Scientific Research Publishing

Open Journal of Obstetrics and Gynecology, 2025, 15(9), 1557-1578
https://www.scirp.org/journal/ojog
ISSN Online: 2160-8806
ISSN Print: 2160-8792

A Randomized Control Trial Comparing Vitamin D Supplementation versus Placebo on Glycemic Control in Gestational Diabetes Mellitus

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How to cite this paper: Sandaruwan, N.K.T.S., Perera, Y.A.G. and Motha, C. (2025) A Randomized Control Trial Comparing Vitamin D Supplementation versus Placebo on Glycemic Control in Gestational Diabetes Mellitus. *Open Journal of Obstetrics and Gynecology*, **15**, 1557-1578.
https://doi.org/10.4236/ojog.2025.159130

Received: August 28, 2025
Accepted: September 22, 2025
Published: September 25, 2025

Abstract

Introduction: The definition of Gestational Diabetes (GD) is glucose intolerance of variable degree with onset or first recognition during pregnancy. Prevalence is 7% - 14% and high in South Asians. It is the most common metabolic disease in pregnancy and is associated with high maternal, fetal, and neonatal morbidity as well as mortality. Increased peripheral insulin resistance and impaired insulin secretion occur in affected individuals. Regulation of insulin secretion is influenced by hormones as well as circulating fuels like glucose. Recent studies have shown that there are other factors which play a role in insulin secretion, such as vitamin D. **Objectives:** This study was carried out to compare the efficacy of Vitamin D (oral) supplementation versus placebo on the control of blood sugar in gestational diabetes. Our hypothesis was that Vitamin D supplementation would increase insulin release and improve glycemic control in pregnant women with gestational diabetes. Monitoring for any adverse effects of vitamin D supplementation was also an objective. **Methodology:** This was a randomized, double-blind, placebo-controlled clinical trial. The study setting was the antenatal clinic of Colombo North Teaching Hospital, Ragama, Sri Lanka (a major obstetric unit in Sri Lanka) during the period of June 2018 to January 2019. Each arm contained 24 persons and the total sample size was 48. After excluding the subjects according to the exclusion criteria, the rest of the patients were recruited to the trial. The randomization was done by block randomization using web-based software and the sealed envelope method was used. For the intervention group, oral Vitamin D (Al-

facalcidol-0.25 mg) was given once daily during the intervention for 4 weeks. A placebo was given to the control arm. FBS and 1h PPBS values were taken before entry into the study and after 4 weeks. The FBS and 1-hour PPBS levels in the two groups' pre-intervention and post-intervention were compared. The results were analyzed by SPSS software using its standard statistical tools. Mothers and newborns were monitored for adverse outcomes until discharged from the hospital after delivery. **Results:** The mean age was 29y, the mean POA at entry was 26 weeks, and the mean BMI was 26 in the study group. The supplementation of oral vitamin D did not show any significant improvement in glycemic control, as measured by the change of FBS (p & lt; 0.26), and the change of 1h PPBS (p & lt; 0.17) in gestational diabetes. There were no adverse reactions found due to vitamin D supplementation during the antenatal period. There were no major congenital defects found in newborns. **Conclusions and recommendations:** Vitamin D supplementation in GD did not improve glycemic control (FBS or 1 h PPBS) significantly. No adverse reactions were observed among patients during vitamin D treatment. Vitamin D did not cause any major adverse effects to the fetus. There is an unmet need for RCTs in this area and large trials with a greater number of cases should be conducted. Future trials should address normal BMI, overweight patients, and obese patients separately in the supplementation of Vitamin D.

Keywords

Gestational Diabetes, Vitamin D, Supplementation in Pregnancy, Pregnancy, Glycemic Control

1. Introduction

The definition of Gestational Diabetes (GD) is glucose intolerance of variable degree with onset or first recognition during pregnancy [1]. This is associated with high maternal, fetal, and neonatal complications during and after the pregnancy, respectively.

Prevalence of GD is roughly 7% - 14% [2] and higher in South Asians and Mediterranean populations, and said to be as high as 14% - 20% [3] according to some studies. Risk factors for GD include high maternal age, obesity, past history of GD, family history of diabetes mellitus, and etc. GD is diagnosed by OGTT. There are other risk factors said to play a role in causing GD, such as vitamin D deficiency, and it is now concluded by several meta-analyses [3] as a well-identified risk factor for causing gestational diabetes.

Among all GD cases identified during pregnancy, about 8% - 10% are caused by preexisting diabetes mellitus. In these cases, insulin (4% - 8%) may also be used for blood sugar control. Medical nutrition therapy as well as oral hypoglycemics like metformin may also be used to control blood sugars during the pregnancy period. Medical nutrition therapy alone will control 80% of cases, and drugs need

to be given to about 20% of cases to optimize blood control.

GD is associated with pre-eclampsia, obstructed labor, high LSCS rate, and also high retinopathy and neuropathy. Adverse fetal outcomes are miscarriages, congenital anomalies, pre-term labor, polyhydramnios (25%), macrosomia (25% - 40%), IUGR, and unexplained IUD. Adverse neonatal outcomes are polycythemia, jaundice, hypoglycemia, shoulder dystocia, other birth injuries, and respiratory distress syndrome. These neonates have a higher risk of developing type 2 DM and metabolic syndrome in later life.

Pathophysiology of GD:

GD is the most common metabolic disease in the pregnancy period. Increased peripheral insulin resistance and impaired insulin secretion occur in affected individuals. However, the exact pathophysiology is not known yet. The body's skeletal muscles become resistant to insulin. Increased insulin secretion occurs as anti-insulin hormones also increase during pregnancy (200% - 250%). This happens to maintain the euglycemia of the mother. GD results when the maternal pancreas cannot secrete an adequate amount of insulin to counteract the glycemic load.

Insulin is a polypeptide having two chains called A (21 amino acids) and B. They are linked by two disulfide bonds. Synthesis of insulin occurs in the cytoplasm in the RER. Two inactive precursors are preproinsulin and proinsulin. These are cleaved to form the active hormone and the C polypeptide. Insulin is stored as granules in the cytoplasm. These are released by exocytosis. The T1/2 of insulin is 6 minutes and it is degraded by enzymes in the liver mainly and by the kidneys.

Regulation of insulin secretion occurs by hormones as well as circulating fuels like glucose. Glucose, amino acids, and GI hormones cause an increase in insulin secretion. Glucose is the primary stimulus for insulin secretion. Beta cells detect high blood sugar levels. They contain GLUT2 transporters and also have the Glucokinase enzyme. Glucokinase is not inhibited by its product, which is glucose-6-phosphate. High glucose-6-phosphate leads to high energy production as ATP, and it increases insulin secretion. Glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP) increase insulin production by Beta cells.

