Impact of gestational diabetes on lipid profiling and indices of oxidative stress in maternal and cord plasma

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ABSTRACT

Objective: To study the effect of gestational diabetes mellitus (GDM) on indices of oxidative stress and lipid profiles in maternal and cord blood samples.

Methods: Blood samples were collected from 40 normal pregnant women and 46 women with GDM during the period 1998 through to 1999 at the Armed Forces Hospital, Riyadh, Kingdom of Saudi Arabia. The GDM patients were subdivided into 2 groups: patients receiving insulin treatment (GDM-I, N=19) and patients under control diet (GDM-D, N=27). Plasma α- and γ-tocopherols were estimated by high-performance liquid chromatography, whereas malondialdehyde (MDA) was analyzed by fluorometry. Serum lipids (low density lipoprotein, high density lipoprotein, total cholesterol, triglycerides, and total lipids) were determined by enzymatic colorimetry using automated clinical analyzer.

Results: The results of lipid profiles in maternal serum showed no significant difference between GDM patients and controls; however, all the lipid constituents except total cholesterol were significantly reduced in the cord blood of GDM patients as compared to control subjects. α-tocopherol levels in the maternal plasma were not significantly different among the 3 groups, whereas, cord plasma α-tocopherol was significantly decreased in both GDM-D and GDM-I. Maternal γ-tocopherol was found to be significantly increased in GDM-D and only insignificantly increased in GDM-I, but the cord γ-tocopherol showed no appreciable changes. The level of MDA was 3-fold higher in maternal plasma as compared to cord plasma. However, neither the maternal plasma nor cord plasma showed significant differences in MDA levels between GDM patients and normal pregnant women.

Conclusion: A significant depletion of α-tocopherol in the cord blood of GDM patients is indicative of a possible oxidative stress in their fetuses. Further studies are warranted to examine a wider range of biochemical parameters to evaluate the potential risks of oxidative damage.

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Women with gestational diabetes mellitus (GDM) are at high risk of maternal complication during pregnancy. Recent studies on experimental animals point towards an important role of oxidative stress and lipid peroxidation in the development of fetal malformations associated with GDM.[1-4] Diabetes-induced oxidative stress might result from the underlying metabolic abnormalities rather than the direct causes of the disease itself.[5] During pregnancy, the synthesis rate of lipoperoxides appears to exceed their decomposition rate, causing oxidative stress. Lipoperoxides are also increased in the fetus as it develops, but to lesser extent than that of mother.[6] However, there is

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little and conflicting information regarding the antioxidant status of pregnant women with GDM and its impact on maintaining proper antioxidant defenses of the fetus.[7-10] We speculated that if there is an imbalance between lipid peroxides and antioxidants in the mother, this might result in decreased vitamin E supply to the fetus. We therefore set out to compare the levels of indices of oxidative stress including vitamin E and malondialdehyde (MDA) in maternal and cord blood of pregnant women with and without GDM. We also analyzed lipid profile of maternal and cord blood to examine a possible correlation between lipids and the free radical indices.

Methods. The subjects were 86 pregnant women who were randomly selected from the antenatal clinic at the Armed Forces Hospital, Riyadh, Kingdom of Saudi Arabia. They were routinely screened between 22nd-28th week of gestation for GDM or at booking for those who attended after the 28th week. The initial screening test consisted of measuring blood glucose level one hour after a 75 g glucose load irrespective of previous food intake. A glucose level of <7.4 mmol/L excludes GDM; glucose level ≥11 mmol/L was considered GDM. Patients with blood glucose level of ≥7.4 and <11 mmol/L underwent a standard 75gm oral glucose tolerance test (OGTT). We followed the NDDG criteria to define GDM.[11] All patients with a diagnosis of GDM were initially put on diet and evaluated by glucose series with an aim of keeping fasting glucose ≤5.3 and 2 hour postprandial glucose ≤6.8 mmol/L. Failure to meet these goals was indicative of insulin treatment. The subjects were divided into 3 groups; those controlled on diet alone (GDM-D), those on diet and insulin (GDM-I), and those who had a normal OGTT (control). Blood samples were obtained from mother by venipuncture during the second stage of labor (maternal sample) and by puncturing the umbilical vein immediately after clamping (cord blood sample). Blood samples were drawn into Vacutainer tubes containing heparin and centrifuged to separate the plasma, which was used to measure tocopherols and malondialdehyde (MDA) levels. Another sample of maternal and cord blood was drown in plain tubes and allowed to clot to separate the serum, which was used for determination of lipid profile. Plasma α- and γ-tocopherol were extracted by a procedure described earlier.[12] Levels of α- and γ-tocopherol were estimated by high-performance liquid chromatography (Varian 5000 pump with pulls damper and Rheodney 7125 injection port connected into MCH-5 reversed-phase column, Alltech, San Jose, California, United States of America (USA). The solvent system was acetonitrile:dichloromethane:methanol (60:20:20

