Gestational Diabetes Mellitus and Iron Supplement; Effects on Pregnancy Outcome

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Abstract- The possible effect of iron supplementation has been investigated in the normal population and patients with gestational diabetes mellitus (GDM). In this study, we survey the risk factors of GDM in pregnant women in contrast with normoglycemic patients in a case control study in patients using iron supplement. This case control study conducted on 52 pregnant women with GDM (25 women with type Al and 27 women with Type A2 of GDM). The control group randomly selected 50 normoglycemic women. Venous blood sampling was done between 24 and 28 weeks of pregnancy for measuring of ferritin, lipoproteins, uric acid and malondialdehyde serum levels. Under study variables including age, gestational age, weight and BMI were gathered. All the women were followed up until the time of delivery and pregnancy outcome were gathered. The serum ferritin levels in GDM group was 31.22+15.44, which is significantly higher than 24.76+8.94, in the control group with (*P*=0.012). Plasma hemogulobin in the control group was 12.2+0.1 compared to 12.9+0.1 in GDM group which was significantly lower (*P*=0.005). Triglycerides was significantly higher in GDM group in contrast with the control group, 275.08+143.17 and 192.30+92.13 (P=0.001), respectively. Finally, our findings indicate the concentration of serum ferritin levels was significantly higher in The GDM group.

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Keywords: Iron; GDM; Pregnancy; Ferritin

Introduction

Gestational diabetes mellitus (GDM) is a form of glucose intolerance which manifests during pregnancy. Most of patients with GDM are diagnosed by tests which require measurement of serum glucose levels following oral intake of glucose, namely the oral glucose challenge test (OGCT) and the oral glucose tolerance test (OGTT). The threshold levels which are used for the GCT and OGTT are relatively lower than the threshold levels which are used to confirm the diagnosis of diabetes mellitus in non-pregnant individuals (1).

An excessive amount of elemental iron has been proposed to induce free radical formation (2). Iron has different effects on the pregnancy outcome. Iron overload is believed to reduce placental perfusion and result in preeclampsia, low birth weight and preterm birth (3). On the other hand, it has been suggested that iron deficiency can increase neonatal morbidity (4,5).

Given the fact that GDM is associated with an increased serum C-reactive protein level, some authors suggest that GDM might be part of an inflammatory process (6,7),.It has been previously reported that iron overload promotes inflammatory processes by inducing free radical formation through an oxidative mechanism (8). Several lines of evidence suggest that the risk of type 2 diabetes in the normal population is increased by iron intake (9,10). However, some studies have shown no association between iron supplementation and GDM (11,12). Interestingly, iron supplementation increases lipid peroxidation possibly through triggering inflammatory processes (13). This indicates that routine iron supplementation should be avoided in women with GDM.

Although the possible effect of iron supplementation has been investigated in the normal population and patients with GDM by many studies, this matter still remains as a controversial issue. In this study, we have

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compared the possible risk factors of GDM in pregnant women with a diagnosis of GDM and normoglycemic pregnant women.

Materials and Methods

This study was held in the Department of Obstetrics and Gynecology of Vali-e-Asr Hospital which is an academic hospital affiliated to Tehran University of Medical Sciences (Iran) during April 2007 to August 2010. Pregnancy was confirmed in all participants of this study with ultrasound examination during the first trimester. Pregnant women with an underlying disease such as hypertension, history of diabetes mellitus, chronic illnesses, thalassemia trait and infections were excluded from the study. All patients received 40 mg of elemental iron supplementation from the 16th week of gestation until the end of pregnancy. Patients which fulfilled the ADA criteria for the diagnosis of types A1 and A2 GDM were considered as the 'case' group in this study (14). An OGCT was performed between the 24th to 28th weeks of pregnancy. Patients were asked to intake 50 g of glucose and their plasma glucose level was measured after an hour. The OGCT was performed regardless of the time of day or the time since the last meal. Patients with a blood glucose level of 135 mg/dL or higher in the OGCT were referred for OGTT. Patients who had been considered to take the OGTT had to maintain a 150 g per day carbohydrate diet and exercise for three days. At the end of the third day, the patients had an overnight fasting which had to be at least eight hours. Their fasting blood glucose level was measured, and they subsequently drank syrup containing 100 g of glucose. Their blood glucose level was measured 1, 2 and 3 hours after oral intake of glucose. If two of the four blood glucose level measurements (Fasting blood glucose, 1, 2 and 3 hours after glucose intake) were above the normal levels indicated by ADA14 the patient was considered to have GDM. Our control group consisted of non-diabetic pregnant women who were matched according to their duration of pregnancy with patients with GDM.

Venous blood sampling was done between 24 and 28 weeks and serum aliquoted and sorted for the batch assay of serum ferritin (Immunoradiometric Assay by Kavoshyar Ferritin IRMA [I125] kit) and Lipid panel, Lipoproteins, Uric Acid were measured by standard tests. Another blood sampling was done for measuring Malondialdehyde (MDA) with thiobarbituric acid based colorimetric test. This assay is based on the reaction of MDA with thiobarbituric acid; forming a MDA-TBA2 adduct that absorbs strongly at 532 nm.

