



## Evaluation of Maternal Vitamin D and Zinc Status and Nutrient Intake Among Gestational Diabetes Mothers

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### Abstract

**Aim:** The current study aims in assessing the levels of Zinc, vitamin D, and nutrient intake among GDM women.

**Methods:** Hospital-based case-control study with n=60 subjects grouped into cases (n=30, women diagnosed with GDM) and control (n=30, healthy pregnant women) were recruited for the study. Vitamin D and Zinc were analyzed, anthropometric measurements and dietary data were obtained. Frequency and percent were calculated for descriptive data. ANOVA was applied to compare the nutrient intake among the groups and Pearson correlation to study the correlation between vitamin D and zinc.

**Results:** The levels of vitamin D ( $32.782 \pm 2.56$  ng/ml and  $34.754 \pm 5.13$  ng/ml) and Zinc ( $70.62 \pm 17.9$   $\mu$ g/dL and  $78.56 \pm 6.82$   $\mu$ g/dL) was found to be at lower end, among cases. Significant positive correlation was observed for vitamin D and Zinc. Significant difference was observed for energy, protein, vitamin D, zinc, iron, and vitamin E compared to RDA.

**Conclusion:** Creating awareness on the importance of each nutrient and addressing the consequences of nutrient deficiencies among reproductive-age and gravid women is crucial in promoting healthy pregnancies and optimal maternal-fetal outcomes. By providing personalized nutritional counseling and emphasizing the significance of proper nutrition, women may be empowered to make informed choices and prioritize their well-being, ultimately contributing to healthier generations to come.

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**Keywords-** Gestational diabetes, Macro and micro-nutrients, Vitamin D, Zinc

### 1. Introduction

Gestational diabetes mellitus (GDM) is a state of glucose intolerance diagnosed for the first time during pregnancy. Various risk factors viz., gestational age, obesity, ethnic background, family history of type 2 diabetes (T2DM) and previous history of GDM have been implicated as the contributors of GDM. The incidence of GDM was reported to be high as 15–20% worldwide. Normal pregnancy is characterized by a significant reduction in maternal insulin sensitivity in the second and third trimesters. [1]

However, the reduced reserve of  $\beta$  cells or their maladaptation to increased insulin demands ~~not~~ result in the development of GDM. [2] Decreased maternal insulin sensitivity, or increased insulin resistance, is the

underlying pathophysiology of GDM, which starts near pregnancy and progresses to the third trimester. [3] Extra calories and increased weight gain during pregnancy induce inflammatory pathways among GDM women, which in turn result in the development of insulin resistance and excessive fetal growth; macrosomia. [4] Evidence suggests that underlying factors like obesity enforce oxidative stress; increased oxidative damage, and reduced antioxidant capacity, in women with GDM, which contributes to the initiation and progression of GDM. [5]

A number of studies focused on putative role of vitamin D deficiency in various pregnancy pathologies including GDM. [5,6] Observational studies revealed the correlation between low vitamin D levels and preeclampsia or GDM. [6] Vitamin D deficiency in pregnancy was related to the incidence of GDM and serum 25(OH) D was significantly lower in women with GDM than in those with normal glucose tolerance. Zinc is a necessary component of over thousand proteins including antioxidant enzymes, metalloenzymes, zinc-binding factors, and zinc transporters. These are essential for a variety of biological processes including carbohydrate and protein metabolism, DNA and RNA synthesis, cellular replication and differentiation, and hormone regulation. [5]

The importance of zinc in the growing fetus is demonstrated by the active transport of zinc across the placenta into the fetal circulation resulting in higher cord blood concentrations compared to those in the maternal circulation. [5] Rodent models of severe maternal zinc deficiency show increased rates of fetal loss and congenital malformations in the surviving fetuses as well as reduced fetal growth, lower implantation rates and impaired placental growth, [2] all highlighting the teratogenic effects of zinc deficiency in pregnancy. [7] Diet is the main factor that determines zinc status. In the United States and Australia, an additional 2–4 mg zinc per day is recommended for pregnant women compared to non-pregnant women. [8] It is widely acknowledged that many pregnant women do not meet this recommendation, particularly in developing countries where diets are often plant-based. [9] Grains and legumes contain a significant amount of phytic acid and phytates binds zinc and limits its absorption in the small intestine, contributing to zinc deficiency. Estimates based on bioavailability of zinc, physiological requirements and predicted zinc absorption suggest the prevalence of zinc deficiency to range from 4% (European countries including the United Kingdom, Sweden, Germany and France) to 73% in Bangladesh, India and Nepal. [10] A more recent evaluation, based on similar estimates, also predicted inadequate zinc intakes in over 25% in populations in Southeast Asia and Africa. [11] The evaluation of maternal vitamin D, zinc, and nutrient intake in gestational diabetes mellitus (GDM) is an important area of research to understand the potential impact of these nutrients on the development and management of GDM. Therefore, understanding the effects of zinc deficiency on pregnancy and fetal growth is very important.