v/v) and the flow rate was one ml/min. Both of the tocopherols were detected at 295 nm, (Varian UV50 detector). Malondialdehyde levels in maternal and cord plasma were estimated by the method of Yagi.[13] Briefly, plasma sample (20 µl) was mixed with 1/12 N H_2SO_4 (0.4 ml) and 10% phosphotungstic acid (0.5 ml). The mixture was incubated at room temperature for 5 min, then centrifuged at 4,000 rpm for 10 min. The sediment was suspended in 2.0 ml of 1/12 N H_2SO_4 and 0.3 ml of 10% phosphotungstic acid, and centrifuged again. The sediment was mixed with 4.0 ml distilled water and 1.0 ml of thiobarbituric acid: acetic acid reagent (1:1) and incubated at 95ºC for one hour. After cooling, 5.0 ml of n-butanol were added and the mixture was vortexed and centrifuged for 15 min. The fluorescence of the butanol layer was measured (λex=515 nm, λem=553 nm) against n-butyl alcohol as blank, using a Perkin-Elmer Model 203 fluorometer. Lipid peroxides were expressed as micromoles of malondialdehyde using a standard absorption curve of 1,1,3,3-tetraethoxypropane. Total cholesterol (TC), high density lipoprotein (HDL)-cholesterol, and triglycerides (TG) were measured in serum samples by enzymatic colorimetric tests using commercially available kits (Boehringer Mannheim Co., Indianapolis, USA) on a BM/Hitachi 917 clinical analyzer. Low density lipoprotein cholesterol was calculated using Friedwald's formula LDL-C (mmol/L) = TC - (HDL-C + TG/2.2)

The data were analyzed by one-way analysis of variance followed by Student's t-test to compare the means. A value of P less than 0.05 was considered significant.

Results. There was no significant difference between maternal serum of GDM patients and control in any of the lipid constituents. However, unlike maternal serum, cord serum showed significant differences between the 3 groups (**Table 1**). Cord serum LDL, TG and total lipids in both GDM-D and GDM-I patients were significantly lower than those of control subjects ($P<0.05$). A significant reduction in cord serum HDL was observed only in GDM-D as compared to controls. Although the level of TC in cord serum of GDM-D and GDM-I was found to be less than control subjects, this difference did not reach the significance level (**Table 1**). A slight reduction in the levels of α-tocopherol was observed in maternal plasma of GDM-D (41.85 ± 2.33 mmol/l) and GDM-I (40.21 ± 5.08 mmol/l) as compared to control subjects (44.30 ± 2.67 mmol/l) (**Table 2**). Whereas, the results of cord plasma showed a significant reduction in α-tocopherol among GDM-D (15.40 ± 2.19 mmol/l) and GDM-I (14.90 ± 1.75 mmol/l) versus controls (22.28 ± 1.38 mmol/l).

Table 1 - Comparison of lipid profile in maternal serum and cord serum among normal pregnant women (control) and pregnant women with gestational diabetes mellitus under controlled diet or insulin treatment.

Lipid profile (mmol/L)	Maternal/ cord	Control (N=40)	GDM-D (N=27)	GDM-I (N=19)
Total cholesterol	Maternal	6.73 ± 0.17	6.81 ± 0.40	6.44 ± 0.34
	Cord	2.03 ± 0.22	1.51 ± 0.09	1.51 ± 0.10
LDL-cholesterol	Maternal	3.71 ± 0.15	4.02 ± 0.30	3.64 ± 0.32
	Cord	1.97 ± 0.49	0.79 ± 0.06*	0.86 ± 0.10*
HDL-cholesterol	Maternal	1.71 ± 0.06	1.59 ± 0.09	1.73 ± 0.09
	Cord	0.72 ± 0.04	0.58 ± 0.03*	0.68 ± 0.05
Triglycerides	Maternal	2.93 ± 0.35	2.86 ± 0.21	3.47 ± 0.34
	Cord	0.64 ± 0.08	0.40 ± 0.07*	0.31 ± 0.03*
Total lipids	Maternal	16.38 ± 0.50	16.49 ± 0.94	16.36 ± 0.90
	Cord	4.70 ± 0.50	3.41 ± 0.18*	3.39 ± 0.19*