Glucose-dependent release of insulin into the bloodstream is Ca^{2+} dependent. For this to happen, it needs a rise in intracellular Ca^{2+} concentration. High ATP leads to the closure of ATP-sensitive K channels and the cell membrane will be depolarized. Then voltage-gated Ca^{2+} ions will open and lead to Ca^{2+} influx. This high intracellular Ca^{2+} level leads to exocytosis of insulin granules.

Recent studies have shown that there are some other factors which play some role in insulin secretion, such as vitamin D [2]-[4]. It has been suggested that the circulating active form of vitamin D binds to vitamin D receptors of the beta cell of the pancreas and modulates insulin secretion. Second, 1,25-dihydroxyvitamin D3 increases insulin receptor expression and hence increases insulin sensitivity. Third, 1,25-dihydroxyvitamin D3 regulates the balance between extracellular and

intracellular calcium pools within the beta cell.

Vitamin D is a fat-soluble vitamin. They are a group of sterols. They have hormone-like functions. The active one is 1,25-dihydroxyvitamin D3. The vitamin D and receptor complex alters gene expression. The most prominent action is that vitamin D regulates plasma calcium and phosphates.

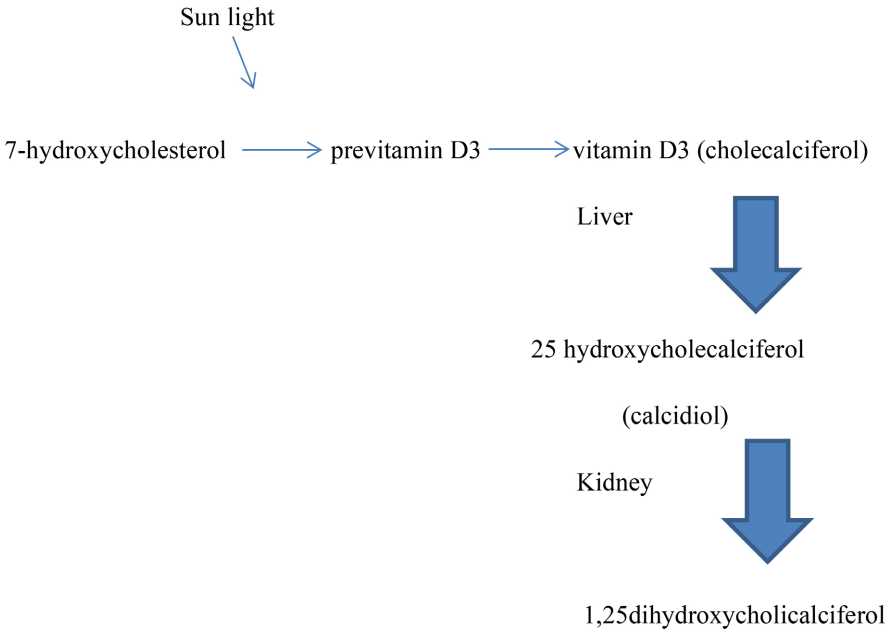

Figure 1. An illustration of vitamin D synthesis in the human body.

Ergocalciferol is vitamin D2 and is found in plants; cholecalciferol is vitamin D3 found in animals. These are preformed dietary vitamin D types and may be given in deficiency. Vitamin D2 and D3 are not active. Calcidiol is the major storage form of vitamin D and the major form of circulating vitamin D. Vitamin D synthesis in the body is shown in **Figure 1**.

Functions of vitamin D are to increase calcium absorption by the intestine, increase calcium reabsorption by the kidney, and increase bone calcium reabsorption. Vitamin D is naturally found in liver, fatty fish, and egg yolk. The RDA recommended daily intake is 15 μg/d, and above 70 y is 20 μg/d. (1 μg is equal to 40 IU).

Vitamin D insufficiency is defined as a 25 (OH) D concentration of 20 to 30 ng/mL (50 to 75 nmol/L). Vitamin D deficiency is defined as a 25 (OH) D level less than 20 ng/mL (50 nmol/L) [5].

Toxicity of vitamin D is due to its ability to be stored in the liver and tissues and its slow metabolism. High doses, such as 100,000 IU for weeks or months, may cause toxicity. Signs and symptoms are LOA, nausea, thirst, stupor, hypercalcemia, and calcification in the organs.

In pregnancy, intestinal calcium absorption is enhanced. Vitamin D toxicity may be manifested through hypercalcaemia and hypercalciuria. So there is a hy-

pothetical concern when secondary hyperparathyroidism follows vitamin D deficiency. Therefore, calcium supplementation given with vitamin D may be associated with transient hypercalcaemia. However, this is self-limiting due to the associated hungry bone and has not become a clinical problem so far.

In hypervitaminosis D, hypercalcemia, and malabsorption, use may be cautious.

Ergocalciferol is in FDA pregnancy category C (it is not known whether ergocalciferol will harm the fetus). Ergocalciferol can be secreted into breast milk and may harm a nursing baby. Cholecalciferol (vitamin D3) is not placed into any pregnancy drug category. Within the recommended dose, it is considered a safe (400 - 600/d) drug.

During T2 and T3 supplementation up to 1000 IU/d did not show any adverse effect on the fetus. Supravalvular aortic stenosis, craniofacial abnormalities, and dental abnormalities in infants and children with idiopathic hypercalcemia can occur.

Hypoparathyroidism women who took a high amount (50,000 - 250,000) of vitamin D have not ended up with bad outcomes. No cardiovascular or craniofacial abnormalities.

RDA dose for lactating mothers is 400 IU - 600 IU, usually given for vitamin D deficiency. It is considered that cholecalciferol or vitamin D3 is safe during breastfeeding by the American Academy of Pediatrics.

2. Literature Review

According to a recently conducted systematic review and meta-analysis [4] of observational studies in 2013, which was published in the British Medical Journal, it was concluded that vitamin D deficiency is a cause of an increased risk for gestational diabetes. Two authors collected data, and among 3,357 studies, 31 studies were selected and analyzed in the research. Two investigators separately selected articles, with acceptance given for analysis. Special concern was taken regarding study design, use of a comparison group, gestational age checked, and quantification methods. In one analysis, GD was found to have an association with vitamin D deficiency compared with the comparison group, odds ratio 1.49 (95% confidence interval 1.18 to 1.88).

The second analysis was also done and showed that mothers with GD had a significantly lower level of vitamin D (25OHD) in comparison to the control group. (Pooled weighted mean difference -7.36nmol/L, 95% CI - 10.16 to -4.56 nmol/L). It also suggested sufficient evidence that vitamin D deficiency is common during pregnancy, and vitamin D supplementation may be a simple intervention to reduce the risk of GD and to improve plasma glucose control in GD patients.

Some studies, like gene array studies in cells, showed that 1.25 dihydroxycholicalciferol (1.25 (OH) 2D) regulates many genes in the body, and it may be around 5% of the human genome.

Some evidence shows vitamin D leads to increased insulin sensitivity by affecting and increasing glucose transport. It is also suggested that vitamin D may play a role

in early placentation and development, as it has a role in gene expression as well.

