
ORIGINAL ARTICLE**Association between Vitamin D, calcium and phosphorous deficiency with increased glycosylated fibronectin levels and susceptibility of early trimester maternal gestational diabetes mellitus**

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Abstract

Background: Gestational Diabetes Mellitus (GDM) is a more often seen long standing disease in pregnancy. It is expected that maternal deficiencies in important micronutrients can have ill effects on fetal development and pregnancy outcome. Glycosylated Fibronectin (GlyFn) is a positive biomarker that has been extensively studied in the context of pre-eclampsia and GDM over the past decade. **Aim and Objectives:** The present study aimed to evaluate the relation between GlyFn and serum levels of calcium, phosphorous, and Vitamin D in pregnant women, to understand potential metabolic links relevant to the pathogenesis of GDM. **Material and Methods:** This case control study was conducted at Rajarajeshwari Medical College and Hospital, Bengaluru. Eighty-eight pregnant women were recruited from the out-patient department. The Oral Glucose Tolerance Test (OGTT) of participants was done at 24 to 28 weeks. Based on OGTT value, participants were divided into GDM group and control group. Blood sample was collected to estimate GlyFn, calcium, phosphorous and Vitamin D. **Results:** The mean value of phosphorous, calcium and vitamin D was lowered while GlyFn was raised in GDM group compared to control group. **Conclusion:** A negative correlations observed in GDM group in this study imply that calcium, phosphorus and vitamin D metabolism has direct influence on variations in GlyFn levels.

Keywords: Gestational diabetes mellitus, oral glucose tolerance test, glycosylated fibronectin, micronutrients

Introduction

Millions of gestating mothers worldwide suffer with Gestational Diabetes Mellitus (GDM), a common chronic illness [1, 2]. Hyperglycemia initially identified in the period of gestation is known as GDM. [3]. Women experienced remarkable inflammatory reactions that results in insulin resistance and macrosomia, or excessive fetal

growth. [4-6]. Pregnancy is an extremely sensitive time that affects the long-term health of both expectant mothers and their unborn offspring [7]. The foetus's primary organs develop during the first trimester, making it a crucial stage of pregnancy [8]. As a result, the growing fetus depends entirely on the mother's surroundings for sustenance [9]. The

regulation of blood calcium levels and the preservation of strong bones are two of vitamin D's most well-known functions [10]. Vitamin D has been associated with a number of critical pregnancy activities, including glucose homeostasis, placental function, inflammatory response, and infection control [11].

Preeclampsia, GDM, and small-for-gestational-age are among the unfavourable pregnancy outcomes that are linked to vitamin D insufficiency [12, 13]. All the macro and micronutrients needed for healthy fetal growth are provided by the mother's diet and nutrient store, which are very much needed for successful pregnancy [14]. Therefore, it is anticipated that maternal deficits in important micronutrients may have detrimental consequences on the growth of the fetus and the outcome of the pregnancy [15]. Gestating mother with GDM have reduced levels of calcium and vitamin D [16,17]. By impairing insulin signal transduction and reducing the function of the Glucose Transporter-4 (GLUT4), changes in calcium in main insulin target tissues may contribute to peripheral insulin resistance [18].

Over the past ten years, Glycosylated Fibronectin (GlyFn) a promising biomarker has been thoroughly investigated in relation to pre-eclampsia and GDM. In particular, glycosylated variations have been connected to impaired endothelial function and vascular remodelling. GDM and preeclampsia are linked to these alterations, especially during spiral artery remodelling in the first trimester [19]. These pathophysiological events are correlated with elevated levels of circulating GlyFn early in pregnancy, which makes it a useful diagnostic tool. Minerals like calcium, phosphorus and hormone vitamin D play a very important role in the maternal health. So, the altered levels of these might result in

GDM. In the present scenario it is very necessary to detect GDM at the earliest and intervene with nutritional supplements. Hence our study aimed to explore the association of vitamin D, calcium, and magnesium levels with the tendency for development of GDM.

