ORIGINAL ARTICLE

Association of Maternal Vitamin C Status with Gestational Diabetes Mellitus

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Abstract:

Background: The role of antioxidants in the etiology of gestational diabetes mellitus (GDM) has been given limited attention. Vitamin C is a nutrient with radical quenching property and has been claimed to influence glucose tolerance. *Aim:* To study the association between vitamin C status (dietary intake and plasma concentrations) and GDM. *Material and Methods:* Using a case-control design with 1:3 ratio we examined 42 pregnant women with GDM and 158 normal glucose tolerant (NGT) gestational age-matched healthy pregnant women at an average of 26 weeks of gestation. Maternal vitamin C intake was determined using detailed semi food frequency questionnaire (SFFQ) and 24 hour diet recall. Plasma vitamin C was determined using a spectrophotometric method in non-fasting samples. GDM was diagnosed by 75 gm oral glucose tolerance test (OGTT) using International Association for Diabetes in Pregnancy Study Group (IADPSG) criteria (fasting $\geqslant$ 92mg%, 1hour $\geqslant$ 180mg%, 2 hour $\geqslant$ 153mg %). *Results:* GDM women had lower median intake of vitamin C (35.0 mg/day vs. 66.7; $p<0.001$) and lower median plasma vitamin C concentration (45.9 µmol/L vs. 95.2; $p<0.001$) compared to NGT women. Plasma vitamin C concentration was inversely related to fasting, 1 hour and 2 hour post glucose plasma glucose concentrations ($p<0.001$). The associations remained significant after adjustment for age, income, pre-pregnancy BMI, and stress. *Conclusion:* Our findings suggest that low vitamin C intake as well as low plasma vitamin C concentration is associated with GDM. This association needs to be tested in a large prospective study and subsequently in a clinical trial.

Keywords: Antioxidant, GDM, Vitamin C

Introduction:

Gestational diabetes mellitus (GDM) is carbohydrate intolerance, first diagnosed during pregnancy [1]. India has a growing problem of GDM, with 10-20% of urban pregnancies diagnosed with GDM in hospital settings [2]. Diabetic pregnancies show an altered antioxidant profile [3] and glycated hemoglobin is inversely associated with reduced antioxidant enzymes [4]. The role of dietary antioxidants in the etiology of GDM has been given limited attention in India. It is possible that oxidative stress plays a role in this context.

Pregnancy is a period of increased metabolic demands, driven by changes in the woman's physiology and requirements of a growing fetus. During this time, inadequate stores or intake of micronutrients and associated oxidant stress can have adverse effects on the mother and the fetus.

Vitamin C is a micronutrient with radical quenching property and may influence glucose tolerance. We investigated vitamin C intake and circulating vitamin C concentrations in GDM and normal glucose tolerant (NGT) pregnancies to evaluate the relationship between vitamin C status and glucose metabolism during pregnancy in Indian subjects.

Material and Methods:

Setting and Subjects:

The study took place in two hospitals in Pune city (King Edward Memorial Hospital {KEM} and Gupte Hospital). Women who attended antenatal clinics between 1st May 2012 and 31st December 2012 and were within 24-28 weeks of a singleton gestation, and otherwise healthy were included (Fig.1). Those who were outside this window of 24-28 weeks of gestation or women with other medical conditions (thyroid disorders, epilepsy, hypertension, known diabetes), or those planning to deliver in other hospitals were excluded (Fig.1). Gestational age was derived from last menstrual period (LMP) and ultrasonography. The study was approved by the KEM Hospital Research Center Ethics Committee. Informed, written consent was obtained from all participants.

Information was collected through structured interview. The key words were translated into Marathi and Hindi, for ease of understanding. In addition to demographic information, risk factors of GDM were included in the questionnaire (i.e. age, family history of diabetes, history of being active/passive-smoker and bad obstetrics history, including abortion, polyhydraminous and previous history of GDM).

Anthropometry:

Pre-pregnancy weight was recorded based on recall. Weight and height were measured at the time of interview (~26 weeks of gestation), and weight was measured again at delivery to calculate weight gain. Weight was recorded to the nearest 0.1 kg (Tanita body analyzer model BC 601) and height to the nearest 0.1 cm (plastic tape). The average of two measurements was used for analysis.