The limitations that can be seen in this analysis are the lack of adjustment for confounding factors, various studies having different cut-off values as a deficiency of vitamin D, and some case-control studies may lead to overestimation of deficiency. This study has conducted a comprehensive review on studies and suggested further research with vitamin D supplementation.

Another recent meta-analysis [3] of observational studies conducted in 2015 has studied 20 researches. The outcome is significant. It provided strong evidence that vitamin D deficiency is associated with increased risk of GD. Women with GD had a decreased level of vitamin D in comparison to the control group, which is 4.93 nmol/L. This also suggested that vitamin D deficiency may be an independent risk factor for GD, considered as other risk factors like maternal age, pre-pregnancy BMI, race, and family history.

Their conclusion was a consistent association between vitamin D deficiency and increased risk of GD. They also suggested an RCT on vitamin D supplementation in gestational diabetes.

One study done recently in Turkey and published in 2015 in African Health Sciences has taken 21 consecutive mothers with GD and 43 pregnant mothers with no GD (age and BMI matched), and vitamin D levels were checked in all. It was found that 76% [6] of GD mothers have severe vitamin D deficiency (below 10 nmol/L). In the discussion, they showed a clear association between vitamin D deficiency and increased risk of GD.

Limitations of that study are having small sample sizes, unequal sample sizes, and not supplementing with vitamin D and following up the vitamin D deficient mothers after diagnosing deficiency can be considered.

Another RCT study conducted [7] in 2014 and published in Diabetes Care investigated vitamin D supplementation (high and low dose) and examined the effect on glucose metabolism. Oral 5000 IU vitamin D3 was given daily to 98 mothers, and 400 IU (recommended dose) was given to the other arm. This study was conducted on women with vitamin D levels < 32 ng/ml, diagnosed before 20 weeks of gestation. Their primary endpoint was OGTT at 28 weeks, and no differences were observed between the two arms.

In that study, both arms were provided with vitamin D, and that may improve any deficiency already present, which might lead to improvement of both groups' OGTT. They could have done this with another set of mothers who would not be provided with vitamin D and could have compared with that arm. That might lead to more controversial results than this.

In this study, only mothers with less than 20 weeks are included as eligible criteria, and increased insulin resistance during the 3rd trimester will not be assessed.

Another drawback of this research is that they cannot obtain vitamin D deficient mothers and cannot use them as a third arm, as it is unethical not to treat them after identification.

Another remarkable finding of this study is that 5000 IU of vitamin D was well

tolerated by mothers and improved neonatal vitamin D deficiency.

Another study done and published in 2013 in the American Journal of Clinical Nutrition [8] evaluated the effect of vitamin D supplementation on glucose metabolism and oxidative stress in GD. Vitamin D3 50000 IU was given orally 2 times during the study versus a placebo given to mothers. Each arm contained 27 mothers and the total was 54. Fasting blood samples were analyzed at baseline and after 6 weeks of treatment and quantified the effect.

As a result of that study, there was a significant reduction in the concentration of fasting blood glucose level (−17 ± 14.8 compared with −0.9 ± 16.6 mg/dL; $P < 0.001$). Serum insulin level also decreased according to their findings.

According to their findings [8], the prevalence of vitamin D deficiency among pregnant women is 18% - 84%.

3. Hypothesis

Vitamin D supplementation will increase insulin release and improve the glycemic control of pregnant women with gestational diabetes.

4. Objectives

4.1. General Objectives

The study is designed to assess the effect of vitamin D supplementation on the metabolic status and glycemic control of pregnant women with Gestational Diabetes.

4.2. Specific Objectives

To measure the FBS levels in two groups and compare the reduction of FBS.

To measure the 1-hour PPBS (post-breakfast) levels in two groups and compare them between the two groups.

To monitor and describe any adverse effects of vitamin D supplementation.

5. Methods and Study Design

5.1. Study Design

This is a randomized, double-blind, placebo-controlled clinical trial.

5.2. Study Setting

Antenatal clinics of Colombo North Teaching Hospital, Ragama, Sri Lanka

5.3. Study Period

2016 July to 2019 January.

5.4. Participants

5.4.1. Interventional Group

Cases will be pregnant mothers aged between 18 - 40 years, diagnosed as GD by

OGTT according to World Health Organization (WHO) and International Federation of Gynecology and Obstetrics (FIGO) recommendations 2015—FBS-92 mg/dl, 2 h 140 mg/dl—during 24 - 28 POG, undergoing BSS, who will be on Medical Nutrition Therapy or on Oral Anti-Diabetics (metformin) and taking vitamin D (Alfacalcidol 0.25 mg daily).

Inclusion Criteria

Pregnant women aged 18 - 40 years, and diagnosed as GD by 75 g OGTT. This will be done between 24 - 28 weeks of gestation. Gestational age will be calculated according to the last regular menstrual period by using Naegele's rule, and it will be confirmed by USS (CRL or HC) within 10 to 14 weeks of gestation. Patients will be only on medical nutritional therapy and/or metformin. Age, BMI, and ethnicity will be matched. Informed written consent will be taken.

Exclusion Criteria

PPROM, existing liver or kidney diseases, placental abruption, PIH or pre-eclampsia, who are already diagnosed as Type 2 DM, who need to be started on insulin during intervention or are already on insulin treatment, who smoke, diagnosed patients with hypothyroidism, calcium or vitamin D metabolism disorders, who were already on vitamin D supplementation.

5.4.2. Control Group

Controls will be pregnant mothers aged between 18 - 40 years, diagnosed as GD by OGTT during 24 - 28 POG (WHO & FIGO recommendations 2015: FBS 92 mg/dl, 2h 140 mg/dl), undergoing BSS, who will be on Medical Nutrition Therapy or on Oral Anti-Diabetics (metformin).

Inclusion Criteria

Pregnant women aged 18 - 40 year, and diagnosed as a GD by 75 g OGTT. This will be done between 24 - 28 week gestations. Gestational age will be calculated according to the last regular menstrual period by using Naegele's rule, and it will be confirmed by USS (CRL or HC) within 10 to 14 weeks of gestation. Patients will be only on medical nutritional therapy and/or metformin. Age, BMI, and ethnicity will be matched. Informed written consent will be taken.

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5.5. Sample Size Calculation

Sample size calculation was done using the previous literature [1] [9].

According to an RCT [1] done in 2013, a 16% difference was shown in the change of fasting plasma glucose between the intervention and placebo groups. To demonstrate a similar change with a study power of 80% at a significance level

of 5%, the sample size calculation was done using Winpepi software.

The SD's in the intervention and control groups were taken as 15.27 and 21.53, respectively [1]. With an intervention to control ratio of 1:1, a total sample size of 48 was calculated, with 24 in each study arm.

5.6. Sampling Method

Forty-eight participants who fulfilled the eligibility criteria and who gave consent were selected from pregnant mothers attending the antenatal clinics of the Colombo North Teaching Hospital for Women.

