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Association of Maternal Vitamin B12 and Folate Levels with Gestational Diabetes Mellitus and Insulin Resistance

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Abstract

Background: The incidence of gestational diabetes mellitus (GDM) has increased in recent decades. Established prevention methods for GDM include diet and exercise. Recent interest has focused on the roles of vitamin B12 and folate in the development of GDM. These micronutrients are essential for one-carbon metabolism, which is involved in many metabolic pathways. However, the study results were conflicting and varied by geographic area.

Objectives: The aim of this study was to evaluate the relationship between GDM and vitamin B12 levels in our region.

Methods: This case-control study enrolled 180 pregnant women at 24 - 28 weeks of gestation. Half of the participants had GDM, and the other half were healthy individuals. All participants attended outpatient pregnancy care clinics at Shiraz University of Medical Sciences from June 2020 to April 2021. Gestational age, age, and body mass index were matched between the two groups. Vitamin B12, folate, insulin, and homocysteine levels were measured in both groups and insulin resistance was calculated. The results were compared between the two groups, and the relationship between vitamin B12 levels and insulin resistance in each group was investigated.

Results: Vitamin B12 deficiency was present in 15.6% of the women with GDM. There were no cases of vitamin B12 deficiency in the control group. The GDM group had lower vitamin B12 levels (365.22 ± 136.82 pg/mL vs. 496.08 ± 156.46 pg/mL, P = 0.001). There was a negative correlation between serum vitamin B12 level and insulin resistance index (P = 0.001, r = -0.62). Folate levels were higher in the GDM group (17.93 ± 3.66 ng/mL vs. 14.60 ± 5.32 ng/mL, P = 0.001), but these levels did not significantly relate to insulin resistance or GDM.

Conclusions: In our region, there is a significant relationship between low levels of vitamin B12 and both insulin resistance and GDM, which suggests the need for larger studies and attention to the detection and treatment of vitamin B12 deficiency during pregnancy.

Keywords: Pregnancy, Gestational Diabetes Mellitus, Vitamin B12, Insulin Resistance, Folate

1. Background

Gestational diabetes mellitus (GDM) is defined as glucose intolerance diagnosed in the second or third trimester of pregnancy. Recognized risk factors for GDM include higher maternal age, obesity, a family history of type 2 diabetes mellitus, and ethnicity (1). Other risk factors comprise a previous history of GDM, having a prior baby weighing ≥ 4.1 kg, maternal birth weight being high or low, polycystic ovary syndrome, multiparity, excessive gestational weight gain, a diet low in fiber and high glycemic load, high fat and low carbohydrate intake, maternal hypertension before or early in pregnancy, and the use of antipsychotic agents (1).

Over recent decades, the urbanization of populations has led to an obesity epidemic, increased maternal age, and, consequently, a higher incidence of GDM. The prevalence of GDM has risen over the past two decades, and globally it stands at 14.0% (2). This prevalence varies according to geographic areas and diagnostic criteria (2). The Middle East and North Africa region report the highest prevalence, with a median of 27.6%, compared to a 7.1% prevalence in North America (2). According to the latest report, the average prevalence of GDM in Iran is 7.9%, but there is a considerable variation across regions;

the highest prevalence is in Tehran province at 23.99%, and the lowest is in Ardabil province at 1.33% (3). If poorly managed, GDM can adversely affect both the mother and fetus, increasing the risk of preeclampsia, preterm delivery, fetal death, neonatal hypoglycemia, macrosomia, and a heightened future risk of developing type 2 diabetes mellitus in the offspring (1, 4).

Given the severe maternal and fetal impacts of GDM, finding preventive measures is crucial. While numerous studies have examined the relationship between macronutrient intake, particularly carbohydrates and fats, and the risk of GDM, less information is available about the role of vitamins in the pathogenesis of GDM. It has been suggested that vitamin B12 and folate may influence the pathogenesis of GDM (5), and numerous studies have explored the relationship between vitamin B12 and GDM, some yielding conflicting results (6, 7). This relationship may vary according to geographic area (6) and genetic background (8).

