C in relation to outcome of the diabetic especially in pregnancies characterized by low levels of vitamin C.

Author contributions

Bente Juhl designed and performed the experiments and wrote the paper.

Conflicts of interest

The author declares no conflict of interest.

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