5.7. Method of randomization and allocation concealment

Block randomization was done using a computer-generated sequence using a web-based application [10]. Block size has been taken as 6. The generated sequence was used with the sealed envelope method. Recruitment and allocation would be done by two different medical officers, leading to allocation concealment.

5.8. Intervention

Vitamin D was supplemented to the intervention arm. That was Alfacalcidol (Vitamin D3) 0.25 mg and given once daily for 4 weeks. This was the vitamin D type available in the government health services.

The interventions had been planned to withdraw if any woman developed an allergic reaction to vitamin D or intolerability to vitamin D, or if she developed any liver or kidney disease.

Placebo was the same size, same color tablet (folic acid—a different tablet from the usual folic acid tablet).

Measures had been taken to improve adherence to the study, where participants were educated about the benefits and additional effects, such as improving immunity, helping to prevent osteoporosis, and preventing cancer and pre-eclampsia, etc.

5.9. Outcome Measures

5.9.1. Primary Outcome

Fasting blood sugar (FBS) value and 1-hour PPBS value {post-breakfast value after an average morning diet that is not high in carbohydrates - PPBS (pb)}.

FBS and PPBS (post-breakfast) values were measured in all participants at the entry to the study on day 1 and after 4 weeks of intervention.

5.9.2. Secondary Outcomes

Educate the women about GD and MNT to increase awareness by the nutritionist.

Monitor any adverse effects of the vitamin D treatment. Craniofacial abnormalities of neonate, any allergic reactions to the vitamin D treatment, side effects such as kidney stones, confusion or disorientation, muscle weakness, bone pain, poor appetite, extreme thirst, nausea, vomiting, or constipation in the patient.

Optimization of glycemic control (Hypothesis) and hence achieving a good perinatal outcome after intervention.

After the intervention and time period, all the data have been collected and computerized for analysis.

5.10. Study Plan

Study plan has been illustrated in Figure 2.

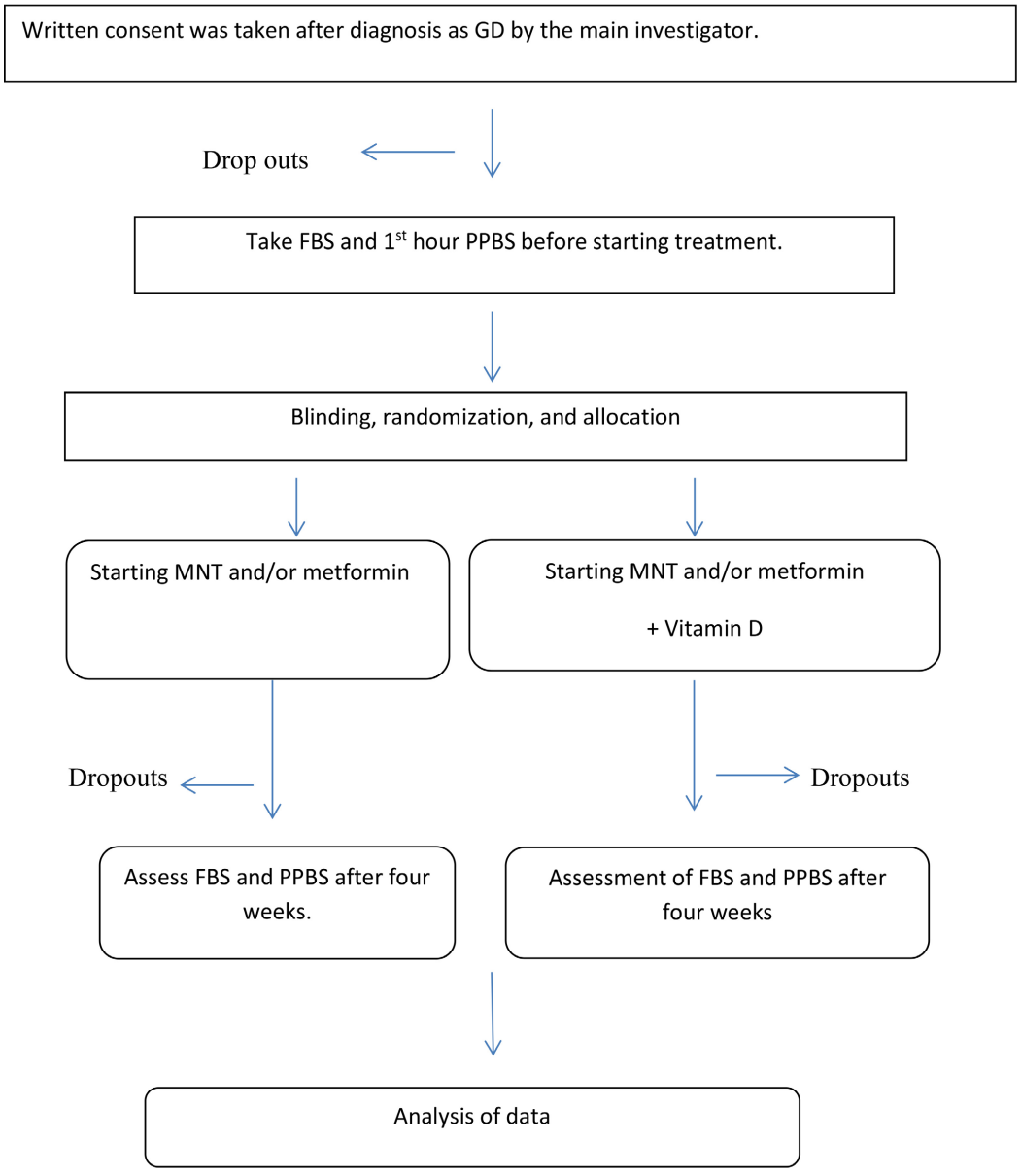

Figure 2. The study plan.

5.11. Definitions

OGTT (WHO and FIGO 2015 International Federation of Gynecology and Obstetrics) One-step diagnostic test. Perform 75 g OGTT with plasma glucose meas-

urement. Test in the morning after the patient has fasted for ≥ 8 hours and test 2 hours after the initial measurement.

Diagnosis was confirmed when plasma glucose levels meet or exceed (FIGO 2017).

Fasting 92 mg/dL (5.2 mmol/L).

1 hour: not taken.

2 hours: 140 mg/dL (7.8 mmol/L).

Table 1 shows the glycemic targets in pregnancy according to the FIGO guidelines.

Table 1. Glycemic targets in pregnancy according to FIGO guidelines.

	Gestational diabetes mellitus (GDM)
Fasting	≤93 mg/dL (5.3 mmol/L)
1-hr postprandial	≤140 mg/dL (7.8 mmol/L)
2-hr postprandial	≤120 mg/dL (6.7 mmol/L)

5.12. Measuring Variables

Study instruments.