Considering the fact, it is important to understand the levels of vitamin D, zinc and nutritional status of pregnant women with gestational diabetes. The present study is first of its kind to determine the levels of zinc, vitamin D and nutrient intake among women with GDM, additional research in this area is warranted.

## 2. Methods

### Sample size

The current study is a hospital-based cross-sectional study. Women with GDM who gave consent and were willing to participate were recruited from three different hospitals in Hyderabad and Telangana states. A total of sixty subjects, were selected for the study and grouped into cases and control. Subjects in any trimester aged between 18-40y with GDM and with complications like hypertension, thyroid, anemia, obesity, and gastric problems were included and grouped as cases and subjects with any other severe metabolic disorders were excluded.

### Data collection

A semi-structured questionnaire was developed by the authors to collect information regarding socio demographic profile, obstetric history. Food records were filled in for three non-consecutive days through a 24-hour dietary recall (two weekdays and one weekend) and food frequency questionnaire (FFQ) was used to measure the dietary intakes, this type of 3-day food record is a gold standard method for assessing diet (Institute of Medicine 2001). [12]

The Anthropometric measurements like height, weight, body mass index (BMI) triceps skinfold (TSF), and Mid-Upper Arm Circumference (MUAC) were included. Decrease in MUAC may reflect either a reduction in muscle mass, subcutaneous tissue or both.

Two ml of venous blood sample was collected from each subject and centrifuged at 3000 rpm for 5 min. Serum was stored at –20°C prior to analysis. Serum zinc levels were assessed by atomic absorption spectroscopy as

described by Molina-López et al., (2016). [13] The serum vitamin D level was measured by the chemiluminescence immunoassay method according to the manufacturer's instructions (LSBio).

Dietary component consists of the assessment of food or nutrient intake which provides information on the current food consumption among pregnant women and is a direct measure of their growth and development of the fetus. This data estimates the individual usual intakes which can be compared among the groups and standards. The FFQ evaluates the frequency of food intakes of food groups rather than intake of specific nutrient. Pregnant women were asked about the meal pattern, food recall of 3 days and frequencies of the various food groups consumed on daily, weekly, monthly or occasional basis. For accurate measurement of portion size of the foods standardized measuring cups and visual guides were shown. Diet Cal software (developed by Kaur 2015; Profound Tech Solutions, New Delhi) was used to calculate nutrient intake and an individual report was generated for each participant.

Diet Cal is a software tool based on Indian Data (National Institute of Nutrition, Indian Council of Medical Research) for dietary assessment and planning of commonly consumed foods. It has an editable central repository of nutrients and their measure units, food items classified based on food groups and their nutritive values, also facility to create customized recipes is provided and permitted the calculation of nutritive values of set of food items and recipes. Additionally, the software also has an option of adding foods into the database wherein local food recipes could be added and estimated for the nutrients content.

### Ethical approval

Written informed consent was obtained from all patients or their relatives. The study protocol was approved by the Institutional Ethics Committee of JSS Medical College, Mysore (ethical code: JSSMC/IEC/050722/32NCT/2022-23). Subjects were recruited based on their willingness through personal acquaintances.

### Statistical analysis

Statistical analysis was performed using the Statistic Software package (SPSS, 16.0, International Business Machines, USA). Frequency and percent were calculated for descriptive data and mean and standard deviation for quantitative data. ANOVA was used to compare the anthropometry, biochemical parameters, and nutrient intake among GDM and normal pregnant women. Pearson correlation was applied to study the correlation between vitamin D and Zinc between the groups.

