EFFECTS OF VITAMIN D SUPPLEMENTATION ON GENE EXPRESSION IN PATIENTS WITH GESTATIONAL DIABETES MELLITUS

ABSTRACT

Background: Gestational diabetic mellitus (GDM) increases maternal and infant morbidity worldwide. The pathogenesis is unknown and believed to result from interactions between genetic and environmental factors. Maternal vitamin D deficiency earlier reported in GDM patients might influence important metabolic processes in GDM.

Objective: This study was undertaken to determine the effect of vitamin D supplementation on the expression of selected genes that are linked to GDM namely insulin release and insulin receptor substrate 1(IRS1), transcription factor 7- like 2 (TCF/L2), Hepatocyte Nuclear Factor 4 Alpha (HNF4A) and Adiponectin, C1Q and Collagen Domain (ADIPOQ) genes.

Methods: This is an interventional study, involving 180 participants between the ages of 18 to 40 years. They were grouped into 3: group A were 60 Glucose tolerant women (Control) ,group B: 60 freshly diagnosed GDM women not to be given Vitamin D as group B, group C: 60 freshly diagnosed GDM patients to be given vitamin D supplements . Women in Group C were given oral Vitamin D supplements (Puritan's Pride Vit.D3) of 1000 IU per day for 8 weeks from the time of recruitment at 24th week. Blood samples of the participants were taken randomly at 32nd week and metabolites quantified by standard laboratory methods.
Results: The result of the study shows that HNF4A and IRS1 and TCF/L2 genes were highly expressed while ADIPOQ gene was poorly expressed ($P<0.05$) in GDM patients when compared with control. However, vitamin D supplementation down regulated significantly the expression of HNF4A, IRS-1 and TCF/L2 genes while it up regulated significantly the expression of ADIPOQ gene in these patients.

Conclusions: This study shows that vitamin D supplementation could play significant role in the management and prevention of GDM by influencing specific genes associated with GDM.

Keywords: Gestation diabetes mellitus, Genes, Vitamin D, Pregnant Women.

1. INTRODUCTION)

Gestational diabetic mellitus (GDM) is a metabolic disorder with chronic hyperglycaemia and carbohydrate intolerance which is first recognised during pregnancy [1]. The global prevalence of GDM was 14.7% in 2021 based on the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria [2]. GDM impact the health of both mother

and child negatively when undiagnosed, pregnant women with GDM often have increased risk of miscarriage, hypertensive disorders or pre-eclampsia, macrosomia, low birth weight of fetus, operative delivery and postpartum haemorrhage [3]. The underlining mechanism of GDM includes impaired beta-cell function and decreased insulin sensitivity. Risk factors of GDM includes family history of type 2 diabetes, advanced maternal age, polycystic ovarian syndrome, and exposure to toxic factors [4]. The offspring often experience premature birth, neonatal respiratory distress syndrome, hypoglycaemia and also impaired glucose metabolism in their early years which make early diagnosis very vital [5].

There are a number of associated genes which are involved in the various stages of glucose regulation with a functional relevance to GDM. Notable among them are transcription factor 7- like 2 (TCF7l2), hepatocyte nuclear factor 4 alpha (HNF4α) which are related to beta-cell function and insulin secretion respectively. Others include, insulin receptor substrate 1(IRS1) which is responsible for regulation of blood glucose level [6], adiponectin, C1Q and collagen domain (ADIPOQ) gene which is expressed in adipose tissue and coded for a protein with similarity to collagen X, VIII and complement factor C1Q which circulates in plasma and influenced metabolic and hormonal processes [7]. Changes to this gene have been reported to cause adiponectin deficiency, a protein that is believed to have anti-inflammatory and insulin-sensitizing properties. Increased level of circulating adiponectin has been linked to lower incidence of diabetes [8]. Mutation to this gene or presence of variants at the gene locus could play key role in the pathogenesis of GDM. Vitamin D deficiency has been reported to be implicated in the pathogenesis of GDM in Nigeria women [9]. A recent study also shows that vitamin D supplementation improved markers of hyperglycaemia via increased insulin secretion and reduced insulin resistance in type 2 diabetes mellitus [10]. Therefore, this study sought to investigate the role of Vit. D supplementation on the genetic expression of genes implicated in the development of GDM.