2. Objectives

The primary aim of our study was to investigate the status of vitamin B12 and folate in pregnant women and their relationship to insulin resistance and GDM in our area. To the best of our knowledge, this is the first study of its kind in Iran.

3. Methods

This is a case-control study, in which 180 pregnant women at 24 - 28 weeks of gestation were enrolled. Half of the participants were diagnosed with GDM, and the other half were healthy individuals. The presence of GDM was assessed during outpatient follow-up between 24 - 32 weeks of gestation. After an overnight fast, blood glucose levels were measured in a fasting state and then at 1 hour and 2 hours after ingestion of 75 g of anhydrous glucose. Gestational diabetes mellitus was diagnosed if any of the following plasma glucose values, as recommended by the American Diabetes Association (9), were met or exceeded: Fasting: 92 mg/dL, 1 hour: 180 mg/dL, 2 hours: 153 mg/dL.

3.1. Inclusion Criteria

Pregnant women in their first or second pregnancy, aged 20 - 30 years, gestational age between 24 - 32 weeks, natural pregnancy, and single gestation.

3.2. Exclusion Criteria

Age below 20 or above 30 years, multiparity (≥ 3 parity), gestational age less than 24 weeks or greater than 32 weeks, pregnancies assisted by fertilization techniques, multiple gestation, any systemic disease including preconception diabetes mellitus, and consumption of vitamin B12 supplements during pregnancy. Taking folate supplements was not an exclusion criterion, as all participants and controls had used folate supplements. To minimize the confounding effect of weight on vitamin B12 levels and the risk of GDM, control participants were matched with patients in terms of body mass index (BMI) within a difference of ± 1 kg/m^2. The patients and controls were also matched by age within a difference of ± 2 years, gestational age within ± 2 weeks, and parity (1 or 2).

3.3. Sampling Process

During the period from June 2020 to April 2021, pregnant women who met our inclusion criteria (pregnant women in first and second pregnancy, age at 20 - 30 years, gestational age at 24 - 32 weeks, natural pregnancy, single gestation) and had GDM based on an oral glucose tolerance test (OGTT) were consecutively referred to maternal clinics of Shiraz University of Medical Sciences (SUMS). For each patient with GDM, the first consecutive pregnant woman with a normal OGTT who met our inclusion criteria and matched in terms of BMI difference within ± 1 kg/m^2, age difference within ± 2 years, and gestational age difference within ± 2 weeks was selected

3.4. Laboratory Measurements

Blood samples for vitamin B12, folate, insulin, and homocysteine were collected in a fasting state and stored at -70°C for a few months. Vitamin B12, folate, and homocysteine levels were measured using the ELISA method (Zell Biokit, Germany). Vitamin B12 deficiency was defined as a plasma B12 concentration of less than 200 pg/mL (< 150 pmol/L). The reference range for vitamin B12 was 200 - 675 pg/mL, and for plasma folate, it was 2 - 20 ng/mL. Plasma insulin was measured using the ELISA method (Monobond Co, USA). The homeostasis model assessment insulin resistance (HOMA-IR) was calculated using the equation (10): HOMA-IR = [Glucose (mg/dL) × Insulin (mU/L))]/405.

3.5. Ethical Considerations

Written informed consent was obtained from all participants. This study was approved by the ethics committee of Shiraz University of Medical Sciences, with the approval ID IR.SUMS.MED.REC.1398.335.

3.6. Statistical Analyses

Our consultant medical statistician calculated the sample size. Based on data reported in reference 19, with a 90% power, a 5% significance level, and an odds ratio for the development of GDM in vitamin B12 deficient patients of 2.59, the required sample size for each group was 80.

