Original Research Article

THE ASSOCIATION BETWEEN MICRONUTRIENTS LEVELS AND GESTATIONAL DIABETES: A CROSS SECTIONAL STUDY IN ASHANTI REGION

ABSTRACT

SAMPLE ABSTRACT:

Aims: Micronutrients such as chromium, iron, magnesium and zinc can serve as good therapeutic and preventive agents for several diabetes complications including Gestational Diabetes Mellitus (GDM). The aim of this study was to compare the serum levels of chromium, iron, zinc and magnesium in pregnant women with and without GDM and to assess the association between the levels of these minerals and GDM.
Study design: A cross-sectional study.
Place and Duration of Study: Antenatal clinics in five selected hospitals in Ashanti region of Ghana between august 2016 to august 2017.
Methodology: A total of 50 pregnant women with GDM and 50 controls of the same gestational age participated in the study. Standard oral glucose tolerance test, fasting blood glucose, blood pressure and urine proteins were measured. Serum levels of chromium, iron, zinc and magnesium were measured using the atomic absorption spectrophotometer (AAS).
Results: The results indicated that 14% of the respondents with GDM had previous history of gestational diabetes. However, there was no previous history among the control group. Serum zinc was not significantly different in the two groups (0.052 ± 0.01 mg/dl; 0.093 ± 0.03 mg/dl, p=0.276). Iron levels in the test group were high (0.956 ± 0.35 mg/dl) when compared with the control group (0.635± 0.41 mg/dl). There was no significant difference of the serum magnesium level in the two groups (p=0.967). Chromium was higher in the test group (0.051±0.05) than the control group (0.012±0.06). There was a significant positive correlation between iron (r = 0.303, p = 0.000) and chromium (r = 0.302, p= 0.002) with the markers of GDM
Conclusion: This study established a positive correlation of iron and chromium with the markers of GDM while zinc and magnesium negatively correlate with the markers of GDM hence it is recommended that micronutrients supplementation during pregnancy needs to be carefully examined and commence only when significant deficiencies are observed.

Keywords: diabetes, gestational, insulin, iron overload, hypertension.

1. INTRODUCTION

Gestational diabetes mellitus (GDM) is a type of diabetes that manifests during pregnancy and normally disappears after delivering the baby. Research has shown that up to 8% of pregnant women between the gestational ages of 24 to 28 weeks develop gestational diabetes [1]. The placenta in pregnancy (the blood source for the baby) synthesizes useful hormones to help the baby grow and develop. Among these hormones are estrogen, cortisol and human placental lactogen that prevent or block the mother's insulin (insulin resistance) from performing its duty of transporting glucose in to cells. In pregnancy, to maintain the

normal levels of blood glucose, the pancreatic cells will have to produce up to 3 times the normal amount of insulin because of this insulin resistance. If the body is not able to achieve this, it might result in the development of GDM [1]. Research has shown that certain micronutrients can serve as good therapeutic and preventive agents for several diabetes complications including GDM. Abnormalities in the metabolism of magnesium, chromium, zinc and copper have been shown to relate to diabetes [2].

Chromium, as a trace element, is a member of the compounds called 'glucose tolerance factor' (GTF) which are required for appropriate metabolism of glucose and lipid sensitivity. It increases insulin sensitivity by inducing the binding of insulin to its receptors in cells and also increasing receptor numbers. Chromium enhances an increased beta-cell sensitivity and glucose utilization. A research conducted by Scott et al [3], has revealed that chromium concentration in serum of pregnant women were considerably lower than normal [3].

Recent research works has revealed that iron supplements and elevated iron stores is associated with increasing oxidative stress and gestational diabetes [4]. Consequently, while iron supplementation may lead to improvement in pregnancy outcomes when maternal iron levels are low, it is also possible that iron supplementation intended to prevent iron deficiency problems may have serious consequences when the woman's iron level is not low or she does not have iron deficiency [4]. There are so many molecular mechanisms that seek to explain these effects. These mechanisms are not completely understood but are said to include modulation of adipokines, oxidative stress and intracellular signal transduction pathways [4].

Zinc as a mineral impact a lot on pancreatic function as well as insulin secretion. It is believed that iron and folic acid supplementation during pregnancy has a negative influence on zinc absorption. Zinc deficiency impact negatively on carbohydrate metabolism. Studies has revealed how zinc deficiency associate with diabetes mellitus, glucose intolerance, cardiovascular disease and insulin resistance. Thus, Zinc supplementation in pregnancy is very important because it has been suggested to reduce the risk of some pregnancy complications [5].