## 3. Results

### General characteristics, anthropometric measurements and biochemical parameters

**Table no.1: General characteristics of pregnant women (n=60)**

Variables	Mean ± SD	
Age (y)	25.95 ± 2.1	
Weight (Kg)	60.3 ± 6.8	
Height (cm)	156.51 ± 5.04	
BMI (Kg/m <sup>2</sup> )	24.86 ± 3.36	
MUAC (mm)	240.82 ± 3.52	
SFT (mm)	18.47 ± 3.11	
Gestational week	26.28 ± 5.65	
Occupation (%)		
	Housewives	83
	Working women	17

The general characteristics of pregnant women, is shown in (Table no.1), the average age and weight of study subjects was 25.95 ± 2.1 years and 60.3 ± 6.8Kg respectively. Though the average BMI was normal (24.87 ± 3.36), overweight was observed among pregnant women. The average MUAC of study subjects were 240.8 ± 3.52mm, it has a comparatively strong association with low birth weight.

**Table no.2: Anthropometric Measurements of Cases and Control**

Variables	Control (mean±SD)	Cases (mean±SD)	F	P
Age(y)	26.167±2.119	25.733±2.116	-	-
Weight (Kg)	59.7±7.679	60.9±5.868	0.462	0.499
Height (cm)	155.363 ± 5.544	157.651 ± 4.268	-	-
BMI(Kg/m <sup>2</sup> )	24.992±3.527	24.745±3.238	0.080	0.778

MUAC (mm)	24.367±3.961	25.267±3.238	0.980	0.326
SFT (mm)	17.867±3.203	19.067±2.947	2.281	0.136

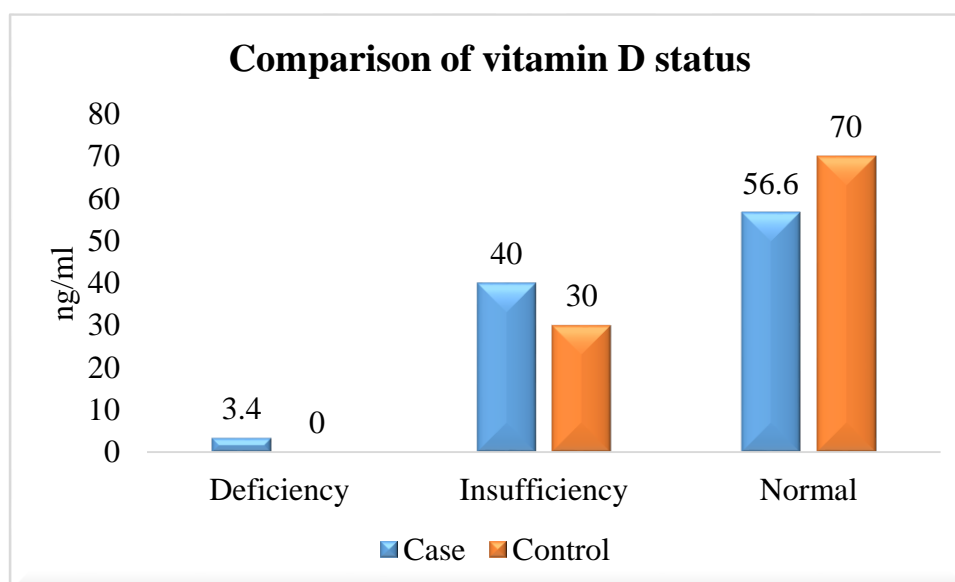
The anthropometric measurements of both cases and control is shown in Table no.2. The mean age of cases was 25.733±2.12y, and control was 26.167±2.12y. The mean weight of cases were greater compared to that of control. The BMI of cases and control were 24.745±3.238 and 24.992±3.527 respectively. According to NIN the BMI for pregnant women is 18.5-23.0 is normal and <18.5 is undernourished, >23.0 is overweight. The majority of subjects from cases and control were overweight. No significant difference observed between the groups for the anthropometric measurements.