3.7. Statistical Methods

The Kolmogorov-Smirnov test was used to evaluate the normal distribution of data. Comparisons of mean values were conducted using the paired T-test. The Pearson correlation test was utilized to assess correlations between parameters. Multiple linear regression analysis was employed to evaluate the association between various factors and insulin resistance. All statistical analyses were performed using SPSS 21, and P values less than 0.05 were considered significant.

4. Results

There were no statistically significant differences between cases and controls regarding age, number of parity (1 - 2), BMI, and gestational age (Table 1). It was found that 15.6% of the GDM group had vitamin B12 deficiency, while no cases of vitamin B12 deficiency were observed in the control group. Folate levels were higher in the GDM group (Table 1). Homocysteine levels were also higher in the GDM group, and there was a significant negative correlation between vitamin B12 levels and homocysteine concentration (P = 0.022, r = -0.53). There was a strong negative correlation between serum vitamin B12 and the HOMA index in the GDM group (P = 0.001, r = -0.62).

There was no significant correlation between the HOMA index and other biochemical parameters in the GDM group. In the control group, the HOMA index did not correlate with serum vitamin B12 and folate (Table 2).

To explore the factors determining the insulin resistance index in the GDM group, associations between specific variables and the HOMA index were tested using multiple linear regression analysis (Table 3). It showed that serum vitamin B12, 1-hour blood sugar, insulin, and fasting blood sugar were responsible for more than 60% of the variation in the HOMA-IR index in the GDM group (R^2 = 0.61, P = 0.002). There was no significant association between the HOMA index and serum folate, B12, 1-hour blood sugar, insulin, and fasting blood sugar in the control group (R^2 = 0.27, P = 0.68), as shown in Table 3.

5. Discussion

The incidence of GDM has increased significantly in recent decades. Given its negative short- and long-term impacts on both the fetus and the mother, finding effective ways to prevent GDM is crucial (11). Current prevention programs emphasize diet and exercise (11). Over the past decade, there has been growing interest in the roles of vitamin B12 and folate in the pathogenesis of GDM (5). Vitamin B12 is critical for single-carbon metabolism (5). A low level of vitamin B12, in the presence of normal or high folate, results in the methyl trap phenomenon, i.e., it inhibits the conversion of inactive tetrahydrofolate to active 5-methyltetrahydrofolate (5). This methyl trapping leads to the inhibition of mitochondrial DNA synthesis (5) and affects the expression of microRNAs involved in lipogenesis in adipose tissue, which are linked to insulin resistance and the development of GDM (12, 13).

Vitamin B12 is also essential for converting homocysteine to methionine (5). A deficiency in vitamin B12 leads to decreased levels of methionine and increased levels of homocysteine. Methionine plays a role in DNA synthesis and its methylation; thus, its deficiency can cause impaired DNA methylation, which is associated with insulin resistance, type 2 diabetes mellitus, and GDM (14). Increased levels of homocysteine are linked to maternal and fetal complications (15). Vitamin B12 is necessary as a cofactor for the conversion of methylmalonyl-CoA to succinyl-CoA, a crucial component of the Krebs cycle (5). A deficiency in succinyl-CoA is linked to the inhibition of fatty acid oxidation, increased lipogenesis, and insulin resistance (5).

Many studies have reported a link between maternal vitamin B12 deficiency and the risk of GDM (6, 16, 17). However, in contrast to these findings, some investigations have reported no link between maternal vitamin B12 levels and GDM (18). One reason for this discrepancy may be the genetic background of the studied population. A recent study reported that certain polymorphisms in genes involved in one-carbon metabolism are linked to susceptibility to GDM in the presence of vitamin B12 deficiency (8).