The study was conducted using one weight scale and one height scale in the clinic to measure weight and height. Patients were asked to wear a minimal number of clothes when measuring weight and to be without shoes. The weight was measured to the nearest 1 kg and height to the nearest 1 cm.

A trained nurse measured the anthropometric measurements at the clinic setup.

Blood samples were collected at baseline and 4 weeks after the intervention in the early morning after an overnight fast and 1 hour PPBS (post breakfast).

Plasma glucose level was measured in the hospital laboratory using the same method and instruments, only by a trained medical laboratory technician.

No occasion led to breaking the Trial code.

5.13. Data Collection

Data had been collected on a specially designed data collection form and also as a softcopy, generated by computer.

5.14. Ethical Clearance

This study was carried out at Colombo North Teaching Hospital, Ragama. The Director's permission was obtained first. Ethical clearance was obtained from the Ethics Review Committee of the Faculty of Medicine, University of Kelaniya. The trial was registered in the Sri Lanka clinical trial registry. Informed written consent was obtained from both the study and control groups.

5.15. Time Frame

Figure 3 shows the time frame for the study. There was a delay compared to the

proposal due to modifications made during ethical clearance and during registration at the Sri Lanka clinical trial registry. Therefore, data collection started in July 2018 and the trial was finalized in March 2019. Recruitment and randomization is shown in Figure 4.

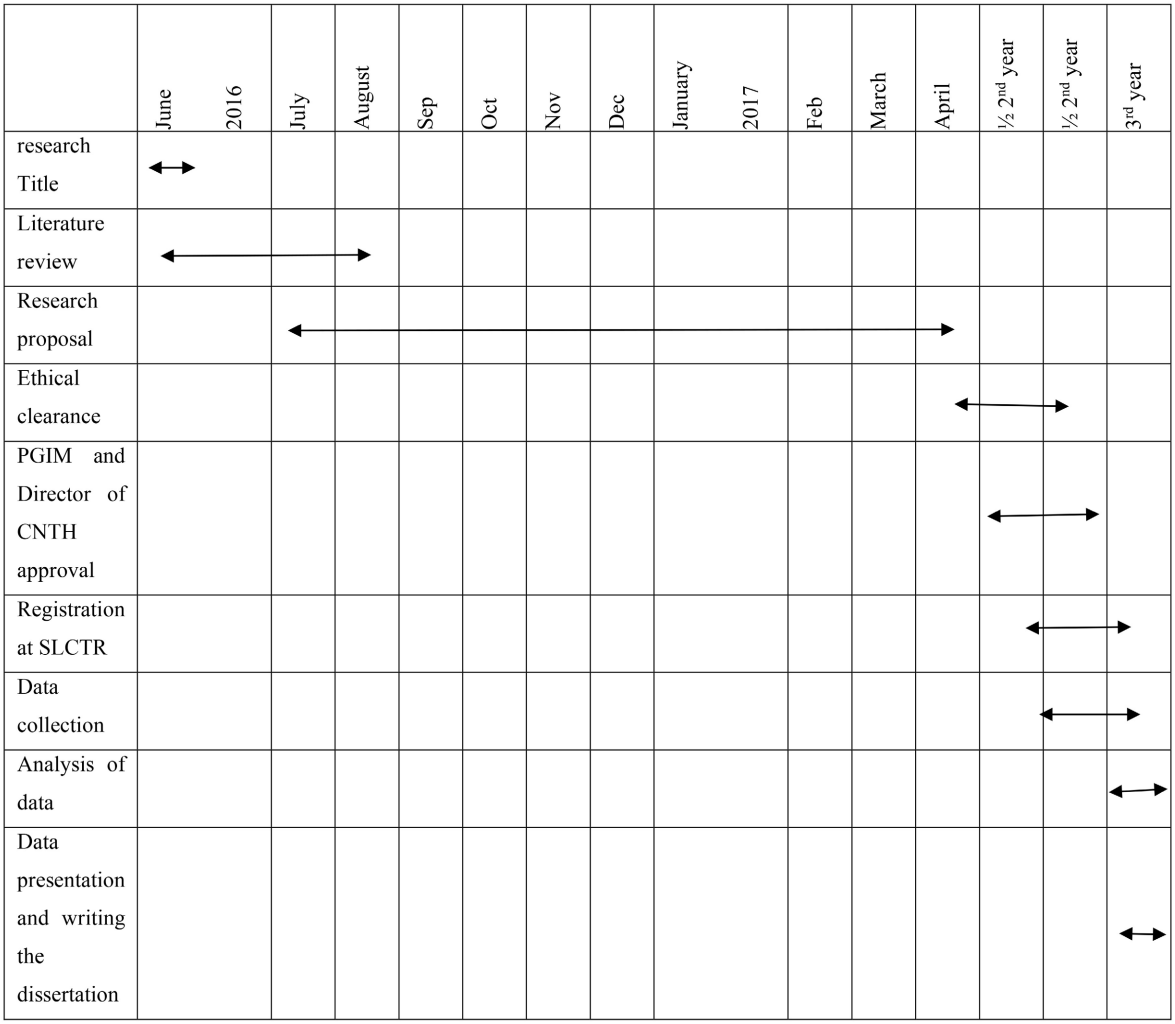

Figure 3. Time frame of the project.

6. Results

6.1. Statistical Analysis

Data were analyzed using SPSS statistical software (version 23). The demographic data, including age and BMI, were analyzed by simple means and standard deviation in the whole group, as well as in cases and controls. Continuous variables such as FBS and PPBS were analyzed by mean and standard deviation in the whole sample, as well as in cases and controls. Changes in FBS and PPBS in cases and controls were analyzed using the independent sample "T" test, and the significance value (p) was taken as 0.05. All documentation was done using Microsoft Word.