2. MATERIAL AND METHODS

2.0 Participants

A total of 180 pregnant women were recruited from the Antenatal clinic of Obstetrics and Gynecology department of University College Hospital, Ibadan, Oyo State, Nigeria. They were randomly grouped into three, 60 Glucose tolerant group (Control) as group A, freshly diagnosed GDM women not given Vitamin D as group B, 60 freshly diagnosed GDM patients to be given vitamin D supplementation as group C. Participant considered to have GDM were subject with values of Oral Glucose Tolerance Test (OGTT) of ≥ 180mg/dL. Fasting ≥ 126 mg/dL (5.3 mmol/L), 1 hour ≥ 180 mg/dL (10.0 mmol/L), 2 hour ≥ 153 mg/dL (8.6 mmol/L), and 3 hour ≥ 140 mg/dL (7.8 mmol/L) , according to World Health Organization (WHO), 2014. Glucose tolerant group (Control) were women with normal serum glucose levels >100 mg/dL (7.2 mmol/L). Blood pressure, weight, height were measured and body mass index (BMI) calculated in all of the pregnant women using a standard analog sphygmometer, weighing balance and meter rule, respectively. Anthropometric measurements were assessed, body weight was measured in an overnight fasting status, without shoes and in minimal clothing state, using a bathroom scale to the nearest 0.1kg. Height was measured using a non-stretched tape measure to the nearest 0.1cm. BMI calculated as weight in kg divided by height in meters squared.

Inclusion Criteria for Cases and Control

Newly diagnosed women with gestational diabetes and apparently healthy pregnant women at the second trimester were recruited for the study

Exclusion Criteria for Cases and Control

Apparently healthy pregnant women with family history of diabetes in first-degree relatives. Women with GDM in previous pregnancy, occurrence of any form of pre-pregnancy diabetes, presence of concomitant systemic disease (chronic or acute or infectious), taking insulin or any oral hypoglycemic medications and/or vitamin D supplementation were not recruited for the study as test subjects.

2.1 Data Collection

Ethical clearance was gotten from the University College Hospital's Ethic and Research committee. Informed consent was obtained from the participants (subjects and control) after the study guidelines had been explained to them before clinical history was taken and anthropometric indices using structured questionnaire.

2.2.Supplementation with Vitamin D

Women in Group C were given oral Vitamin D supplements (Puritan's Pride Vit.D3) of 1000 IU per day for 8 weeks from the time of recruitment at 24th week. They were closely monitored and followed up all through the period of supplementation to ensure usage of the supplement.

2.3 Sample Collection

Blood samples of the selected participants were randomly taken after supplementation at 32nd week. Fasting Venous blood (5mls) was collected from each participant by phlebotomist using a syringe after issuing them the consent form. 5mls of the blood was dispensed into plain vacutainer and 2ml of DNA/RNA shield was added to the plasma in order to preserve the DNA/RNA from rupturing and the blood from clotting. Then all the samples refrigerated and gene expression analysis was done 24hours later.

3.0 Biochemical Analysis

3.1 Extraction of RNA

The Total RNA was extracted from the stored blood sample using commercially-available RNA extraction Kit, Direct-Zol™ RNA MiniPrep as described by [11].

3.2 Gene Expression Analysis

The gene expression of ADIPOQ, IRS-1, HNF4A, TCF7L2 in all the three studied groups, were analyzed using reverse transcription-PCR (RT-PCR) assay, the assay was performed using an optimization Template (cDNA) .Reactions (50 µL final volume) contained 5 µL of cDNA, 2 µL each of sense and antisense primers, 200 Mm of each deoxynucleotide, 5 µL of 10×Taq polymerase buffer, and 1.25 U Taq polymerase. The cycling conditions were : 94°C pre-denaturation for 5 min, 94 °C for 30 s, annealing 55 °C for 30 s and Extension 72 °C for 30 s and then 5 min at 72 °C by 30 cycles using Eppendorf Master cycler Hamburg. PCR products were separated by electrophoresis in 2% agarose gels and visualized under blue light transilluminator. The product intensities were quantified by computer using image mRNA expression levels and normalized to that of the housekeeping gene, β-actin.

Table 1: Showing the Forward and Reverse Primers of the Selected Genes.

Gene	Forward primers	Reverse primers
IRS1	5'-ACCGTCAGTAGCTCAACTGGACAT-3'	3'–GGGTACCCATGAGTTAGAAGAGGA -5'
TCF7L2	5'-GGGACGCTACTCAACACTTAAT-3'	3'-GACGACATACAGGTACGACAAG-5'
HNF4A	5'-TACTCCCAGGCACTGTCTTA-3'	3'-GGGAGGGAGGAGGAGAATAAA-5'
ADIPOQ	5'-GAACTCCTGACCTTGTGATCTG-3'	3'-CTCACCTCTTCATCCCTCTCTA-5'

4. STATISTICAL ANALYSIS

The distribution of the analyzed anthropometric and expression data gotten from gel images were quantified by densitometry method using Image J launcher (version 1.4.3.7). Data were subjected to statistical analysis using Graph pad prism version 7. The results obtained were also grouped and expressed as mean ± Standard Deviation (SD). Two-way analysis of variance (ANOVA) was used to compare variable across the three groups. Student t-test was used to compare variables between two groups and p value < 0.05 was considered significant.