**Table no.3: Biochemical parameters of case and control**

Blood parameters	Control	Cases	F	P
Haemoglobin (mg/dL)	11.27±1.162	10.937±1.579	0.867	0.356
HbA1C (%)	5.08±0.49	6.010±0.639	33.027	0.000*
FBS (mg/dL)	81.9±8.014	109.459±13.610	91.339	0.000*
PPBS (mg/dL)	121.167±12.610	148.874±24.908	29.851	0.000*
Total cholesterol (mg/dL)	176.477±10.246	179.683±17.529	0.748	0.391
HDL (mg/dL)	50.013±10.872	39.592±8.062	16.553	0.000*
LDL (mg/dL)	109.473±16.185	110.049±18.742	0.016	0.899
VLDL (mg/dL)	25.445±6.286	24.328±6.653	0.447	0.506
Triglycerides (mg/dL)	95.103±25.50	72.097±22.937	13.494	0.001*

\*P<0.01 is statistically significant.

Table no.3 represents the biochemical parameters of both cases and control. The mean hemoglobin content of cases was 10.94±1.58 mg/dl, as the hemoglobin values of both cases and control were below normal it is considered that they were at mild risk of anemia. Significant difference was observed for HbA1C, FBS, PPBS, Total cholesterol, HDL and triglycerides between the groups indicating the changes in the biochemical parameters among cases and control. The mean HbA1c of control was 5.08±0.49 and of cases was 6.01±0.63 significant (p<0.01) difference was observed between the groups.



**Figure no.1: Comparison of vitamin D status**

**Table no.4: Mean Zinc & Vitamin D level**

Variables	Control (mean±SD)	Case (mean±SD)	P
Zinc (µg/dL)	78.56±6.82	70.62±17.9	
Vitamin D (ng/ml)	34.754 ±5.13	32.78 ±2.56	0.320*

\*Correlation is significant at the 0.05 level (2-tailed)

Although, the mean vitamin D levels (Table no.4) were within the reference range (34.73±9.87 ng/ml among cases and 38.74±12.61 ng/ml in the control). Individually, majority of the cases were found to be deficient (3.4%)

and were having insufficient (40%) Vitamin D levels. Similarly, with that of zinc values though the mean values were within the reference range but borderline deficiency existed among the pregnant women. Pregnant women with serum zinc levels  $\leq 45.5 \mu\text{g/dL}$  are 3.2 times more likely to experience pre-eclampsia compared to pregnant women with serum zinc levels  $> 45.5 \mu\text{g/dL}$ . [18] A significant positive correlation was observed between the groups for both Zinc and Vitamin D.

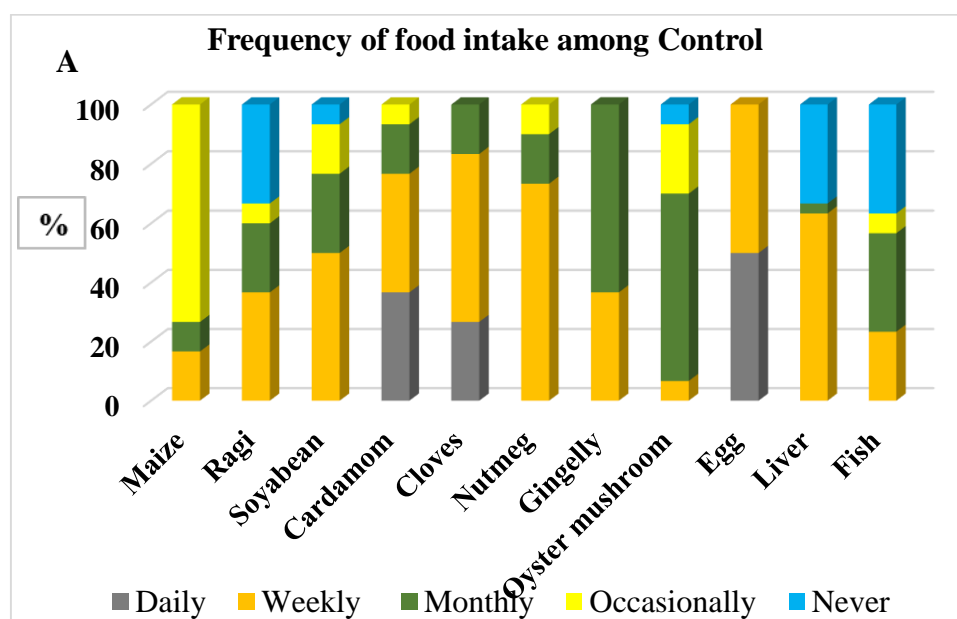
### Nutrient intake and frequency of food intake among both the groups