Material and Methods

This case-control study was conducted at Raja Rajeswari Medical College and Hospital, Bangalore, Karnataka, India, with institutional ethical clearance no. RRMCH-IEC/220/2023. In the year 2023, on the basis of average patient inflow to the Outpatient Department (OPD), the sample size was calculated using Yamane's formula. The estimated total sample size was 90. Participant recruitment occurred from January 2024 to February 2025.

Women who were less than 45 years of age presenting with healthy singleton pregnancy which was conceived spontaneously were recruited following written informed consent. Subjects with prior bariatric surgery, known pre-existing diabetes or current metformin treatment, chronic infections (e.g., hepatitis, HIV), or chronic kidney/liver/heart disease, history of hypertensive disorders treated with prophylactic aspirin, fetal abnormalities requiring intervention and inability to comprehend participant information were excluded [20].

Between 6th and 7th month of gestation, participants underwent an Oral Glucose Tolerance Test (OGTT). Blood glucose levels ≥ 140 mg/dL two hours after consuming 75g of glucose were classified as GDM according to the diabetes in pregnancy study group criteria (DIPSI) [21]. Forty-four women were assigned to the GDM group and 45 to the control group based on the sample size of 90. One GDM participant was removed from the study because she declined to provide a sample. The

laboratory expert collected 5 mL of venous blood in a serum Separator Tube (SST tube), allowed it to clot, and spun it at 3000 rpm for 20 minutes after receiving written consent and filling out a questionnaire with demographic data. Serum was isolated and kept at -80°C . Vitamin D, calcium, and phosphorus quantities were measured using an automated analyzer, while GlyFn was measured using an ELISA method. Glycosylated fibronectin was estimated by ELISA method whereas Vitamin D, Calcium and phosphorus was estimated by Automated analyzer. (Roche Cobas)

Statistical analysis

The control and GDM groups' mean values for several parameters were compared using the unpaired t test. The chi-square test was used to evaluate the relationship between the amounts of GlyFn and the biochemical parameters calcium, phosphorus, and vitamin D. The negative association between GlyFn and calcium, phosphorus, and vitamin D was examined using the Pearson correlation coefficient test.

Maternal characteristics

Mean age of GDM group was 24.61 years, while the control group was 23.82 years. The mean OGTT of GDM group was 166.43 mg/dL, while the control group was 116.57 mg/dL. For both groups, the mean duration of pregnancy was around 25.6 weeks. The GDM group had raised amount of GlyFn ($p < 0.001$). The GDM group had less amount of calcium, phosphorus, and vitamin D than the control group; however, the levels of calcium were not statistically significant ($p = 0.503$), whereas the quantities of vitamin D and phosphorus were statistically significant ($p < 0.001$) (Table 1). Chi-square test was used to find the relationship between the levels of calcium, phosphorus, and vitamin D with normal and increased GlyFn levels (Table 2). The chi square test results with p value, chi square value and degree of freedom are summarized in (Table 2). According to chi square analysis based on chi square value, degree of freedom and p value the association between glycosylated fibronectin and calcium, phosphorus was statistically significant.

Table 1 : Maternal characteristics by GDM status

Parameters	GDM (n = 44)	Control (n = 45)	p
Age (years)	24.61	23.82	0.0479
OGTT (mg/dL)	166.43	116.57	0.0001*
Gestational weeks	25.70	25.55	0.5895
GlyFn (ng/mL)	31.65	27.03	<0.001*
Vitamin D (ng/dl)	20.86	29.66	<0.001*
Calcium (mg/dL)	1.414	1.570	0.5033
Phosphorus (mg/dL)	2.875	3.945	<0.001*

*Statistically significant; OGTT – oral glucose tolerance test

Table 2: Chi-square test and Pearson correlation coefficient between GlyFn, calcium, phosphorous and vitamin D