5. RESULTS

Table 2 : Anthropometric Data of GDM and Glucose Tolerant women

Parameters	Glucose Tolerant Group (n=60)	GDM Group (n = 120)	P Value
Age (years)	32.60±3.66^{a}	31.00±2.36^{a}	0.775
Height (m)	1.62±0.06^{a}	1.63±0.06^{a}	0.624
Weight (kg)	70.30±0.40^{a}	82.70±9.90^{b}	0.022**
BMI (kg/m^2)	26.83± 0.91^{a}	31.20± 3.71^{b}	0.031**
PPW(kg)	66.10 ±19.36^{a}	75.30±12.19^{b}	0.240
Waist Circumference (cm)	115.00±12.97^{a}	112.90±7.06^{a}	0.658

Values are mean± Standard Deviation. Values of the same subscript within the same column are not statistically different at (p>0.05) between control and the case groups, while values with different subscript are significantly different at (p<0.05). PPW- Pre pregnancy weight.

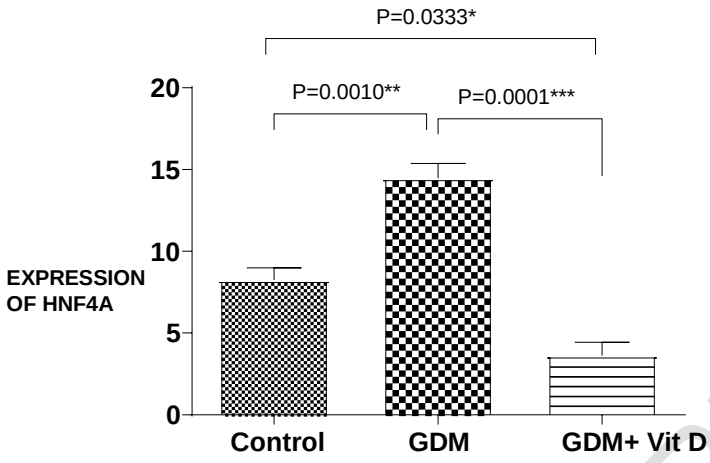

Figure 1: Expression of HNF4A gene in the Control, unsupplemented and Supplemented Groups. GDM +Vit D = GDM patients given vitamin D supplementation

Expression of HNF4A was significantly higher ($p<0.05$) in the GDM group when compared with control. There was a significant reduction ($p<0.05$) in the expression of HNF4A in the Vit. D supplemented GDM group when compared with the nonsupplemented GDM group.

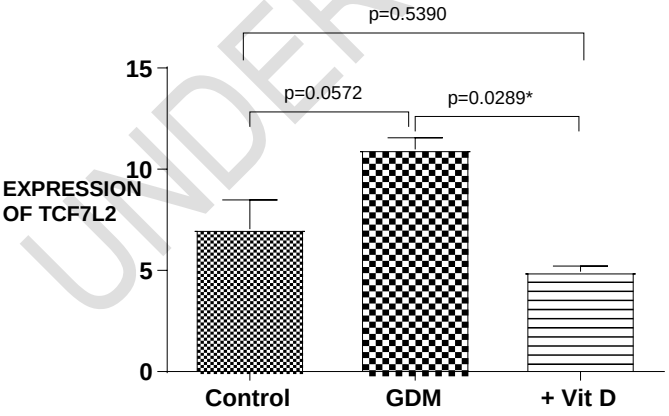

Figure 2: Expression of TCF7L2 gene in the Control, unsupplemented and Supplemented Groups.

There was a significant increase in expression of TCF7L2 gene ($p<0.05$) in the GDM unsupplemented group when compared with control. There was also a significant reduction($p<0.05$) in the expression of TCF7L2 gene in the Vit. D supplemented GDM group when compared with the nonsupplemented GDM group.