Dietary Intake:

The conventional 24 hour diet recall method was modified and made more objective by incorporating information on portion sizes. In addition to 24-hour diet recall, a Semi Food Frequency Questionnaire (SFFQ) was administered to obtain frequency of consumption of Vitamin C rich foods during the preceding 12 month period on a six point scale ranging from "never" to "daily" [5]. The SFFQ included locally available rich sources of vitamin C. The amount of vitamin C in individual food sources was calculated using values for Indian foods [6] and the total amount for each participant was reported as daily vitamin C intake.

Biochemical Measures and Plasma Vitamin C Status:

A 75g oral glucose tolerance test (OGTT) was performed at an average of 26 weeks of gestation after an overnight fast. GDM was diagnosed according to International Association of Diabetes in Pregnancy Study Group criteria (Fasting $\geqslant$ 92mg%, 1 hour $\geqslant$ 180 mg%, 2 hours $\geqslant$ 153mg%) [7].

Maternal plasma vitamin C concentrations were measured on a non-fasting sample using the principle of spectrophotometry [8, 9]. This method has been reported to have a high degree of sensitivity, specificity and precision. Samples were protected from light and transferred within 1 hour to the laboratory where samples were centrifuged at 3600 RPM for 12 minutes at 4˚C [8]. Plasma was separated. Fresh 0.4 ml plasma was mixed with 1.6 ml of 10% Trichloroacetic acid (TCA) and spun again at 8000 RPM for 10 minutes. Pellet was discarded and clear supernatant was frozen at -80ºC till further analysis. TCA extract of plasma was mixed with DNPH reagent and incubated at 37°c for three hours, mixed with 65% H_2SO_4 and optical density was measured at 520nm [9]. A duplicate measurement was performed for all samples. All laboratory analyses were performed within 3 months of blood collection.

Assessment of Stress:

To evaluate stress level, an international standardized questionnaire (Social Readjustment Rating Scale; SRRS) was used [10]. Life changing events in the past year, both positive (job promotion, increase in salary, marriage, etc) and negative (divorce, long job hours, etc), in an individual's life were added and the total life change unit (TLCU) score was considered an estimate of stress. A score > 300 was considered as severe stress, 200-299 as moderate stress, 150-201 as mild stress and <150 as low stress.

Physical Activity:

Information regarding usual physical activity prior to the present pregnancy was measured using a standard questionnaire [11]. The mother was asked about the type of activity and duration of each activity for the preceding seven days prior to confirmation of present pregnancy.

Statistical Analysis:

A 1:3 ratio of women with GDM to controls was assumed to detect a minimum effect size of 3 (odds ratio), at 80% power and 5% significance level. With the probability of low vitamin C in the controls estimated at 0.25 [12], the required sample size was calculated using the formula: $N=(r+1/r)\ \overline{P}\ (1-\overline{P})\ (Z_{\beta}+Z_{\alpha/2})^2 / (P_1-P_2)^2$ in which r is ratio of controls to cases, $z_{\alpha/2}$ is the desired level of statistical significance, Z_{β} is the desired power, $\overline{P}$ is a measure of variability (standard deviation), and (P_1-P_2) is the effect size. A sample size requirement of 42 GDM women and 126 NGT controls was obtained.

Data were presented as mean (SD), or median (IQR) if skewed (vitamin C intake, plasma vitamin C concentration, stress score and income). For skewed data, log transformation was undertaken for conversion to normality before further analysis. Difference between groups (GDM and NGT) was analyzed by t-test and chi-square test as applicable.

Relationships between exposures (maternal dietary intake and plasma vitamin C concentration) and outcomes (glycemic status) were examined using linear regression, taking into account potential confounders. Two-tailed are P values and confidence interval were estimated at the 95% level. All statistical analyses were performed using Statistical Package for Social Sciences (SPSS IBM version 21) software.

Results:

General Characteristics:

Of 855 pregnant women who attended the antenatal clinics of the two conveniently selected study hospitals between 1st May 2012 and 31st December 2012, 475 were not in the window of 24-28 weeks of gestation and hence were excluded from the present study. In addition, women with other medical conditions (thyroid disorders, epilepsy, hypertension, known diabetes), and planning to deliver in other hospitals were also excluded (Fig. 1). Of the remaining 314 eligible for the present study, 200 (63%) [42 GDM, 158 NGT] agreed to participate. Non participants were similar to participants in terms of age, BMI, gestational age and income.