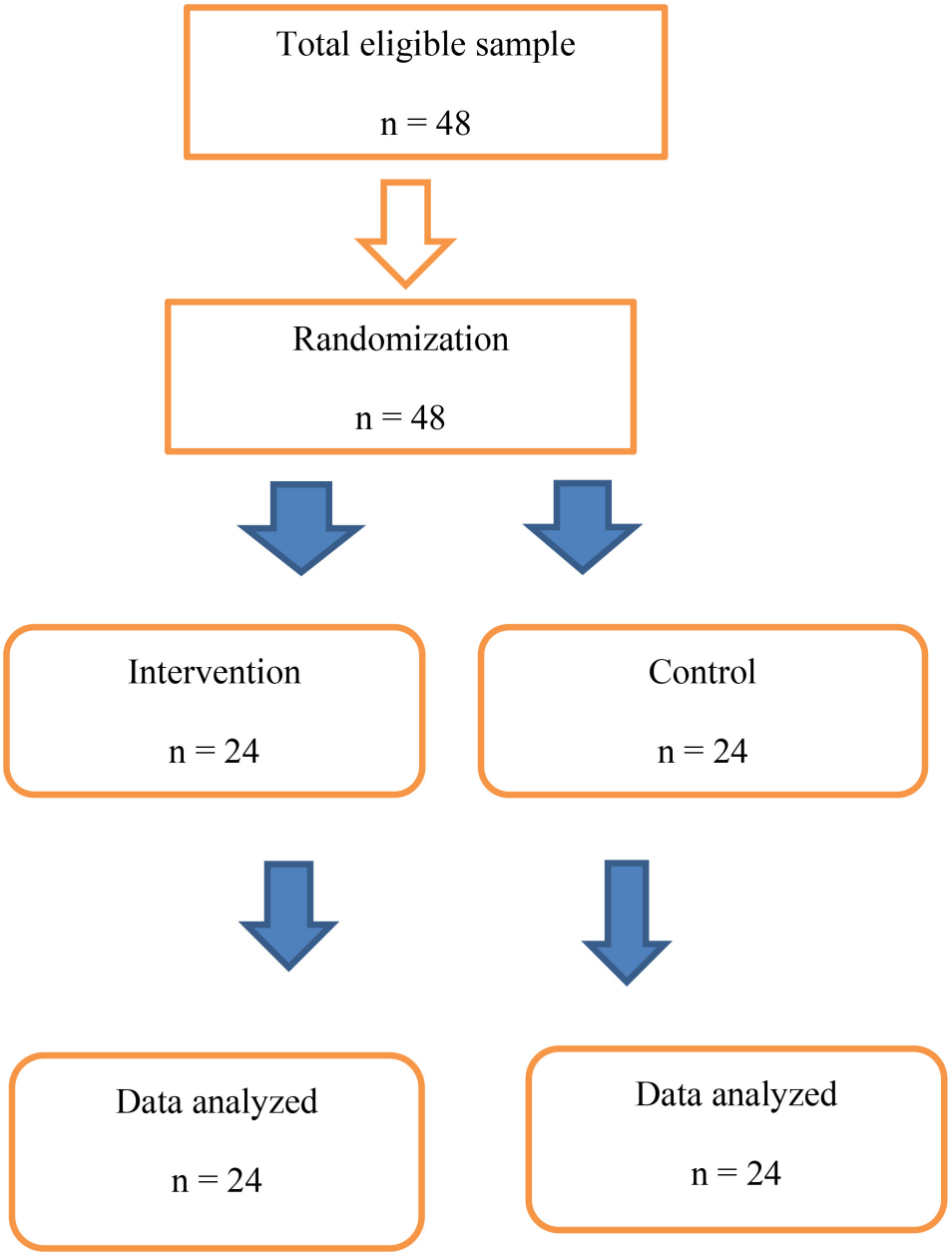

Figure 4. CONSORT flow diagram for recruitment and randomization of samples.

6.2. Demographic Data

A total of 48 subjects were recruited from patients who attended the antenatal clinic and fulfilled the recruitment criteria. There were 24 subjects randomized to the intervention arm and 24 to the control arm. The process has been shown in the CONSORT diagram in **Figure 4**.

6.2.1. Age

The mean age of the sample was 29.6 ± 5.5 years, which followed a near-normal Gaussian curve. The graph below in **Figure 5** represents the distribution of the age.

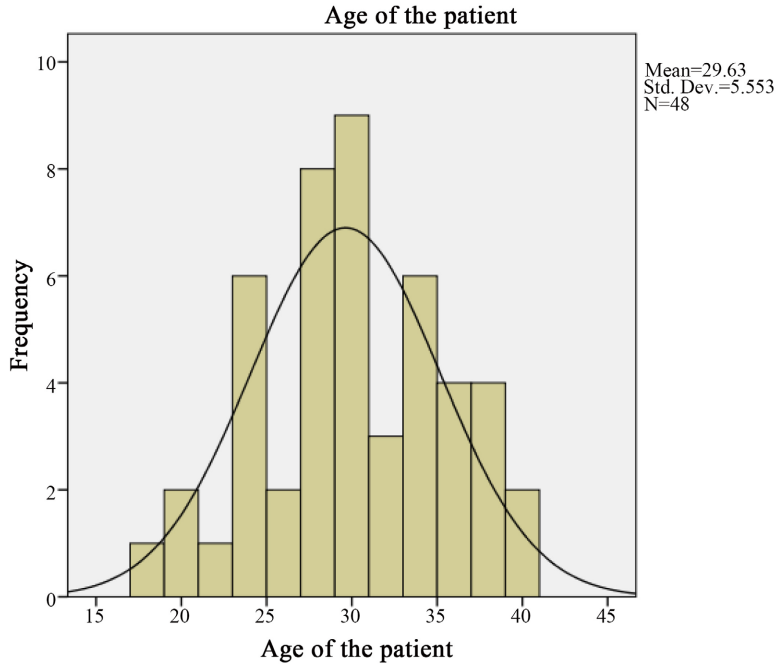

Figure 5. Distribution of age in the total sample (n = 48).

6.2.2. BMI

Mean BMI was 26.1 ± 4.7, and it is a slightly skewed distribution shown in Figure 6, which may reflect the pregnancy changes and/or the present demographic changes of the whole population towards obesity.

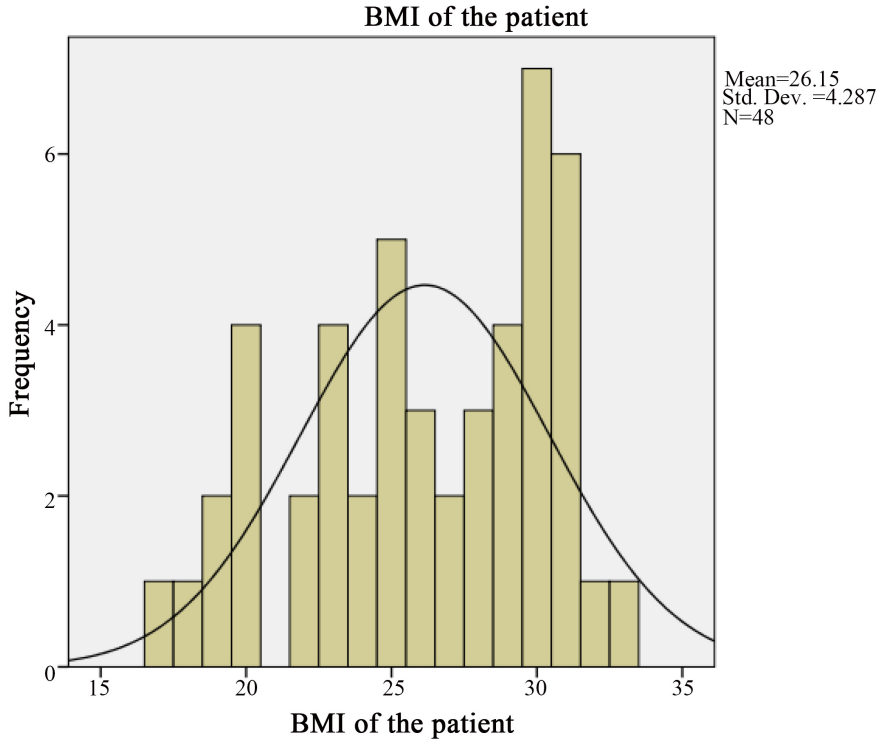

Figure 6. Distribution of the BMI of the total sample (n = 48).