LDL - low density lipoprotein, HDL - high density lipoprotein
GDM-D - diabetes mellitus under controlled diet
GDM-I diabetes mellitus under insulin treatment
Values are expressed as mean and standard error of mean (SEM).
The numbers of subjects are represented in parenthesis.
*P<0.05, significant compared with normal controls

Table 2 - Comparison of oxidative stress indices in maternal plasma and cord plasma among normal pregnant women (control) and pregnant women with gestational diabetes mellitus under controlled diet or insulin treatment.

Parameter (µmol/L)	Maternal/ cord	Control (N=40)	GDM-D (N=27)	GDM-I (N=19)
α - Tocopherol	Maternal	44.30 ± 2.67	41.85 ± 2.33	40.21 ± 5.08
	Cord	22.28 ± 1.38	15.40 ± 2.19*	14.90 ± 1.75*
γ - Tocopherol	Maternal	1.78 ± 0.14	2.77 ± 0.39*	2.08 ± 0.25
	Cord	1.56 ± 0.22	1.44 ± 0.21	1.41 ± 0.11
Malondialdehyde	Maternal	7.87 ± 0.57	7.55 ± 0.82	8.82 ± 0.89
	Cord	2.88 ± 0.28	3.07 ± 0.38	3.61 ± 0.37

GDM-D - diabetes mellitus under controlled diet
GDM-I diabetes mellitus under insulin treatment
Values are expressed as mean and standard error of mean (SEM).
The numbers of subjects are represented in parenthesis.
*P<0.05, significant compared with normal controls

The levels of γ-tocopherol in maternal plasma were found to be increased in both GDM-D (2.77 ± 0.39 mmol/l) and GDM-I (2.08 ± 0.25 mmol/l) as compared to control (1.78 ± 0.14 mmol/l), however, the difference was significant only in the former case. There was no significant difference in the cord plasma γ-tocopherol levels among the 3 groups (**Table 2**). **Figures 1 & 2** show the ratios of α-tocopherol/total lipids and α-tocopherol/γ-tocopherol, in maternal and cord plasma. Maternal plasma MDA levels were found to be, on average, 3 times higher than that of cord plasma in all groups (**Table 2**). The mean values of maternal and cord plasma MDA levels of GDM-I patients were insignificantly higher than those of respective controls. Statistical analysis revealed no significant differences between the MDA levels observed in controls and GDM-D or GDM-I for the sets of either maternal or cord plasma (**Table 2**).

Discussion. The results of this study showed a significant depletion of vitamin E in the cord plasma of GDM patients (**Table 2**), which is in agreement with earlier study[14] suggesting excessive oxidative stress in GDM patients. A slight reduction in Vitamin E level in the maternal plasma of GDM patients is supported by the findings of Kharb[7] but in contrast with the study of Bates et al[8] who have shown an increase in serum vitamin E in pregnant diabetic women. Depletion of vitamin E in the cord blood might result due to progressive consumption of this vitamin during excessive lipid peroxidation. However, this could not be explained solely on the basis of plasma MDA levels that appeared to be