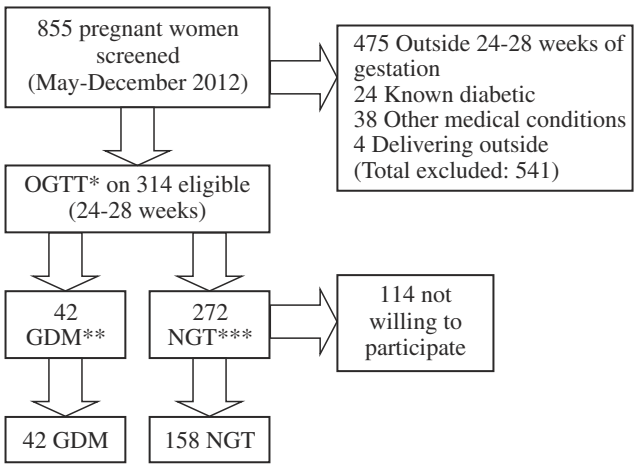

Fig.1 Flow diagram of participants

OGTT-Oral Glucose Tolerance Test, ** GDM-Gestational Diabetes Mellitus, * NGT=Normal Glucose Tolerant*

Women with GDM were older, had higher pre-pregnancy weight and BMI, higher weight gain during pregnancy and higher stress score (Table 1). They were also more likely to have a family history of diabetes and bad obstetric history (abortion, polyhydroamnios, and previous history of GDM). Almost all women were taking multivitamin supplements.

p<0.001) (Table 2). More than 90% of GDM women consumed less than the RDA for vitamin C. Frequency of consumption of vitamin C rich fruits and vegetables were also lower in GDM women compared to NGT women (p<0.001). In a continuous analysis, lower frequency of consumption of vitamin C rich foods was associated with higher fasting, 1 hour and 2 hour post glucose plasma glucose concentrations (p<0.001); these relationships persisted after adjustment for confounding factors (age, pre-pregnancy BMI, income, stress).

Table1: Characteristics of Study Population

Parameters	GDM (N=42)	NGT (N=158)	P values
Age (years)	29.2 ± 4.6	26.9 ± 3.8	0.001
Education in years	14.8 ± 2.4	14.5 ± 3.1	0.65
Occupation (Employed) (%)	23.8	31	0.36
Family history of diabetes (%)	61.9	39.3	<0.001
Height (cm)	156.2 ± 5.9	156.3 ± 4.8	0.87
Pre-Pregnancy weight (kg)	63.7 ± 10.6	54.4 ± 10.3	<0.001
Pre-Pregnancy BMI (kg/m^2)	26.5 ± 4.3	22.2 ± 3.9	<0.001
Weight at 26 weeks (kg)	72.6 ± 11.0	61.3 ± 10.7	<0.001
Weight gain at 26 weeks (kg)	8.8 ± 2.1	6.8 ± 1.6	<0.001
BMI at 26 weeks (kg/m^2)	29.8 ± 4.4	25.1 ± 4.1	<0.001
Total weight gain during pregnancy (kg)	13.8 ± 5.3	11.5 ± 2.9	<0.001
Passive smoking (%)	7.1	3.8	0.28
Stress score	60.0 (50.0, 80.0)	35.0 (35.0, 45.0)	<0.001
Bad obstetrical history			
Abortion (%)	26.2	20.3	0.32
Polyhydraminous (%)	2.4	0	0.11
History of previous GDM (%)	4.8	0.6	0.06

Values are presented as mean ±SD, or median (IQR), or %
P values represent significance for differences between groups

Dietary Intakes of vitamin C:

The estimated median dietary intake of vitamin C was significantly lower in GDM women compared with NGT (35.0 vs 66.7 mg/day;

Table 2: Dietary Intake (24 Hour Diet Recall) and Plasma Vitamin C Concentrations in GDM and NGT Pregnant Mothers

Parameters	GDM (n= 42)	NGT (n=158)	P value (two tailed)
Dietary intake (mg/day)	35.0 (29.2, 46.3)	66.7 (34.8, 132.5)	<0.001
Intake < RDA (60mg/day)* (%)	90.5	48.8	<0.001
Plasma vitamin C (µmol/l) (GDM=42, NGT=134)	45.9 (28.3, 54.3)	95.2 (47.2, 124.1)	<0.001
Plasma level <45 µmol/l (%) (GDM=42, NGT=134)	46.2	24.4	<0.001

Values are presented as median (IQR) and %. P values represent significance for differences between groups
**ICMR 2010 for Indian pregnant mothers*

Circulating Vitamin C Concentrations:

Circulating vitamin C concentrations were strongly associated with vitamin C intake by quadratic association for the whole group (p<0.001) (Fig.2).