The comparison of demographic parameters between two groups is shown below in Table 2.

Table 2. Comparison of age and BMI of the intervention and control groups with the total study sample.

	Mean age	Mean BMI
Total sample n = 48	29.63	26.15
Intervention n =24	30.46	27.50
Control n = 24	28.79	24.79

6.3. Clinical Parameters

The comparisons of the clinical parameters between the two groups are shown below. The diagnostic OGTT values were compared in the two groups in Table 3.

Table 3. Comparison of diagnostic OGTT values (FBS and 2h value) in Intervention and Control with the total study sample.

	Mean FBS in OGTT	Mean 2h value in OGTT
Total sample n = 48	95.08	149.88
Intervention n =24	95.67	157.67
Control n = 24	94.50	142.08

This table (Table 4) summarizes the values of the intervention arm and the control arm. FBS and PPBS of pre-intervention and post-intervention were compared.

Table 4. FBS and PPBS of pre-intervention and post-intervention were compared.

	Pre FBS (mg/dL)	Post FBS (mg/dL)	Pre PPBS (mg/dL)	Post PPBS (mg/dL)
Total sample n = 48	92.02+/−10.24	93.42+/−12.91	120.60+/−16.12	119.88+/−12.59
Intervention n =24	93.67+/−9.00	92.17+/−11.40	123.46+/−17.43	118.63+/−14.48
Control n = 24	90.37+/−11.30	94.67+/−14.40	117.75+/−14.51	121.13+/−10.54

The glycemic control:

The improvement of FBS and PPBS in the intervention group and control group was assessed at 4 weeks. Table 5 shows the mean change of FBS and PPBS in both cases and controls.

Table 5. The changes of FBS and PPBS in a table.

	Mean Change of FBS (mg/dL)	Mean Change of 1h PPBS (mg/dL)
Intervention n = 24	−1.50+/−9.29	−4.38+/−13.87
Placebo n = 24	1.54+/−9.39	0.88+/−12.75

Two groups were analyzed by independent "t" test. Please see **Table 6**.

Table 6. T-test for Equality of means.

	t	df	Sig. (2-tailed)	95% Confidence Interval of the Difference
Change of FBS	−1.127	46	0.266	−8.474 to 2.390
Change of 1h PPBS	−1.365	46	0.179	−12.994 to 2.494

Change of FBS df (46) = −1.12, p = 0.26.

Change of PPBS df (46) = −1.36, p = 0.17.

Change in FBS and PPBS was not significant. The P value for change in FBS was 0.266 and the P value for change in PPBS was 0.17. So, there were no significant improvements in glycemic control measured with FBS or PPBS.

6.4. Adverse Effects

Adverse reactions in patients were not noted during this trial.

No congenital defects were detected in the neonates.

7. Discussion

7.1. Methodology and Statistical Analysis

This randomized clinical trial (double-blind) was conducted to compare the effectiveness of vitamin D (oral) supplementation versus placebo on glycemic control of GD subjects. Stratified block randomization was used for the two arms. Strict inclusion criteria for the diagnosis of GD were used and recruited. The exclusion criteria were formulated to neutralize any effect on insulin secretion by external factors such as diseases, because it was the action that was tested in the intervention arm by providing the treatment of vitamin D.

The patients were recruited from diagnosed GD subjects who attended antenatal clinics. The sample size was calculated by study power of 80% at a significance level of 5%. The sample size calculation was done using Winpepi software. The total sample size was 48, with 24 allocated to each arm.

The consent was taken at the recruitment of subjects to the trial on a voluntary basis. The sealed envelope method was used to allocate the patients to both arms. A questionnaire was prepared to collect data. It was available in two languages. SPSS software was used to analyze the data.

7.2. Results

The hypothesis was "providing vitamin D to GD leads to an increase in the secretion of insulin and hence improvement of glycemic control". The objective was to assess the effect of vitamin D supplementation on glycemic control in gestational diabetes. However, the supplementation of oral vitamin D did not show any significant improvement in glycemic control, as measured by the change in FBS ($p < 0.26$), and the change in 1h PPBS ($p < 0.17$) in gestational diabetes.

Reasons for the results could be due to the high mean BMI of the Intervention arm, which was 27.5 versus 24.79 in the control arm (mean BMI of the total study group was 26.1 ± 4.7). The selection of the GD population may have automatically chosen a high BMI population (high BMI is a risk factor for GD). This might even indicate the current obesity epidemic, especially in urban areas of Sri Lanka as well as worldwide. When BMI is higher, there is a high chance of impaired glycemic control and a requirement for insulin. In that sense, the Intervention group (due to high mean BMI) was more likely to develop more impaired glycemic control than the control group. That was reflected in the results as a minor improvement of FBS and PPBS values in the Intervention arm, even though it was not statistically significant.

The mean of the FBS change in the Intervention group was −1.50 mg/dL, and the mean of the 1 h PPBS change in the Intervention group was −4.38 mg/dL. This indicated that there was a reduction of both FBS and PPBS values in the Intervention group. The observed reduction was greater in PPBS than in FBS. If there had been no difference in the mean BMI of cases and controls, this reduction could have been much more significant {p (PPBS) = 0.179 versus p (FBS) = 0.266}.

7.3. Relevance to Current Knowledge

At present, the belief is that Hypovitaminosis D is associated with impaired glucose tolerance and diabetes in the general population. However, the evidence for an association between low levels of vitamin D and GD is conflicting. There is no evidence to support routine screening for vitamin D deficiency in pregnancy. However, some clinicians do recommend screening tests for those who have dark skin colour or coverage, obesity, or are at risk of pre-eclampsia. This test for Hypovitaminosis is expensive, and offering it as a screening is not cost-effective, especially in a country like Sri Lanka.

The NICE guideline recommended vitamin D supplementation to all without a screening test during pregnancy and breastfeeding. Daily vitamin D supplementation is considered safe in pregnancy.

The categories to whom vitamin D supplementation is recommended at present according to Scientific Impact Paper (No. 43) [11] issued by the Royal College of Obstetricians and Gynecologists are:

1. In general, vitamin D (400 units) a day is recommended for all pregnant women.

2. High-risk group—women with increased skin pigmentation, reduced expo-

sure to sunlight, or those who are socially excluded or obese. Women at high risk of pre-eclampsia.