insignificant between control and GDM patients (**Table 2**). Alternatively, the lower cord vitamin E level in GDM patients might be a reflection of preferential mobilization of this vitamin toward tissues with the greatest requirement. A novel α-tocopherol-binding protein in human placenta might control the flow of tocopherols between fetus and mother.[15] Increased oxidative stress in pregnant women and their fetuses has been associated with congenital malformations or other serious complications.[9,10] Inadequate antioxidant defense has been postulated as a prime factor in pathologic states of many premature infants.[15] This study showed that the α/γ tocopherol ratios in cord or maternal plasma of control group were significantly higher than that of GDM patients regardless of the treatment they received (**Figure 2**). However, this ratio was always higher in maternal plasma than cord plasma. Our earlier study showed different profiles in maternal plasma α- and γ-tocopherols during normal human pregnancy.[16] It is possible that utilization of γ-tocopherol is higher than α-tocopherol, particularly in GDM patients or there might be a competition between the 2 forms of tocopherols, or both. In fact, elevated plasma α-tocopherol level can depress plasma γ-tocopherol concentration.[17] The concurrent reduction in cord plasma LDL together with α-tocopherol in GDM patients suggests a close relationship between vitamin E and lipoprotein in the cord blood. It has been proposed that low vitamin E in cord plasma is due to the placental permeability barrier for lipids and other fat-soluble vitamins[18,19] or to the neonatal restricted lipoprotein metabolism.[20] The lipoprotein system of the newborn is unique; neonatal plasma
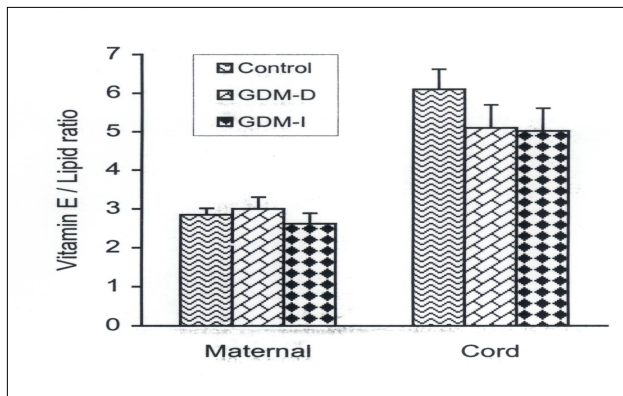
Figure 1 - Vitamin E/total lipids ratio in healthy normal pregnant women and women with gestational diabetes mellitus under controlled diet (GDM-D) or treated with insulin (GDM-I).
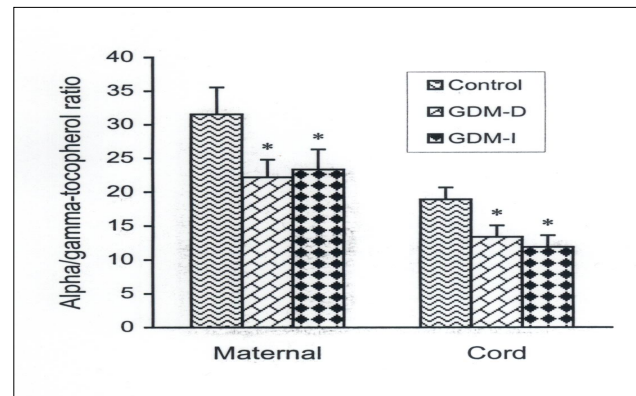

Figure 2 - α/γ tocopherols ratio in healthy normal pregnant women and women with gestational diabetes mellitus under controlled diet or treated with insulin. *$P<0.05$ versus respective control group using t-test.

lipoprotein levels are lower than in adults, HDL in neonates is only 30% of that in adults, whereas LDLs are also present at low concentration.[20,21] Tocopherols concentrations are markedly influenced by plasma transport capacity and their interpretation in relation to plasma lipids has been suggested earlier.[22] In this study, α-tocopherol/total lipid ratio was remarkably constant (**Figure 1**) and was not significantly influenced by GDM. The increased level of vitamin E in pregnant women and in some diabetics has been considered as a manifestation of the increased plasma lipids.[23] Previous study on GDM patients had shown that vitamin E level of GDM patients was higher than control subjects during the first trimester but lower in the third trimester.[12] Thus, hikes in the maternal requirements of vitamin E during first trimester would have a direct role in protection against lipid peroxidation generated by increased lipid concentrations. Moreover, the differences in vitamin E levels may also be linked to the rate of fetal oxidative metabolism; fetus of GDM mother has higher rate of oxidative metabolism than normal fetus due to the persistent maternal hyperlipidemia and increased fuel delivery to the fetus.[24]

In conclusion, our results showed no significant differences in maternal lipids, α-tocopherol and MDA levels between GDM patients and normal pregnant women. However, significant depletion of α-tocopherol in the cord blood of GDM patients points towards a possible risk of oxidative stress in their fetuses. Further studies are warranted to examine the levels of other markers of oxidative stress including antioxidant enzymes, glutathione, lipid hydroperoxides and conjugated dienes in concert with α-tocopherol and MDA, to have more clear understanding of the extent of oxidative stress in GDM maternal and cord blood.

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