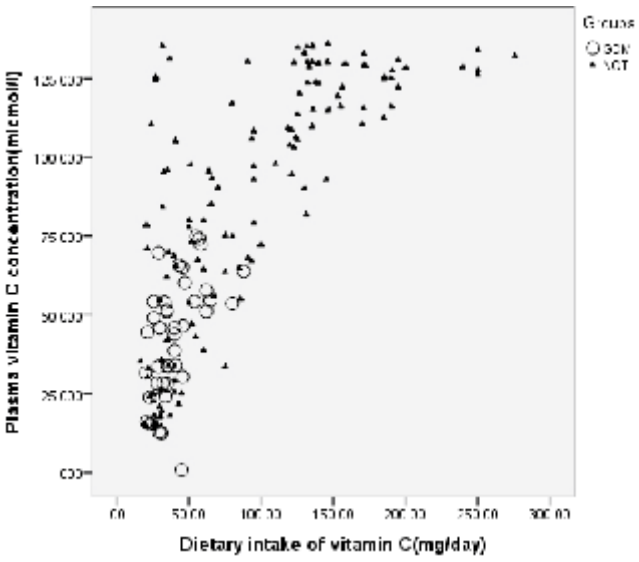

Fig. 2: Correlation of Dietary Intake and Plasma Concentration of Vitamin C

Vitamin C concentrations were widely distributed (0.9 to 135.8 µmol/L) and the distribution was skewed. However the distribution of vitamin C levels in the GDM women was substantially shifted to the left compared to that in the NGT women. Median plasma vitamin C concentrations were significantly lower in GDM women compared to NGT women (45.9 vs 95.2 µmol/l; p<0.001). Following Kartz et al and Knight et al [13, 14], we used a plasma vitamin C cut-off of < 45µmol/l to define deficiency; 46.2% of GDM women and 24.4% of NGT women were deficient according to these criteria.

Table 3 shows the characteristics of the study population, according to quartiles of plasma vitamin C concentrations. Age, income, and total weight gain during pregnancy were no different in the four quartiles. Increasing vitamin C concentrations were associated with lower levels of pre-pregnancy BMI and lower levels of stress. There was a strong inverse relationship between vitamin C concentrations and plasma glucose levels at all 3 time points as well as with prevalence of GDM (p<0.001 for all). These relationships remained of similar strength after adjustment for age, pre-pregnancy BMI, income and stress.

Discussion:

The association between vitamin C intake and its circulating concentrations with glycemic status during pregnancy was studied. Increasing intake and higher concentrations of plasma vitamin C were associated with lower plasma glucose

Table 3: Characteristics of the Study Population according to Quartiles of Plasma Vitamin C Concentrations

Quartiles of Maternal Plasma Vitamin C Concentrations					
Parameters	**Q_1 (<33.9 µmol/l) (n=41)**	**Q_2 (-72.3 µmol/l) (n=48)**	**Q_3 (-116.0 µmol/l) (n=43)**	**Q_4 (>116.0 µmol/l) (n=44)**	**P value**
Plasma vitamin C concentration (µmol/l)	21.9 ± 7.3	54.0 ± 11.9	95.9 ± 13.7	127.9 ± 5.2	-
Dietary vitamin C intake (mg/day)	29.93 (18.75, 75.18)	42.27 (16.75, 100.1)	95.04 (20.79, 185.02)	143.01 (27.17, 275.81)	-
Age (years)	27.5 ± 4.4	28.0 ± 3.8	28.3 ± 4.0	27.4 ± 4.0	p=0.84
Income/month (Rupees)	21000 (7800, 40000)	22000 (9500, 95000)	22000 (8000, 50000)	22500 (7000, 65000)	p=0.13
Pre-pregnancy BMI (kg/m^2)	24.7 ± 4.3	24.6 ± 4.5	22.9 ± 4.1	22.3 ± 4.3	**p=0.002**
Total weight gain during pregnancy (kg)	11.9 ± 3.4	13.4 ± 4.8	11.5 ± 3.2	12.2 ± 3.3	p=0.43
Stress score (TLCU)	45 (35.0, 62.5)	45 (35.0, 58.7)	35 (35.0, 50.0)	35 (35.0, 45.0)	**p<0.001**
Bad Obstetric History (BOH) (%)	43.9	43.8	46.5	27.3	p=0.24
GDM (%)	36.6	50.0	4.7	0	**p<0.001**
FPG (mg/dl)	87.0 (78.0, 99.5)	86.5 (77.5, 99.8)	79.0 (71.0, 86.0)	79.0 (71.0, 85.0)	**p<0.001**
1 hour PG (mg/dl)	163 (152.5, 220.0)	181 (158.3, 278.8)	159 (149.0, 170.0)	155 (145.0, 161.0)	**p<0.001**
2 hour PG (mg/dl)	118.0 (107.5, 167.0)	122.0 (107.0, 180.5)	112.0 (102.0, 127.0)	113.5 (101.3, 127.3)	**p<0.001**