In this current work we investigated and established the association between levels of micronutrients in serum and gestational diabetes among pregnant women.

2.0 Materials and Methods

2.1 Participants

This cross-sectional case-controlled study was restricted to pregnant women attending antenatal clinic at the St. Michael Hospital, Ejisu Government Hospital, Kumasi South Hospital and the KNUST Hospital all in Ashanti region of Ghana. These pregnant women were within the gestational age of 24 to 28 weeks. Convenient sampling technique was used till the required number was obtained. All those who qualify to participate in the study signed a consent form. Pregnant women who had already been diagnosed with gestational diabetes for the current pregnancy were included. Oral Glucose Tolerance test was done for the participating pregnant women. All participants were asked to have an unrestricted diet rich in carbohydrate for three days. They were then asked to fast overnight before tests were conducted. First, they were tested for fasting blood glucose after the 8-10 hours overnight fasting using glucometer. The participants were made to drink 100 g/ml of glucose solution. They were then tested for glucose levels using glucometer at 1, 2 and 3 hours afterward. The following were considered as abnormal values of glucose according to the American Diabetes Association [6].

I. Fasting blood glucose levels ≥ 95 mg/dL (5.33mmol/L).
II. 1 hour post-prandial blood glucose level ≥ 180 mg/dL (10mmol/L).

III. 2 hour post-prandial blood glucose level ≥ 155 mg/dL (8.6mmol/L).
IV. 3 hour post-prandial blood glucose level ≥ 140 mg/dL (7.8mmol/L).

For the participants who recorded abnormal values of glucose, the test was repeated after one week to rule out false positives. Those who had abnormal OGTT values were chosen for the study as those with GDM. Those that were normal and matched with those with diabetes were chosen as the control group. A total of 50 pregnant women with GDM and 50 controls of the same gestational age were recruited for study. A structured, pre-tested questionnaire were used to solicited information such as age, occupation, educational background, tobacco use, alcohol intake, previous history of GDM, family history of diabetes and nutritional supplementation of the participants. Ethical approval for this project was sought from the Committee on Human Research Publication and Ethics of Kwame Nkrumah University of Science and Technology (CHRPE/AP/015/17).

2.2 Micronutrients analysis

Four (4) ml of blood was taken from each participant in to vacutainer tube by trained hospital phlebotomist. The serum was separated by centrifuging at 3500 g for 10 min and stored at -20^{0}C prior to micronutrient analysis. After the total number of participants was reached, the samples were digested by mixing (0.5) ml of serum with deionized water in a digestion flask. Five milliliters of Nitric-perchloric acid (5 ml, 1:1 v/v) and 5 ml of sulphuric acid were added to each sample in a fume chamber. The samples were then heated on a heat plate at 2000^{o}C±5 for 30 minutes and allowed to cool. Measurement for chromium, iron, magnesium and zinc was done using the atomic absorption spectrophotometer. The sensitivity of methods for measuring the macronutrients was 1ppm.

2.3 Statistical analysis

The data was analyzed using the SPSS 20 (IBM, USA). Continuous variables were expressed as means and standard deviation while categorical variables were expressed as percentages. The significance of the differences in the mean values between study group and controls for normally distributed parameters were determined using the independent t-test for continuous variables and Chi-square test for categorical variables at 95% confidence level. Pearson correlation coefficients were calculated to signify the association between different quantitative variables. P values <0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

3.0 Results

A total of 100 pregnant women participated in the study of which 50 were with GDM as the study group and 50 were without gestational diabetes mellitus (NGDM) as the control group. Table 1 summaries the demography and clinical characteristics of the participants. With the study group, age, family history of GDM, previous history of diabetes, the use of alcohol and tobacco, systolic and diastolic blood pressures compared to the control group were not significantly different. The urine glucose level of the participant presented in Table 3 shows that the control group had no trace of glucose in their urine. In the GDM group 30% had no trace while 38% of them have 2+ of urine glucose. About 2%, 18% and 12% had trace, 3+ and 4+ respectively of urine glucose. The urine glucose levels were significantly different in the two groups (P=0.000). The urine protein of the participants is presented in Table 4. The findings revealed that 80% of the test group and the entire control group had no proteins in their urine. It was also shown that 12%, 6% and 2% of the participant with GDM had trace, 2+ and 3+ of urine protein respectively. There was statistically significant difference between the two groups (p=0.001).