**Table no.5: Comparison of nutrient intake of both cases and control with RDA**

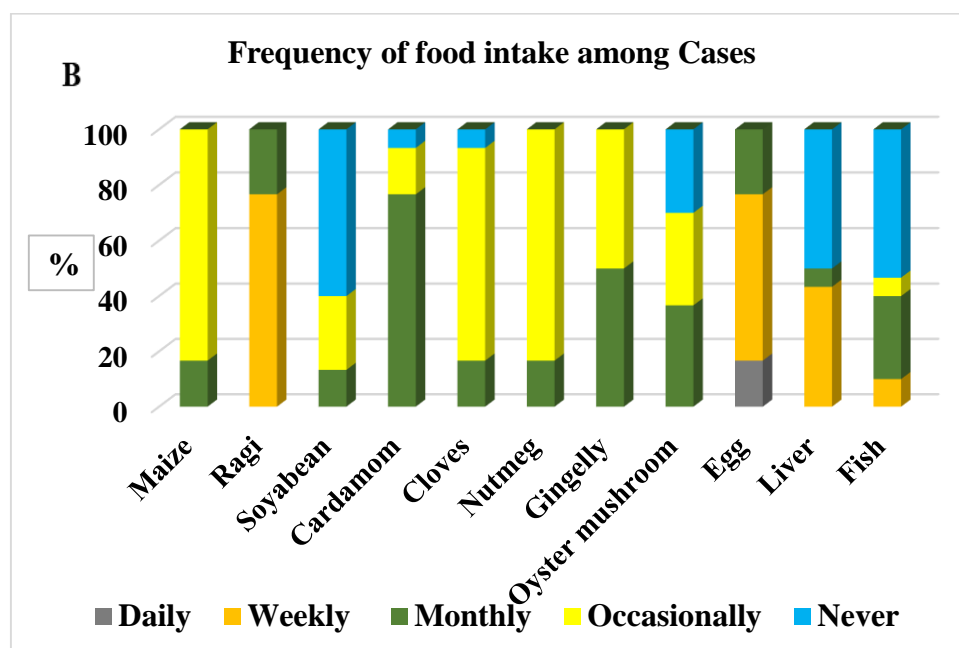
Nutrient intake	RDA	Control (mean $\pm$ SD)	Case (mean $\pm$ SD)	Comparison Between the groups		Comparison with the RDA (Nutrient Adequacy)	
				F	P	t- value	P
Energy (kcal)	2010	1810.83 $\pm$ 221.01	1262.88 $\pm$ 217.76	0.883	0.351	-9.589	0.0001*
Carbohydrate (g)	175	150.30 $\pm$ 30.34	193.36 $\pm$ 121.91	0.225	0.637	1.877	0.65
Protein (g)	55	74.21 $\pm$ 10.68	36.64 $\pm$ 5.95	1.044	0.311	-16.82	0.0001*
Fat (g)	30	45.9 $\pm$ 7.49	44.85 $\pm$ 7.68	0.302	0.585	-0.549	0.584
Fiber (g)	40	19.76 $\pm$ 4.4	19.78 $\pm$ 4.44	0.000	0.989	0.014	0.988
Vitamin D (mg)	600	223.43 $\pm$ 239.78	102.93 $\pm$ 127.97	1.817	1.817	-2.428	0.018*
Zinc (mg)	14.5	8.0 $\pm$ 1.18	4.63 $\pm$ 1.21	0.000	0.987	-10.94	0.0001*
Calcium (mg)	1000	424.65 $\pm$ 86.02	427.99 $\pm$ 98.40	0.020	0.889	0.140	0.888
Iron (mg)	27	17.66 $\pm$ 3.0	13.34 $\pm$ 4.51	0.029	0.865	-4.309	0.0001*
Folic acid (mcg)	570	17.4 $\pm$ 3.99	17.23 $\pm$ 2.64	0.044	0.835	-0.209	0.834
Omega-3-FattyAcids (mg)	650	317.5 $\pm$ 99.75	319.55 $\pm$ 93.40	0.007	0.935	0.081	0.935
Iodine (mg)	220-250	181.08 $\pm$ 41.78	174.79 $\pm$ 16.44	1.799	0.185	-0.767	0.446
Selenium (mcg)	60	43.92 $\pm$ 12.16	44.10 $\pm$ 11.42	0.004	0.953	0.059	0.952
Magnesium (mg)	440	281.99 $\pm$ 155.59	292.24 $\pm$ 161.35	0.063	0.803	0.250	0.803
Potassium (mg)	3510	1575.01 $\pm$ 1495.98	1584.59 $\pm$ 1419.76	0.001	0.980	0.025	0.979
Vitamin C (mg)	80	45.33 $\pm$ 24.31	49.82 $\pm$ 31.67	0.379	0.541	0.615	0.540
Vitamin E (mg)	10	6.38 $\pm$ 2.40	4.95 $\pm$ 1.85	0.057	0.813	-2.58	0.012*