Understudy variables including age, gestational age, weight and BMI were gathered. All the women were followed up until the time of delivery and pregnancy outcomes were gathered as well.

This study was confirmed by the ethical committee of Tehran University of Medical Sciences and was undertaken in accordance with good clinical practice and the Declaration of Helsinki. Written informed consent was obtained from all the participants of this study.

All data were analyzed with SPSS version 16 (SPSS Inc. Chicago, IL, USA). The chi-square test was employed to compare the categorical variables. Student's t-test was performed to compare the different measured variables between the case and control groups. Wilcoxon and Mann–Whitney tests were performed on data that did not have a normal distribution according to the Kolmogorov–Smirnov test. *P* value <0.05 was considered statistically significant.

Result

One hundred and two women were recruited in this study. They consisted of 52 patients with GDM (25 Type A1 & 27 Type A2) and 50 control normoglycemic pregnant women. Their demographic data is shown in Table 1. There was no significant difference between the two groups in terms of age, BMI, gestational age and Weight.

Table 1. Demographic Data

	$A_{1(N=25)}$	$A_{2(N=27)}$	P Value	$Control_{(N=50)}$	$GDM_{(N=52)}$	P Value
Age(Years)	30.04	32.44	0.287	28.88+6.58	31.24+ 6.71	0.061
Gestational age(Week)	34.69+3.45	33.16+3.19	0.107	34.08+3.19	34.06+3.49	0.349
Gravidity n (%)	2.65+1.53	3.42+1.58	0.145	2.46+0.29	2.96+1.46	0.624
BMI(KG/M^2)	26.81+ 2.98	27.05+3.13	0.724	25.69+4.36	26.94+3.84	0.072
Weight(KG)	67.60+10.82	68.96+7.85	0.613	65.42+10.41	68.28+9.38	0.056

Results showed in mean + SEM

Neonatal birth weights in the GDM group were significantly higher than the control group (3324 ± 22.45 g and 3115 ± 20.65 g, respectively, P < 0.001). However, there was no significant difference in the mode of delivery, gestational age at delivery, preterm delivery rate, risk of neonatal respiratory distress syndrome, risk of

having a small for gestational age fetus and risk of preeclampsia between the two groups (Table 2).

Table 2. Delivery and Infant outcome

	$A_{1(N=25)}$	$A_{2(N=27)}$	P Value	$Control_{(N=50)}$	$GDM_{(N=52)}$	P Value
Birth weight (g)	3370+28.74	3295+20.12	0.073	3115+20.65	3324+22.45	0.001
Caesarian Section n (%)	5(16%)	9(29.6%)	0.147	12(24%)	14(26.9)	0.456
Instrumental delivery n (%)	4(16)	8(29.6%)	0.131	9(18%)	12(23%)	0.349
Small for gestational age n (%)	2(8%)	2(7.4%)	0.665	2(4%)	4(13%)	0. 358
Gestational age (weeks)	37.8+0.14	37.4+0.17	0.142	38.7+0.12	37.6+0.23	0.092
Preterm delivery n (%)	2(8%)	2(7.4%)	0.665	2(4%)	4(13%)	0.358
Respiratory distress syndrome n (%)	1(4%)	1(3.7%)	0.12	0	2(3.8%)	0.257
Preeclampsia n (%)	1(4%)	2(7.4%)	0.471	1(2%)	3(5.7%)	0.324

Results showed in mean + SEM or n (%)

Serum ferritin levels were significantly different between the GDM and control groups (31.22 + 15.44 (ng/mL) and 24.76 + 8.94 (ng/mL), respectively, P = 0.012). Blood hemoglobin concentration was significantly higher in the GDM group in comparison to the control group (12.9 + 0.1 g/dL and 12.2 + 0.1 g/dL, respectively, P = 0.005). Additionally, plasma TG levels were significantly higher in the GDM group in comparison to the control group (275.08+143.17 mg/dL and 192.30+92.13 mg/dL, respectively, P = 0.001, Table 3). Moreover, MDA was significantly higher in the GDM group in comparison with the control group (1.22 + 0.69 (mM) and 0.57 + 0.38 (mM), respectively, P < 0.001, Table 3) Total cholesterol, HDL (high density lipoprotein) cholesterol, LDL (low density lipoprotein) cholesterol, uric acid and hematocrit didn't significantly differ between the two groups (Table 3).