Table 4 shows the serum micronutrients including chromium, iron, magnesium and zinc levels in the study group and control group. There was no significant difference in the levels of zinc and magnesium in the two groups (p=0.278; p= 0.967). However, there was significant difference in the iron and chromium levels for the two groups (p=0.000; p=0.002). Table 5 shows the correlation of the measured minerals with the markers of GDM. The results show a weak and an inverse relationship between FBG and serum zinc level (r = -0.041, p=0.276). There was a weak and insignificant correlation with zinc. Magnesium also showed a weak and an inverse or negative relationship (r = -043, p= 0.967) with FBG. There was positive correlation between serum chromium and fasting blood glucose (r = 0.302, p= 0.002). Serum iron also correlated positively with fasting blood glucose (r = 0.303, p = 0.002).

Table 1: Demographic and clinical characteristics of the participants.

Subjects	**GDM Group N (%)**	**Normal group N (%)**	**P-value**
Age	30.98 ± 4.89	30.66 ± 5.14	0.682
Family history of GDM			
YES	7 (14.0)	1 (2.0)	0.059
NO	43 (86.0)	49 (98.0)	
Previous history of GDM			
YES	5 (10.0)	0 (0.0)	0.056
NO	45 (90.0)	50(100.0)	
Alcohol use			
YES	2 (4.0)	5 (10.0)	0.436
NO	48 (96.0)	45 (90.0)	
Tobacco use			
YES	0 (0.0)	0 (0.0)	
NO	50 (100.0)	50 (100.0)	
SBP	121.3 ±18.6	122.0 ± 15.1	0.823
DBP	68.2±17.7	68.9 ± 13.8	0.816

Table 2: Markers of GDM in the participants.

Classification	**Women with GDM n (%)**	**Women without GDM n (%)**	**P-value**
WC	**109.0 ± 6.93**	**97.18 ± 5.13**	**0.000**
OGTT			
1-hr	14.40 ± 2.26	8.98 ± 0.54	0.000
2-hr	12.51 ± 2.01	7.00 ± 0.67	
3-hr	10.42 ± 1.74	5.02 ± 0.61	
FBG	7.90 ±1.8	4.39 ± 0.41	0.000

Table 3 Urine glucose and protein level in the participant's urine

Classification	**Women with GDM**	**Women without GDM**	**P-value**

	n (%)	n (%)	
Glucose			
Normal	15 (30)	50 (100)	0.000
Trace	1 (2)	0 (0)	
2+	19 (38)	0 (0)	
3+	9 (18)	0 (0)	
4+	6 (12)	0 (0)	
Protein			
Normal	40 (80)	50 (100)	0.001
Trace	6 (12)	0 (0)	
2+	3 (6)	0 (0)	
3+	1 (2)	0 (0)	

Table 4 Serum micronutrients levels in the participants.

Macronutrients	GDM Group	Control Group	P–value
Chromium	0.05057	0.0124	0.002
Iron	0.9561	0.6348	0.000
Magnesium	1.9792	1.9740	0.967
Zinc	0.05152	0.09302	0.276

Table 5 Pearson correlation of the measured minerals and gestational diabetes

	Zinc	Magnesium	Chromium	Iron
FBS	-.041	-.043	.302**	.303**

3.1 DISCUSSION

The main aim of this study was to find out the possible association between the serum micronutrients concentration in the development of gestational diabetes. This will help to better understand their role in the development and complication of gestational diabetes. Gestational diabetes is a very complex metabolic disorder that can be associated with several factors. The causes of GDM are multifactorial which include environmental factors affecting insulin sensitivity and genetic factors.

In this study information on socio-demographic characteristics in Table 1 showed that the mean age for pregnant women with gestational diabetes was 30.98 years while that of the controls was 30.66 years. These findings were consistent with two previous studies [5, 7], which revealed higher mean ages for the people with diabetes compared to the controls. This study also shows that the family and previous history of diabetes was high among the study group compared with the control group (Table 1). Risk factors such as previous macrosomic neonates, family history of GDM and previous medical history of GDM increase the rate of developing diabetes and hence pregnant women with these factors should be screened early for gestational diabetes mellitus [7]

Information from the standard oral glucose tolerance test (OGTT) conducted revealed that 40% of the women with GDM had severe diabetes and 60% were mild diabetic (Table 2). All the participants used as controls recorded normal blood glucose levels. These findings

agree with others who report increasing OGTT values in pregnant women with GDM [8]. This study showed no significant differences in the blood pressures of the study group and the control group (Table 1). This finding contradicts with that of Monique et al. [9], who showed that the blood pressure of persons with GDM is higher than controls.

