Diagnostic Utility of Oral Glucose Tolerance Test to Identify Gestational Diabetes Mellitus in Expectant Mothers

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Abstract

Apart from poverty and political conflicts, India faces another major challenge that is termed as 'Diabetes.' It is an everlasting health challenge and not only affects the older aged ones, but also it can affect the Children and Pregnant females. The topic of discussion in this study is restricted to Gestational Diabetes Condition (GDM), a condition where the expecting mother's blood exceeds the normal glucose levels due to various physiological and pathological factors which ultimately affects the unborn child too. The patients are fed with 75 gm oral glucose and their blood is collected thrice at an interval of 1-hour, followed by assessment of their blood glucose levels using GOD-POD (Glucose oxidase peroxidase) method and analyzing them to rule out GDM. As per WHO guidelines, those patients were considered gestationally diabetic whose FBS was > 92 mg/dL or 2-hour OGTT value was > 153 mg/dL. Among all the patients taken for the study, around 30% of them are affected with the disease. However, more detailed study with larger population is required to lay a strong conclusion and throw light on unexplored areas like connection between hypertension and GDM.

Keywords

OGTT, GDM, Blood glucose, Semi-autoanalyzer, GOD-POD, Vials, FBS.

Introduction

Pregnancy, a phase changing moment in the life of a woman that not only alters the physical state but also interferes with her emotional and mental well-being. In humans, it last for 280 days or 9 months 7 days to be precise. There are a humungous number of changes occurring in the mother's body that includes hormonal changes, mood swings, inconsistent bladder, hair fall, and variety of others. The certain endocrine alterations in the levels of catecholamines, TSH, LH, FSH, beta-hCG, hPL leads to altered glucose metabolism and reduces insulin sensitivity to the cells [1]. These leads to consequences like Gestational Diabetes Mellitus [2].

Gestational diabetes mellitus (GDM) is one of the most common metabolic complications of pregnancy, characterized by glucose intolerance with onset or first recognition during gestation. It affects approximately 5–20% of pregnancies worldwide, with prevalence varying depending on population characteristics and diagnostic criteria [3]. It typically manifests in the second or third trimester and is driven by a combination of increased insulin resistance and inadequate compensatory insulin secretion by pancreatic beta cells. The pathophysiology of GDM involves complex hormonal changes during pregnancy, including elevated levels of placental lactogen, progesterone, and cortisol, which interfere with insulin signalling and glucose homeostasis. These metabolic disturbances can have significant consequences for both the mother and the developing foetus [4].

Early and accurate diagnosis of GDM is crucial for preventing short- and long-term complications such as preeclampsia, polyhydramnios, macrosomia, neonatal hypoglycaemia, preterm labour, an increased likelihood of caesarean delivery, and an increased lifetime risk of type 2 diabetes mellitus (T2DM) for both mother and child. Long-term, children born to mothers with GDM are more likely to develop obesity, insulin resistance, and type 2 diabetes in later life, highlighting the intergenerational consequences of this condition [5]. The oral glucose tolerance test (OGTT) remains the cornerstone for

diagnosing GDM, providing a dynamic assessment of maternal glucose metabolism and insulin response. Despite its widespread use, variability exists among international guidelines regarding glucose load, timing, and diagnostic thresholds, which may influence detection rates and management strategies. Screening and early detection are critical to mitigate these risks. Guidelines recommend that pregnant women undergo glucose tolerance testing between 24 and 28 weeks of gestation, with earlier testing for high-risk populations [6]. Management of GDM typically begins with lifestyle modifications, including tailored dietary plans and physical activity programs. Pharmacological interventions, such as insulin therapy or selected oral hypoglycaemic agents, are implemented when lifestyle measures are insufficient to achieve glycaemic control. Effective management has been shown to significantly reduce maternal and neonatal complications [7].

In recent years, research has focused on understanding its molecular and physiological mechanisms related to GDM, refining the diagnostic accuracy of OGTT and exploring its association with biomarkers of insulin resistance, inflammation, and placental function. Studies have highlighted the role of placental dysfunction, chronic low-grade inflammation, oxidative stress, and epigenetic modifications in the development of GDM [8]. Additionally, genetic predisposition and environmental factors, including maternal obesity and nutrition, contribute to susceptibility [9]. Advances in identifying biomarkers for early detection and monitoring glycaemic control hold promise for improving clinical outcomes and reduce the burden of adverse outcomes associated with GDM [10].