### RDA- Recommended dietary Allowances

It was observed that the pregnant women were insufficient in both macro and micronutrients (Table 5). The nutrient intake among both the groups were less compare to the RDA, no significant ( $P < 0.005$ ) difference between the groups, significant difference was observed for energy, protein, vitamin D, zinc, iron, and vitamin E compared to RDA. The GDM women had lesser nutrient intake for fiber, calcium, iodine, folic acid, omega-3, selenium, magnesium, vitamin C, and vitamin E but no significant difference was observed for the same when compared to that of RDA because the adequate intake of both the groups were equal which were found to be below than the recommended levels.







**Figure no.2 (A- Cases; B- Control): Frequency of food intake which are good sources of Vitamin D.**

The frequency consumption of foods which are good sources of vitamin D is shown in Figure no.2. The consumption of vitamin D<sub>2</sub> (Ergocalciferol) foods like maize, ragi, soyabean, cloves, nutmeg and gingelly, oyster mushroom was more frequent among normal pregnant women compare to GDM subjects. Similar trend was observed for foods rich in Vitamin D<sub>3</sub> (Cholecalciferol) like egg, liver and fish. Also, the frequency consumption of commonly consumed foods showed that the intake of rice, roots and tubers along with other fast food consumption was high and intake of fruits was less among GDM compare to the control.

#### 4. Discussion

The MUAC is a proxy measure of nutrient reserves in muscle and fat that are unaffected by pregnancy and independent of height. MUAC cutoffs for severe acute malnutrition (SAM) varied from < 210 mm to < 230 mm in national nutrition protocols among pregnant women. [14] The average SFT of study subjects was  $18.47 \pm 3.11$  mm and the average gestational week of study subjects was  $26.28 \pm 5.65$ . Total skinfold measurement, the preferred method for assessing obesity, is often impeded by the dearth of trained staff, time, and costly equipment. [15] The majority of study subjects were housewives.

The biochemical parameters have revealed higher HbA<sub>1c</sub> in GDM women, the reference ranges below 5.7% indicates normal and 5.7%- 6.4% indicates pre-diabetes, [16] indicating the cases as pre-diabetic. Managing blood lipid levels is vital because women with GDM are at a higher risk of developing gestational hypertension, preeclampsia, and type 2 diabetes later in life. These conditions are associated with an increased risk of cardiovascular diseases. [17] Additionally, maintaining a healthy lipid profile can help reduce the risk of complications during pregnancy and promote overall maternal and fetal well-being.

Maternal Vitamin D deficiency can lead to abnormal fetal development. A neonate with insufficient vitamin D stores is more likely to suffer from morbidity and mortality. [19] Vitamin D seems to have several extra skeletal functions including regulation of glucose metabolism through influencing insulin sensitivity, although the mechanisms is not fully understood. The pancreatic  $\beta$  cells express both vitamin D receptor and enzyme  $1\alpha$ - hydroxylase which enables them to produce 1, 25 (OH)<sub>2</sub>D locally. [20] The effect of vitamin D on regulation of pancreatic  $\beta$  cell function and insulin secretion could be mediated through intracellular changes in calcium pool. Vitamin D could also enhance insulin sensitivity by stimulating insulin receptor gene expression thereby enhancing insulin mediated glucose transport. [21] In addition, vitamin D may also be needed to ensure a normal rate of calcium flux across cell membranes and maintenance of an adequate cytosolic calcium pool, which is important for insulin-mediated intracellular signaling in insulin-responsive tissues. [22] Studies have shown that vitamin D could play a vital role in the pathogenesis of type 2 diabetes mellitus by affecting insulin sensitivity of  $\beta$  cell function. [23,24] Vitamin D is also essential for proper fetal programming and its deficiency during pregnancy may lead to low birth weight and increased susceptibility to chronic disease later in life. [25]