Chromium is a micronutrient that is required in small quantity in the body and plays a very important role in human nutrition. Regulation of blood glucose levels is among the several importance of chromium in the body. In our study, it was revealed that there was a significant decrease in the levels of chromium in the pregnant women without GDM (Table 4). This was different from other findings by Ghosh et al. [10], which show that chromium levels in non-diabetic Indians people where higher compared to those with diabetes. Scientist believe that chromium is a co-factor that assist insulin to get to the cell membrane and aid in the transport of glucose in to the cell [11]. Long before now, scientist used to believe that to achieve this, the body will have to convert chromium to glucose tolerance factor which is a large chemical. However current research has shown that there is no glucose tolerance factor but chromium acts with a protein known as low molecular weight chromium –binding substance to aid in the action of insulin. This means that chromium's role is to assist insulin to bind to its receptors and its deficiency in pregnancy can result into abnormal glucose tolerance and insulin resistance leading to GDM.

The study revealed that the serum iron level in pregnant women with GDM was significantly higher than the pregnant women without GDM. These findings were in line with the study conducted by Muhammad et al. [12] that showed that serum iron levels were significantly higher in pregnant women with abnormal glucose tolerance when compared with those with normal glucose tolerance. A study by Ford and Cogswell [13], has shown that elevated iron stores are associated with high frequency of diabetes. Lao et al. [14], has shown in an observational study consisting of 762 Chinese women without diabetes and with singleton pregnancies, selected between 28 to 30 weeks, that the group with a higher haemoglobin level had a significantly elevated incidence of gestational diabetes as well as greater iron concentration. These findings confirm the observation of our study of higher iron levels in the women with GDM than the control group. In another study involving 1023 women revealed a double increase in the risk of GDM for women with high level of serum ferritin at entry and almost three times higher in the third trimester [6]. This positive association shows that iron store may contribute to the development of gestational diabetes mellitus by inducing oxidative stress on the pancreatic cell.

Magnesium is an important metal that is involved in several levels of insulin production, its binding and activity. Magnesium is also a critical co-factor of several enzymes involve in carbohydrate metabolism. In this study, there was no significant difference in the serum magnesium levels for the pregnant women with and without diabetes. These findings are similar to the finding of Walter et al. [15], which indicated no significant difference in the plasma magnesium levels of people with diabetes and the control group. Previous studies have shown low levels of magnesium in pregnant women with GDM than control group [16, 17, 18]. In the current study, there was also a negative correlation between the serum magnesium levels with fasting blood glucose. Karim et al. [19] and Mishra et al. [20], also found a negative correlation between serum magnesium levels and fasting blood glucose in diabetes. This association indicates the role it plays in the development and progression of diabetes. The differences in the results regarding the correlation of serum magnesium levels and glycaemic control may be as a result of difference in study design and also population characteristics.

This study showed that the serum zinc levels of pregnant women with gestational diabetes are significantly lower when compared with the pregnant women without gestational

diabetes. These findings agree with other studies [21]. The decreasing levels of zinc in diabetes may be associated with hyperzincuria and also lower gestational absorption of zinc.

4. CONCLUSION

This cross-sectional study evaluated the serum levels of some micronutrients in pregnant women with gestational diabetes. Base on the standard oral glucose tolerance test that was conducted, 40% of the study group were having severe forms of poor glycaemic control. The study also identified family history of diabetes, previous history of gestational diabetes and alcohol usage as risk factors to the development of gestational diabetes mellitus. Family and previous history of diabetes which was recorded in the study group also increase the chances of GDM. The study showed that serum iron was significantly higher in the pregnant women and correlated with the development of gestational diabetes mellitus ($p=0.00$). Our study also revealed that the serum iron levels correlated with the development of gestational diabetes. There were no significant differences of the serum magnesium level between the study group and the controls ($p=0.967$). Magnesium also showed a very weak and negative correlation with the development of gestational diabetes mellitus. Serum chromium levels was significantly high in the study group compared to the control group ($p=0.002$) and positively correlated with the development of GDM. The findings of this work have revealed that serum zinc levels were not significantly different in the two groups ($p=0.276$). Serum zinc levels inversely correlate with the development of gestational diabetes mellitus.

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

AAS: Atomic Absorption Spectrophotometry
GDM: Gestational Diabetes Mellitus
GTF: Glucose Tolerance Factor
IGT: Impaired Glucose Tolerance
NDSS: National Diabetes Service Scheme
NGDM Non-Gestational Diabetes Mellitus
OGTT: Glucose Tolerance Test