Therefore, this study aims to compare OGTT profiles among pregnant women to rule out the ones with and without GDM. Findings from this research may contribute to improving the screening and management of gestational diabetes in clinical practice and lay impact on translational research.

Materials and Methods

Sample collection:

The pregnant females are asked to consume 75gm oral glucose in fasting mode. Their blood samples are collected three times: once before glucose consumption (fasting blood sugar-FBS), one hour after consumption and finally two hours after consumption of glucose (2-hour OGTT). The samples are collected in gray capped vials (EDTA-NaF) so that the blood does not coagulate and plasma could be easily separated for glucose level estimation. The NaF prevents the action of enolase enzyme which further prevents glycolysis and ensures accurate measurement of glucose level [11]. The phlebotomists are well trained in blood collection and gloves are worn by them while dealing with patients. After collection from one patient, they sterilized their hands by 70% ethanol and wore fresh gloves while collecting blood and this process continued. The syringes without needle and other biomedical wastes are disposed of in red bins containing red autoclavable bags.

Sample processing:

After blood sample collection, they are processed within 3-4 hours to prevent any damage. The samples are centrifuged at 3500 rpm for 10 minutes to collect the plasma. The supernatant formed contains the plasma, which is collected for further analysis.

Glucose estimation:

The plasma glucose levels are analyzed using a semi-autoanalyzer machine [12]. Three test tubes are prepared namely – Blank (B), Standard (S) and Test (T). 1ml of Reagent is added to all the three tubes, followed by addition of 0.01mL standard glucose solution to S and 0.01mL plasma sample to T. The tubes are incubated for 15 mins at 37ºC and the results are checked on the analyzer. The glucose present in the plasma reacts with oxidase enzyme of the reagent to form gluconic acid, an oxidized product. O-dianisidine present in the reagent reacted with peroxidase and the peroxide formed reacted along with gluconic acid to form a coloured product whose absorbance is measured at 540nm.

Calculation:

The autoanalyzer assessed the absorbance (OD) of samples present in all three test tubes. The following formula is applied for the calculation.

[OD (T) – OD (B)/ OD(S) – OD(B)] * 100

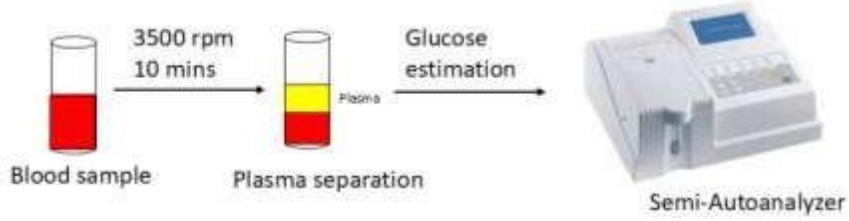

Figure 1: Diagrammatic overview of the methods to be followed. [made in PowerPoint]

Statistical analyses:

The mean age and blood glucose levels are expressed in Mean ± SD. Two groups have been classified as GDM vs non-GDM and Mann-Whitney U test has been performed to check whether the FBS levels statistically varying between the groups. The same is used to check the difference in 2hr OGTT value between the groups. A p value of less than 0.05 is statistically significant. Shapiro-Wilk test is applied to check the normality of the data and thereby, Spearman ratio is applied to rule out the correlation between the variables- age vs FBS and age vs 2-hour OGTT. R software is used to study statistics and MS Excel to plot the graphs.

Results and Discussion

The glucose levels of all the recruited pregnant females are analyzed and as per the "WHO" (2018) guidelines, they are classified as Gestationally Diabetic and Non-diabetic. As per the guideline, any expecting mother's FBS greater than 92 mg/dL or 1hr OGTT value greater than or equal to 10 mmol/L or 2-hour OGTT value > 153 mg/dL is considered to be gestationally diabetic (GDM). The ones who do not fulfill these criteria are gestationally non-diabetic (non-GDM)

77 expectants at their fifth or sixth month of gestation visiting the OPD are recruited within a span of 15 days. The mean age of the recruited pregnant females is 23.42 ± 3.98 years and they belonged to a lower-middle class family. After analysis of blood glucose levels and applying the WHO guidelines, 23 expectants out of 77 are found to be affected with Gestational Diabetes Mellitus. The following table [**Table 1**] depicts the various parameters related to both the group of pregnant females.