Data presented as mean ± SD, Median (25^{th}, 75^{th} centiles), and % ¶ TLCU= Total life change unit

concentrations. GDM mothers had lower dietary vitamin C intake (fruits and vegetables) as well as lower plasma vitamin C concentration compared to NGT women. These associations were significant after adjusting for confounders such as age, pre-pregnancy BMI, income and stress.

Ours is one of the few studies to measure dietary vitamin C intake and also circulating vitamin C levels in pregnant Indian women. From the information obtained from the food questionnaires, it was apparent that the majority of the women were vegetarian but had low intakes of fruits and vegetables. Vitamin C rich foods in this population included local fruits and vegetables. Intake of these foods was influenced by socioeconomic condition, personal likes and dislikes, cultural taboos, availability and seasonality.

Our findings are consistent with those of Cuilin *et al* [12] and Bazzano *et al* [15] who found an association of lower vitamin C intake and lower circulating vitamin C concentrations with GDM. In a meta-analysis of 14 studies, the effect of dietary vitamin C and E supplementation on plasma glucose, HbA1c and insulin concentration was assessed [16]. Vitamin C did not have a significant effect on plasma glucose levels and

insulin concentrations, but was associated with reduction of HbA1c, suggesting that "antioxidants may have some benefit in protecting against the complications of T2DM". A 6 week vitamin C supplementation study with a dose of 1000 mg/day suggested that vitamin C helps reduce fasting and postprandial oxidative stress and may help in preventing diabetes related complications [17].

Vitamin C is a reducing agent [5, 18] that functions in the body as an antioxidant, scavenging free radicals. About 60 mg vitamin C intake per day will meet the adult requirement. Additional intakes (20-40% increase) are recommended for women during pregnancy and lactation [19]. It is interesting that the efficiency of enteric absorption of ascorbic acid is high (80-90%) at low intake but declines markedly at intakes greater than about 1g/day [19]. Cooking, storage, and chopping reduce vitamin C content of food. Hence availability of vitamin C depends not only on content but also on processing of foods. Loss of vitamin C during conventional cooking process has been reported to the extent of 52-82% [20].

Plasma volume expansion during pregnancy results in a lowering of circulating levels of different nutrients. Thus, lower circulating levels of many nutrients in pregnancy are partly contributed by physiological adaptations and disorders which affect these adaptations. High glucose levels also increase oxidative stress and therefore reduce levels of antioxidants including vitamin C. Thus, the inverse association between vitamin C and glycemia may be partly contributed by 'reverse causality'. Machlin [21] found that diabetic patients have an increased turnover rate and decreased vitamin C status compared to non-diabetic individuals. Jhankar *et al* [22] found that progressive improvement of glycemic status in newly diagnosed diabetic patients was associated with reduced oxidant stress and increasing vitamin C concentrations.

Some of the strengths of our study include simultaneous measurements of dietary intake and circulating levels of vitamin C in normal and glucose intolerant pregnant women. Our dietary assessment extended to a period before conception, and preceded measurement of glycemic status, thus allowing an interpretation in the direction of causality between intake and glucose intolerance. Selection bias is unlikely as non-participants were no different to participants in age, income, BMI and gestation at enrollment. We studied adequate numbers to satisfy the power calculations and are therefore able to interpret the results with good confidence. Despite these strengths, we have accepted that there are some weaknesses: we have relatively small numbers and it would be important to expand the numbers to confirm these findings. In addition, we had only a single assessment of dietary intake and circulating nutrient concentration. Finally, in an observational study we cannot be sure of causality nor can we rule out reverse causality.

In summary, ours is a preliminary investigation to elucidate an association between maternal vitamin C intake, circulating vitamin C status and the risk of gestational diabetes. GDM mothers had lower vitamin C intake and lower vitamin C concentrations compared to glucose tolerant mothers. Our study suggests that all women preparing for pregnancy and during pregnancy need to consider increasing their dietary intake of vitamin C. The association between vitamin C status and GDM needs to be tested in a large prospective study and subsequently in a clinical trial.

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