3. Treatment. To whom vitamin D is deficient (Therapeutic doses).

According to these trial results, there was no significant improvement in glycemic control in subjects who had GD. Therefore, prescribing vitamin D for the sole purpose of controlling blood sugar is questionable. The study population had a mean BMI of 26.1, and this was an overweight population. As there was no effect on the overweight population, there may not be an effect on the normal BMI population as well. In that sense, prescribing vitamin D to a normal BMI population who does not have other risk factors may not be effective.

The other possibility was that the obese population might have a more significant effect than the normal BMI population, which was not interpreted by this trial, and prescribing vitamin D for them routinely would be justifiable to someone. Therefore, more trials need to be conducted in patients with various BMI groups.

An alternative argument would be that the subjects who have gestational diabetes are at increased risk of hypertensive disorders. On that basis, someone may still justify vitamin D during pregnancy.

If patients are confirmed with hypovitaminosis, Vitamin D is indicated irrespective of GD status to prevent hypocalcemic effects.

7.4. Methodology; A Comparison with Previous Research

There was no such study done earlier. Only a few studies have been available up to now on vitamin D supplementation for GDM. Table 7 has summarized these studies. In this study, we directly analyzed the effect of vitamin D on subjects with GD without knowing their vitamin D status.

Table 7. The methodology comparison with past studies.

Study	Sample size	Study method	Sample groups	randomization	Primary outcome	Secondary outcome
Zatollah A *et al.*; 2013	54 (total)	Randomized trial	Cases-50000 IU, 2 tablets during study Control-received placebo at the same frequency	Not mentioned	Serum 25OH Vitamin D concentration, FBS improvement	Total cholesterol and LDL lipid reduction
Constance Y *et al.*; 2011	n = 179	Randomized trial Whose vitamin D level < 32 ng/ml	Cases-high dose Vitamin D Control-Low dose Vitamin D	Block randomization	Glucose level on OGTT	Neonatal 25OHD, obstetrics, and other neonatal outcomes
Meng-Xi *et al.* 2014	n = 9209	Meta-analysis			Association between Vitamin D deficiency and GDM	

7.5. Results Comparison with Previous Research

An RCT conducted in 2013 (Zatolla A *et al.*) found that vitamin D megadose supplementation led to an improvement in FBS by 17.1 mg/dl ± 4.1 (Table 8). However, they used 50,000 IU dose tablets twice during the trial period. These high doses may have influenced glycemic control. However, these types of high doses are usually recommended for vitamin D-deficient subjects.

Another RCT done in 2011 (Constance Y *et al.*) on vitamin D deficient subjects by giving high or low doses of vitamin D had not changed the OGTT values. That trial showed that vitamin D supplementation had no influence on pancreatic insulin secretion even in confirmed deficiency. In the current trial also, there was no effect of vitamin D on insulin secretion and hence glycemic control.

A recent meta-analysis (done by Meng-Xi *et al.*) [3] has concluded that Vit D deficiency is associated with an increased risk of GDM, with an OR of 1.53, which is not very significant.

Table 8. Results comparison with the previous studies.

Study	Study method	Sample groups	Primary outcome	Results
This trial	RCT (n = 48)	Cases Oral Vitamin D alfacalcidol 0.25 mg Vs placebo	Improvement of FBS and 1 h PPBS value	No significant improvement of PPBS or FBS {P (PPBS) = 0.179 versus p (FBS) = 0.266}
Zatollah A *et al.*; 2013	Randomized trial (n = 54)	Cases-50000 iu, 2 tablets during study Control-placebo	Serum 25OH vitamin D concentration, FBS improvement	Vit D supplementation led to a reduction of FBS −17.1 +/−14.8 (p < 0.001)
Constance Y *et al.*; 2011	Randomized trial Whose Vitamin D level < 32 ng/ml (n = 179)	Cases-high dose Vitamin D Control-Low dose vitamin D	Glucose level on OGTT	No difference in glucose levels in OGTT (p = 0.25)
Meng-Xi *et al.*	Meta-analysis (n = 9209)		Association between Vitamin D deficiency and GDM	Vit D deficiency is associated with an increased risk of GDM (OR - 1.53) CI 1.33 - 1.75.

8. Strengths and Limitations

8.1. Strengths

1) This trial was a double-blind randomized clinical trial, and hence the bias would be minimal.

2) Sample size has been calculated for statistical power of 80% and for a 95% confidence interval according to a 16% change demonstrated in previous RCT[18]. Even though this study was not able to demonstrate such a difference, if the sample size had been larger, there would have been a chance to achieve some significance as the BMI factor would be neutralized in the Intervention arm.

3) The questionnaire used for data collection was well prepared by describing

exclusion criteria. Inclusion criteria were much narrowed to minimize the variability in subjects.

4) Variables were matched between the two arms.

5) Data analysis was done using standard methods such as means and t-tests by using SPSS 23 version.

8.2. Limitations

1) If the analysis was done separately on GD on MNT only, on MNT and Metformin, these results would be more descriptive.

2) Drug compliance might have been suboptimal due to daily vitamin D supplementation.

3) If the serum Vitamin D values could have been analyzed in all participants, this would be a very descriptive study. Due to financial restrictions, that was not offered.

4) Only FBS and morning PPBS 1h values were taken for the easiness of the patient as well as due to limited resources. Patients who attended clinics may not have taken their routine diet on the day before and the clinic day due to social reasons, even though they were educated.

5) This was conducted only in one center in an urban setting. If there were recruitment from a center situated in a rural setting, it would be more representative of the whole Sri Lankan population.

6) There was some delay in analyzing collected blood samples from the clinic. This could have led to some blood sugar value changes.

9. Conclusions

1) Vitamin D supplementation in GD did not improve glycemic control (FBS or PPBS 1h) significantly.

2) No adverse reactions were observed during vitamin D treatment in pregnancy.

3) Vitamin D did not cause any major adverse effect to the fetus.

4) There is a demographic change of BMI toward overweight in the Sri Lankan population.

10. Recommendations

1) Vitamin D should not be offered to all GD patients to improve glycemic control, as it did not show any improvement in FBS or PPBS 1h value.

2) There is an unmet need for RCTs on these subjects, and larger trials with a greater number of cases should have been conducted.

3) If vitamin D is prescribed during pregnancy, it can be considered safe.

4) Future trials should address normal BMI, overweight patients, and obese patients separately in the supplementation of Vitamin D.

In such trials, assessment of Vitamin D will provide more descriptive statistics.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Appendix

List of abbreviations:

GD	Gestational Diabetes
OGTT	Oral Glucose Tolerance Test
LOA	Loss of Appetite
RCOG	Royal College of Obstetricians & Gynecologists
RCT	Randomized controlled trial.
FBS	Fasting blood sugar
PPBS	Postprandial blood sugar
WHO	World Health Organization
CRL	Crown round length
BSS	Blood sugar series
USS	Ultrasound Scan
BMI	Body mass index
PPROM	Premature preterm rupture of membranes
PIH	pregnancy-induced hypertension
DM	Diabetes mellitus
MNT	Medical nutrition therapy