Table 1: Tabular depiction of various parameters found in GDM vs non-GDM expecting mothers.

Pregnant female type	Number of expectants	Mean age (Mean ± SD)	Mean FBS (Mean ± SD)	Mean 2hr OGTT (Mean ± SD)
GDM	23	24.86 ± 4.35 years	108.65 ± 32.21 mg/dL	204.87 ± 60.08 mg/dL
Non-GDM	54	22.79 ± 3.69 years	84.98 ± 9.56 mg/dL	119.04 ± 18.70 mg/dL

The blood glucose levels are very high in diabetics as compared to the ones without as reflected in the graph (**Figure 3&4)**. Around 30% of the recruited expecting mothers are Gestationally diabetic and since the number of participants are

very less along with a narrow age group (19-28 years), no strong conclusion can be drawn. A very high significant difference has been observed in FBS levels for both GDM group and non-GDM group and same for 2-hour OGTT levels.

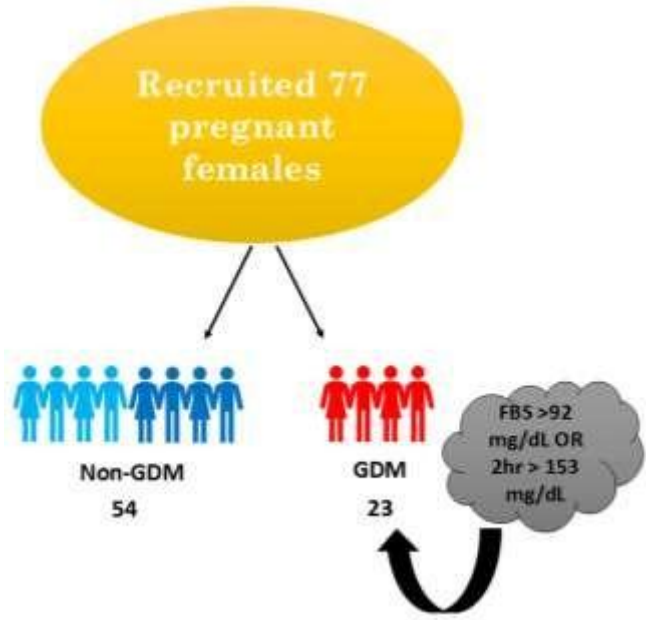

Figure 2: Figure portraying classification of recruited expectants into Gestationally diabetic vs non-diabetic.

As per Shapiro-Wilk test, the Fasting blood glucose level data is not normally distributed for both the groups ($p<0.001$) indicating very high statistical significance. The same is observed in case of 2-hour OGTT and age data for both the groups. Hence, Spearman ratio is applied to check the correlation between age and blood glucose levels (FBS & 2-hour OGTT) in the two groups- GDM vs non-GDM. A weak positive correlation has been observed between age and FBS (rho= 0.08 and $p = 0.4$), suggestive of the fact that this correlation is not significant statistically. A weak negative correlation (rho=-0.11) has been observed between age and 2-hour OGTT levels with a p value of more than 0.35.