Two recent meta-analyses have found that vitamin B12 deficiency increases the risk of GDM (6, 7). Chen et al., in a systematic review, reported that vitamin B12 deficiency in the first trimester of pregnancy had no relation with GDM, but in the second and third

Table 1. General Characteristics and Biochemical Studies in Both Gestational Diabetes Mellitus and Control Groups and the Related Comparisons

Variables	Control (N = 90)	GDM (N = 90)	P-Value
BMI (kg/m ^{2})	23.60 ± 1.80	24.28 ± 1.84	0.72
Gestational age (w)	28.23 ± 1.22	27.52 ± 1.53	0.62
Parity 1 or 2	1.56 ± 0.20	1.45 ± 0.30	0.75
Age	24.44 ± 2.47	25.23 ± 2.12	0.67
Folate (ng/mL)	14.60 ± 5.32	17.93 ± 3.66	0.001
Vitamin B12 (pg/mL)	496.08 ± 156.46	365.22 ± 136.82	0.001
1-hour blood sugar (mg/mL)	127.86 ± 27.88	194.06 ± 22.91	0.001
2 hours blood sugar (mg/dL)	98.51 ± 17.66	146.24 ± 21.41	0.001
FBS (mg/dL)	87.21 ± 7.92	103.86 ± 12.95	0.001
HOMA-IR	1.99 ± 1.61	6.88 ± 4.53	0.001
Insulin (mIU/mL)	19.57 ± 13.61	41.05 ± 24.77	0.001
Homocysteine (μmol/L)	5.06 ± 1.23	7.82 ± 1.72	0.0001

Abbreviations: BMI, Body Mass Index; HOMA-IR, homeostasis model assessment insulin resistance; GDM, gestational diabetes mellitus.

Table 2. Correlation Between Serum Biochemical Parameters in the Gestational Diabetes Mellitus and Control Groups

Group and Variables	B12 (pg/mL)	Folate (ng/mL)	1-hour BS (mg/dL)	FBS (mg/dL)	HOMA-IR	Insulin (mIU/mL)
GDM						
B12 (pg/mL)	-	P = 0.92; r = 0.01	P = 0.39; r = -0.13	P = 0.08; r = 0.26	P = 0.001; r = -0.62	P = 0.81; r = -0.03
Folate (ng/mL)	P = 0.92; r = 0.01	-	P = 0.08; r = 0.25	P = 0.6; r = 0.07	P = 0.15; r = -0.21	P = 0.49; r = -0.1
1-hour blood sugar (mg/dL)	P = 0.39; r = -0.13	P = 0.08; r = 0.25	-	P = 0.44; r = 0.11	P = 0.8; r = 0.02	P = 0.3; r = 0.15
FBS (mg/dL)	P = 0.08; r = 0.26	P = 0.6; r = 0.07	P = 0.44; r = 0.11	-	P = 0.19; r = -0.19	P = 0.14; r = 0.22
HOMA-IR	P = 0.001; r = -0.62	P = 0.15; r = -0.21	P = 0.8; r = 0.02	P = 0.19; r = -0.19	-	P = 0.86; r = 0.02
Insulin (mIU/mL)	P = 0.81; r = -0.03	P = 0.49; r = -0.1	P = 0.3; r = 0.15	P = 0.14; r = 0.22	P = 0.86; r = 0.02	-
Control						
BMI (kg/m^2)	P = 0.12; r = 0.11	P = 0.132; r = 0.978	P = 0.12; r = - 0.104	P = 0.185; r = -0.280	P = 0.273; r = 0.233	P = 0.262; r = -0.238
B12 (pg/mL)	-	P = 0.52; r = 0.09	P = 0.43; r = -0.14	P = 0.33; r = 0.07	P = 0.43; r = -0.12	P = 0.21; r = -0.24
Folate (ng/mL)	P = 0.52; r = 0.09	-	P = 0.33; r = 0.14	P = 0.95; r = 0.008	P = 0.38; r =0.13	P = 0.27; r = 0.16
1-hour blood sugar (mg/dL)	P = 0.43; r = -0.14	P = 0.33; r = 0.14	-	P < 0.001; r = 0.6	P = 0.56; r = 0.08	P = 0.24; r = 0.17
FBS (mg/dL)	P = 0.33; r = 0.07	P = 0.95; r = 0.008	P < 0.001; r = 0.6	-	P = 0.32; r = 0.15	P = 0.16; r = 0.21
HOMA	P = 0.43; r = -0.12	P = 0.38; r =0.13	P = 0.56; r = 0.08	P = 0.32; r = 0.15	-	P = 0.22; r = 0.18
Insulinm (IU/mL)	P = 0.21; r = -0.24	P = 0.27; r = 0.16	P = 0.24; r = 0.17	P = 0.16; r = 0.21	P = 0.22; r = 0.18	-