Glycosylated fibronectin (GlyFn)	Calcium		Phosphorous		Vitamin D		
	Normal	Deficient	Normal	Deficient	Normal	Deficient	Insufficient
Normal	1	44	44	1	25	19	1
Increased	11	33	26	18	36	8	0
Chi-square	9.8947		19.83		4.9976		
df	1		1		2		
p	0.00165		0.0000084		0.0241		
Significance	Significant		Highly significant		Significant		
Interpretation	Strong association with GlyFn		Very strong association with GlyFn		No statistically meaningful association with GlyFn		

Table 3: Pearson correlation coefficient results

GlyFn	Calcium	Phosphorous	Vitamin D
r	-0.12991	-0.187	0.0133
p	0.4039	0.224	0.931

But the association between Glycosylated fibronectin and vitamin D was not statistically significant. The negative correlation between GlyFn and calcium, phosphorus, and vitamin D was examined using the Pearson correlation coefficient test; the r values for the calcium and phosphorus groups were -0.12991 and -0.187, respectively, indicating a strong negative correlation, while the r value for the vitamin D group was 0.0133, indicating no correlation. The calcium and phosphorus group's negative r value indicates that as GlyFn rises, the GDM group's calcium and phosphorus levels fall. However, the vitamin D group did not reveal this type of negative relation, indicating that there was no correlation (Table 3).

Discussion

According to our research, there is a statistically significant ($p < 0.05$) correlation between calcium levels and GlyFn. This implies that fluctuations in GlyFn are strongly correlated with changes in calcium levels. Additionally, a robust correlation between phosphorus levels and GlyFn was found, with a highly statistically significant result ($p < 0.001$). GlyFn and vitamin D levels were significantly correlated negatively ($p < 0.05$). The study shows that compared to healthy controls, those with GDM had noticeably lower levels of calcium, phosphorus, and vitamin D. Elevated levels of GlyFn accompany these decreases, indicating a possible inverse relationship between these crucial

biochemical markers and GlyFn. These results emphasize how crucial it is to keep an eye on and control micronutrient levels in GDM patients in order to potentially enhance outcomes for both the mother and the developing foetus. It has been demonstrated that normal pregnant women have far greater serum vitamin D levels than pregnant women with GDM [22]. Pregnancy-related vitamin D insufficiency may impact insulin secretion and raise insulin resistance, which raises the risk of GDM [23]. After controlling for seasonal variables, Australian cohort research revealed that serum 25-(OH)-D levels < 30 nmol/L in the middle of pregnancy were more prone to develop GDM [24]. According to a study by Khagorika *et al.* (2023) health education and simple access to vitamin D testing and supplementation at various healthcare facilities are essential because vitamin D levels were found to be below normal even in adults [25]. In contrast to pregnant women in good health, women with GDM discovered to have considerably reduced serum levels of both calcium and vitamin D [26, 27]. According to these studies, cosupplementing with calcium and vitamin D might be capable of treating GDM. As demonstrated by the lower levels of fasting plasma glucose, serum insulin, and LDL, a meta-analysis verified that vitamin D-

calcium supplementation might enhance the metabolic profile for the treatment of GDM [28]. Phosphorus is an essential micronutrient involved in a number of required biological processes. A study demonstrated a significant association between mineral levels and the risk of GDM, particularly for iron, magnesium, manganese, phosphorus, and zinc. However, folate and phosphorus showed only a negligible impact on GDM risk when evaluated using odds ratios. Additionally, the mean levels of magnesium and phosphorus were not found to be statistically significant [29]. Research conducted by Masoodi *et al.* (2015) further indicated that both low dietary intake of vitamin C and reduced plasma vitamin C concentrations are associated with an increased risk of GDM [30].

Conclusion

To the best of our knowledge, this study simultaneously evaluates vitamin D and micronutrients such as calcium and phosphorus within a single investigation. It also examines the likelihood of developing GDM and highlights the role of micronutrients during pregnancy. Therefore, it provides guidance on nutritional requirements in pregnancy that may help reduce and prevent the incidence of GDM.

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