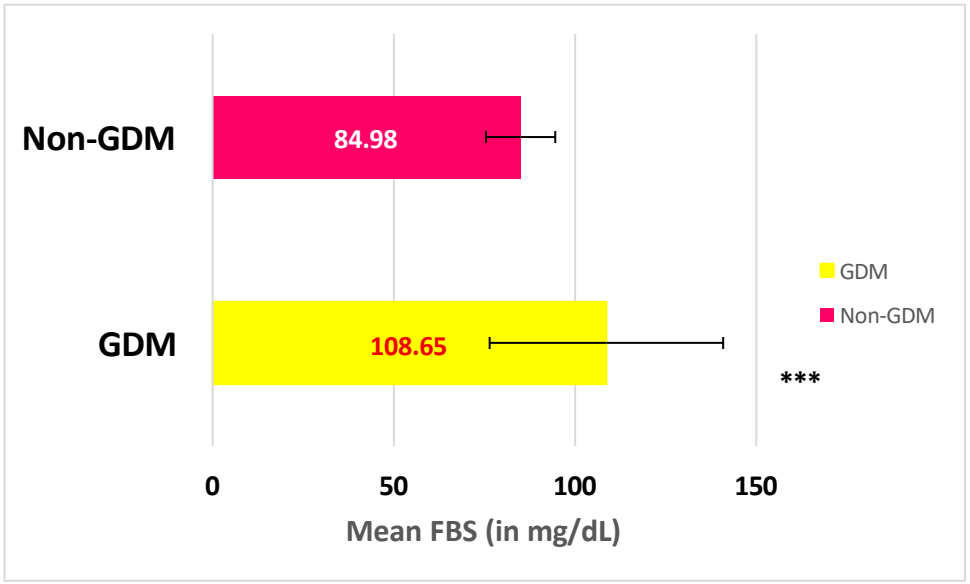

Figure3: Bar plot reflecting the mean fasting blood glucose levels for both the groups. The Standard Deviation is shown using the black bars and mean value is displayed on the bars.

Mann-Whitney U test is applied and a significance difference has been observed ***($p<0.001$). Significance is highlighted in stars.

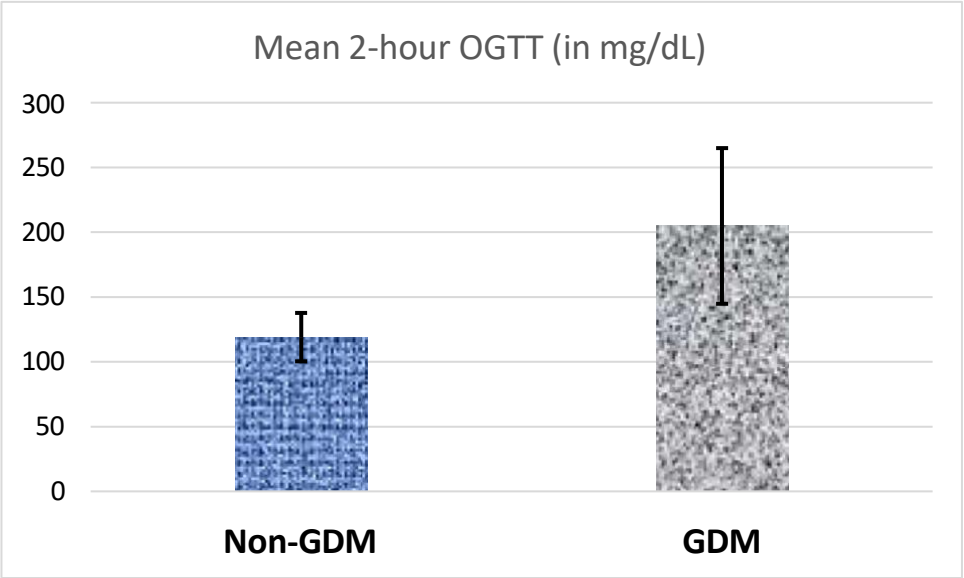

Figure 4: Bar plot reflecting the mean 2-hour OGTT levels for both the groups. The Standard Deviation is shown using the black bars and mean value is displayed on the bars.

Mann-Whitney U test is applied and a significance difference has been observed **($p<0.01$). Significance is highlighted in stars.

Out of the 23 GDM females, 10 of them are expecting their first child, 6 of them their second, and 3 of them will give birth to their third child. The FBS levels of the three females (those expecting their third child) are found to be between 95mg/dL and 119mg/dL with a mean value of 108mg/dL and the 2-hour OGTT value ranged between 99mg/dL to 211mg/dL with a mean value of 145.3 mg/dL.

Conclusion & Future directions

From this study, it can be decoded that only 30% of the recruited expectants are suffering from GDM and a significant difference has been observed between the blood glucose levels of the two groups. No significant correlation has been observed between age and being diabetic. Consequently, a strong conclusion cannot be made as the recruited participants are very less. Increasing the recruited ones till 500 or more can yet give a clear-cut notion and strongly validate the study. However, a large study group with wide age range is required to make a comparative analysis. Patients with pre-existing hypertension, kidney disease and other factors should be considered to make a more evident study and to this study the blood pressure factor can be considered in future to estimate that how many GDM ones are hypertensive too. Molecular studies can be conducted to compare the cytokine levels in both the groups, check gene expression analysis, bioinformatic analyses to extract genes and molecular pathways exacerbating GDM, and molecular docking to design a novel drug targeting the condition.

Conflict of interest

The authors have declared no conflict of interest.

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