Abbreviations: BMI, Body Mass Index; HOMA-IR, homeostasis model assessment insulin resistance; GDM, gestational diabetes mellitus.

trimesters, it was linked to an increased risk of GDM (7), and this relation was stronger among Asians (7). Another systematic review of 10 studies associated vitamin B12 deficiency with an increased risk of GDM, noting that this risk varied geographically. Elevated vitamin B12 levels decreased the risk of GDM by 23% (6).

The results of our study align with most other similar studies and represent the first of their kind in Iran. A limitation of our study was the relatively small sample size, while its strength was that the BMI between patients and controls was matched, thereby removing the confounding effect of BMI on vitamin B12 levels, insulin resistance, and GDM risk. Vitamin B12 levels tend to be lower in obese pregnant women (19). The prevalence of vitamin B12 deficiency in our entire study population was 7.8%, and 15.6% in the GDM group, which is lower than in some other studies (19).

The GDM group exhibited lower mean vitamin B12 levels and higher folate levels, similar to some other studies (7, 20); however, the relationship between high folate levels and GDM remains controversial (6). The homocysteine level was higher in the GDM group. There was a significant negative correlation between vitamin B12 levels and homocysteine concentration because vitamin B12 is required for the conversion of homocysteine to methionine (5). Higher homocysteine

Table 3. Multiple Linear Regression Analysis of Covariates of Homeostasis Model Assessment in Both the Case and Control Groups

Group and Associated Factor	Beta	P-Value
GDM (R^2 = 0.61) (P = 0.002)		
B12 (pg/mL)	-0.01	0.001
Folate (ng/mL)	-0.26	0.11
1-hour blood sugar (mg/dL)	0.003	0.92
FBS (mg/dL)	-0.01	0.82
Insulin (mIU/mL)	-0.002	0.93
Control (R^2 = 0.27) (P = 0.68)		
B12 (pg/mL)	-0.001	0.43
Folate (ng/mL)	0.042	0.39
1-hour blood sugar (mg/dL)	-0.005	0.69
FBS (mg/dL)	0.038	0.36
Insulin (mIU/mL)	0.012	0.54

levels are associated with increased risks of intrauterine growth retardation, placental infarction, preeclampsia, neural tube defects, and insulin resistance (15).

Homeostasis model assessment insulin resistance, which is an index of insulin resistance, was negatively correlated with vitamin B12 levels in the GDM group. The mechanism of this correlation involves the requirement of vitamin B12 in one-carbon metabolism, which is involved in many metabolic pathways that influence lipogenesis and insulin resistance (5).

5.1. Conclusions

In pregnant women in our region, there is a significant relationship between lower levels of vitamin B12, insulin resistance, and GDM. This underscores the need for further, larger studies and attention to the detection and treatment of vitamin B12 deficiency during pregnancy. The definition of vitamin B12 deficiency in pregnancy may also need to be revised.

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Footnotes

Authors' Contribution: SZ did study design, data collection, statistical analysis, and manuscript preparation. MS studied concept and design, data collection, and preparing the manuscript and correspondence.

Conflict of Interests: The authors declare that they have no financial interest related to the materials in the manuscript.

Data Availability: The dataset presented in the study is available on request from the corresponding author during submission or after publication. The data are not publicly available due to ethical and privacy concerns.

Ethical Approval: Shiraz University of Medical Sciences Ethics Committee and the Vice-Chancellor of Research at this university approved this study (approval code: IR.SUMS.MED.REC.1398.335).

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