Soheilykhah et al. reported that prevalence of vitamin D deficiency was higher among women with impaired

glucose tolerance or GDM among Iranian women but they did not find correlation with BMI or fasting plasma glucose. [26] Significantly lower 25(OH) D levels in GDM women was observed in Australian women compared to control; however, no association was observed between 25(OH) D levels and GDM when 4 ethnic subgroups were analysed separately. [2] Our results are in agreement with the studies reporting lack of association between vitamin D levels in pregnancy and GDM which may be due to insufficient sample size.

Zinc is a crucial mineral with multiple biological functions that play a vital role in various physiological processes, including protein synthesis, cell division, and nucleic acid metabolism. One of its significant contributions is its involvement in the oxidative/antioxidant balance within the body. Perturbations in this balance can lead to various pathogenesis processes, including preeclampsia (PE), a serious pregnancy complication. [19] The zinc supplementation reduced the risk of spontaneous preterm birth (sPTB) by 14% but no effect on other outcomes such as stillbirth/neonatal death, birth weight and pregnancy-induced hypertension was observed. [27] The association between dietary zinc intake and GDM was assessed in the 24–28 weeks gestation, and found an 11% reduction in the risk of gestational hyperglycaemia with every 1 mg/day increase in dietary zinc intake. [28] Another study, women in 14–20 weeks' gestation period, found no association between maternal dietary zinc intakes below 50% of the recommended daily allowance and GDM. [29]

Inadequate energy intake leads to increased risk of preterm birth, growth restriction, and insulin resistance. Adequate energy intake is required during pregnancy for the growth and maintenance of the foetus, placenta, and maternal tissues. [30]

Higher frequency of potato intake increased the risk of GDM. Replacing two servings of potatoes per week for other vegetables types, legumes or wholegrain food resulted in a 9%, 10% and 17% GDM risk reduction respectively. No significant association was observed between potato crisps and GDM risk after adjustment of confounding variables including age, parity, race, and family history of diabetes, energy intake, diet quality and BMI. [31]

Studies have reported on caffeine intake and risk of GDM. Coffee consumption was reported to have a protective affect against GDM in one study, but failed to reach a statistical significance in the other. Consumption of decaffeinated coffee was not associated with risk reduction. Increasing frequency of tea intake indicated a potential protective effect against GDM risk. [32] Increasing frequency of fast food intake prior to pregnancy was associated with a significant increased risk or incidence of GDM. [33] Interestingly, Schoenacker et, al., observed fruit and low-fat dairy intake found be beneficial among GDM subjects by reducing the risk. [34]

## 5. Conclusion and Recommendation

Study findings revealed that deficiency of vitamin D and Zinc co-existed along with dyslipidemia and impaired glycated hemoglobin levels among GDM women. The macro and micro nutrient intakes were essentially similar between the groups exhibiting a range of 10-70% nutrient adequacy when compare with the RDA. However, carbohydrate and fat consumption was higher than the recommendations. Deficiencies and excesses in these nutrients can lead to a range of adverse outcomes, including preterm birth, low birth weight, developmental delays, and long-term health issues for both the mother and the child. Therefore, it is important to create awareness and provide personalized nutritional counselling to the mother before pregnancy to make informed choices, and optimize their overall well-being. This proactive approach not only reduces the risk of GDM and related cardiovascular complications but also sets a foundation for a healthy pregnancy and improved outcomes for both mother and child.

Comprehensive diabetes care should be available in the hospitals to the mothers, encompassing both nutritional education and psychosocial assistance. In order to address the needs of diabetic care in the mothers, our healthcare system should involve dietitians and nutritionist professionals with a diabetes expertise and training. To educate the mothers, their families, and the general public, continuing education is essential on many levels.

## 6. Strength and Limitation

The present study is first of its kind to study the vitamin D status and Zinc levels along with nutrient intake among pregnant women. One of the main limitations of the current study is selection of subjects using a convenient sampling technique and opting for small sample size and it's a short-term and area-based study. To study the long-term effects and complications of Vitamin D and Zinc status on both materno-fetal health longitudinal studies are warranted.

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**9. Conflict of Interest-** The authors declare that they need no conflict of interest.

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