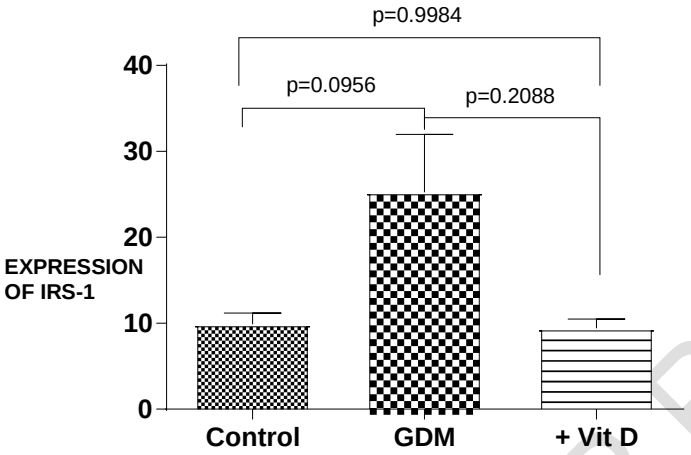

Figure 3: Expression of IRS-1 gene in the Control, unsupplemented and Supplemented Groups.

There was an insignificant increase in the Expression of IRS-1 in the unsupplemented GDM group ($p>0.05$) when compared with control. There was an insignificant reduction ($p>0.05$) in the expression of IRS-1 in the Vit. D supplemented GDM group when compared with the nonsupplemented GDM group.

6. DISCUSSION

Till date, the pathogenesis of GDM has not been fully understood but its risk factors has been linked to both genetic and environmental factors. The results of this study as shown figure 1, 2 and 3 reveals significant expression of Hepatocyte Nuclear factor 4 alpha (HNF4α), IRS-1 and TCF/L2 genes in GDM patients. This result is similar to the work of [12] who reiterated the notion that TCF7L2 encodes a transcription factor, which is involved in Wnt signaling, an important pathway that regulates glucose homeostasis [12].
Vitamin D supplementation down regulated the expression of these genes in the supplemented group. HNF4α is a nuclear transcription factor which has been found to increase the risk and susceptibility to GDM as reported by [13]. In pancreas, HNF4A directly influences the expression of genes for insulin, and glucose transporter, GLUT2 (Glucose Transporter 2) [13]. Significant expression of IRS1 and TCF7L2 genes have earlier been reported in type 2 diabetes mellitus patients which share similar mechanism with GDM and also linked to beta-cell function and insulin secretion [14]. These genes are reliable predictor of Type 2 diabetes mellitus and may likely increase susceptibility to GDM considering the similarity in the underlining mechanism of both diseases [15]. Down regulation of this gene as a result of vitamin D supplementation could improve beta-cell function and therefore increase insulin secretions which can subsequently help in the management of these patients and associated complications[16].

Interestingly, vitamin D supplementation up regulated the expression of ADIPOQ gene in GDM patients to a significant level. This gene encodes adipocytes-derived protein adiponectin which has antioxidant, insulin sensitizing, anti-inflammatory and atheroprotective properties and subsequently decrease the risk of macrovascular complications and also enhance the absorption of glucose in muscles of diabetic patients [17]. Adiponectin was also found to inhibit Ucp1 gene expression in mice adipocyte which is associated with the risk of diabetic complications [18]. Up regu-lation of this gene as shown in this study supports the probable role of Vitamin D in the management of GDM complications. The potential role of vitamin D in the man-agement of GDM is proposed to be through its role in calcium homeostasis and insulin secretion which might be facilitated by the presence of a VDRE in the human insulin receptor gene promoter region hence influencing insulin action [19].

Another possible mechanism by which Vitamin D exerts its effect is by binding as a ligand to vitamin D receptor (VDR), that functions as a transcription factor thus producing an heterodimer with the retinoid X receptor (RXR). The VDR/RXR complex recognizes vitamin D-responsive elements (VDRE), a direct tandem repeat of two hormone response element in the regulatory regions of target genes [20].

6.1. CONCLUSION AND RECOMMENDATION

The positive effects of vitamin D supplementation on the expression of genes associated with GDM ,as revealed by this study is a pointer that Vitamin D is implicated in the pathogenesis of GDM and also has the potential to modulate progression of GDM at genetic level. Diet and supplements rich in Vitamin D are encouraged before conception, during pregnancy and after childbirth. More studies involving large population sizes are important to establish the findings of this present research work.

7. STATEMENTS

7.1. ETHICAL APPROVAL

Written informed consent was duly signed by all participants. The study protocol has been approved by the research institute's committee on human research and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki." Ethical Clearance was issued by Institute of Medical Research and Training (IMRAT) in the University College Hospital. Ibadan. Nigeria.

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