Table 3. Para Clinical Data

	GDM	Control	P Value	A1	A2	P Value
HCT (PCV)	37.60+4.20	37.02+3.94	0.482	36.68+3.67	38.52+4.57	0.124
Hb (g/dL)	12.9+0.1	12.2+0.1	0.005	12.9+0.1	129+0.1	0.427
TG (mg/dl)	275.08+143.17	192.30+92.13	0.001	270.92+105.34	279.24+175.26	0.840
Total Cholesterol (mg/dl)	202.56+81.62	260.25+78.456	0.381	209.68+95.46	195.44+66.21	0.543
HDL-C (mg/dl)	57.10+36.77	92.55+17.96	0.288	58.65+28.75	55.56+43.92	0.769
LDL-C (mg/dl)	129.18+ 13.35	78.98+23.71	0.133	143.54+21.50	114.82+64.179	0.445
Uric Acid (mg/dl)	5.12+1.91	5.61+1.38	0.145	4.76+1.69	5.48+2.07	0.185
Serum Ferittin (ng/mL)	31.22+15.44	24.76+8.94	0.012	30.64+14.92	31.81+16.17	0.792
Malondialdehyd (mM)	1.22+0.69	0.57+0.38	0.0001	1.14+0.53	1.30+0.23	0.215

Results showed in mean + SEM

There was no significant difference between patients with type A1 GDM and those with type A2 GDM in terms of the different factors which were studied (Tables 1, 2 and 3).

Discussion

The association of GDM with supplemental iron use and iron overload remains as a controversial topic. In a randomized placebo-controlled clinical trial, the incidence of GDM was not significantly different between iron supplement users and the placebo group.15 Furthermore, it has been demonstrated that iron deficiency animal is not associated with a reduced prevalence of GDM, and it was related to the duration and timing of anemia.16 On the other hand, there are a number of studies which suggest a significant association between iron overload and GDM (2,3,9,10). Some authors suggest that iron overload can cause insulin resistance. An interesting study demonstrated that iron can affect insulin synthesis and secretion by decreasing glucose utilization in muscles and increasing hepatic gluconeogenesis (17). Furthermore, it has been shown that elevated serum ferritin levels are associated with a two-fold increase in the risk of developing type 2 diabetes mellitus (18). In accordance with these studies, we found that patients with GDM have higher plasma ferritin levels.

It has been suggested that in an iron overload status, lipid peroxidation is increased due to free radical formation (13). Our study might provide further evidence for this fact. MDA results from the degradation of polyunsaturated fatty acids in the presence of reactive oxygen species which cause toxic cellular stress (19). Its noteworthy that MDA is an indicator of the level of oxidative stress in different organisms (20) Patients with GDM in our study had significantly higher plasma MDA levels in comparison to euglycemic controls. Since patients with GDM have higher plasma ferritin levels, the higher oxidative stress in these patients might be

partially attributable to their higher iron levels. In agreement with this assumption, Lachili *et al,* reported that patients who used iron supplements had higher plasma thiobarbituric acid levels. Plasma thiobarbituric acid results from lipid peroxidation and is another indicator of oxidative stress (13).

The results of our study show that patients with GDM are probably at an increased risk of delivering neonates with higher birth weights. Lao *et al.,* reported that higher neonatal birth weights in women with GDM are probably attributable to the higher maternal weight in patients with GDM in comparison to normoglycemic pregnant women (21). In contrast to their study, our findings suggest that higher neonatal birth weights in women with GDM is not solely due to higher maternal weights in patients with GDM since the difference in neonatal birth weight was still present when the GDM and control groups were matched by weight, BMI, gestational age, maternal age and etc. Moreover, in contrast to their study, our study showed that except for neonatal birth weights there was no significant difference in other pregnancy outcomes (*i.e.* rate of small for gestational age, rate of preterm deliveries, modes of delivery and gestational age at delivery) between the two groups.

Herein, we demonstrated that there was no significant difference in total cholesterol, HDL cholesterol and LDL cholesterol between patients with GDM and normoglycemic pregnant women. This finding is in contrast with the findings of Savvidou *et al.,* who reported that patients with GDM had higher cholesterol levels. In accordance with our study, they found that patients with GDM had a higher TG level in comparison to normoglycemic pregnant women. In contrast, Savvidou *et al.,* enrolled a control group that was not matched with patients with GDM in their study. This might justify the difference in the results of the two studies (22).

Our study suggests that regardless of differences in lipid profile in GDM patients in contrast with the control group, serum ferritin level and plasma Hb were significantly higher in GDM patients. These data support by induction of MDA as a marker of oxidative stress, which had stated that iron overload leads to induction of oxidative stress and free radical damages.

One of the drawbacks of our study is that inflammatory cytokines weren't measured. This is needed to establish a clear relation between iron supplementation and inflammation. Moreover, by measuring different inflammatory cytokines, we can determine the specific cytokines responsible in this inflammatory process. Additionally, future studies can also focus on measuring anti-inflammatory cytokines which possibly inhibit the detrimental inflammatory processes in pregnant women who use iron supplementation but have not developed GDM. These studies can prove very helpful in understanding the pathophysiology of GDM and preventing GDM in iron supplement users. One of the advantages of our study is that it lacks confounders such as BMI, hypertension, previous history of diabetes mellitus, chronic illnesses, thalassemia trait and infections.

In conclusion, our study showed that, when supplemental iron is administered, plasma TG, hemoglobin, ferritin and MDA levels are significantly higher in patients with GDM in comparison to the control group. Further studies are required to elucidate the influence of iron on GDM.

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