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Glycosylated haemoglobin in Gestational Diabetes Mellitus".

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Gestational Diabetes (GDM) is defined as ` any degree of glucose intolerance with onset or first recognition during pregnancy`[1].The incidence of gestational diabetes is increasing worldwide and varies in direct proportion to the prevalence of type-2-diabetes mellitus for a given ethnic group or population. Detection of gestational diabetes is crucial as it is associated with diverse range of adverse maternal and fetal outcome. It also carries a long-term implications for subsequent development of type-2-diabetes in the mother and an increased risk for obesity and glucose intolerance in the offspring.

In India, according to a random national survey, the prevalence of GDM was 16.55%[2]. And the prevalence of gestational diabetes in different parts of India has been reported as 3.8% to 17.9%[3].

Pregnancy is associated with alterations in regulation of glucose metabolism caused by actions of various hormones[4] and substances that antagonize the action of insulin. As a result, a relative state of insulin resistance occurs as the pregnancy progresses. Glycated haemoglobin (HbA1c) is formed by a non-enzymatic glycation pathway by haemoglobin exposure to plasma glucose and is measured as the ratio of glycosylated to non-glycosylated haemoglobin[5]. As the average amount of plasma glucose increases, the fraction of glycated haemoglobin increases in a predictable way. The purpose of this study was to determine the role of HbA1c in predicting cases of gestational diabetes.

I. MATERIALS AND METHODS

1.1 Setting and participants

This was a cross-sectional hospital based study conducted at a tertiary care hospital. Thirty pregnant women between 24th to 28th weeks of pregnancy who were diagnosed as gestational diabetes after an oral glucose tolerance test without any other medical conditions complicating the pregnancy were included in the case. For control groups, thirty age- matched pregnant women with normal glucose tolerance test without any other medical conditions presenting in the out-patient department of the hospital and fulfilling the inclusion criteria were included for the study. An informed consent was taken from all the patients participating in the study.

1.2 Screening and diagnosis of case of Gestational diabetes

A two step procedure was applied for screening and diagnosis of cases of gestational diabetes. Glucose challenge test (GCT) was done and 1 hour blood glucose was measured. All patients with 1 hour glucose level ≥ 140 mg/dl were further taken up for confirmation of diagnosis by performing OGTT test at 24th to 28th weeks of pregnancy. Gestational Diabetes was diagnosed after 100 gms Oral Glucose Challenge Test (OGTT) using the American Diabetes Association Criteria.

1.3 Patient evaluation

A detailed clinical history along with meticulous general physical examination was conducted. Anthropometric measurements including height and weight were taken. Also pre-pregnancy weight was taken and pre-pregnancy body mass index (BMI) was calculated for all the patients using the formula BMI=weight (Kg)/ [height (meter)]^2.

1.4 Sample collection and handling

8ml of fasting venous blood was collected under aseptic precaution and distributed to EDTA vial for complete haemogram and HbA1c, fluoride vial for blood glucose and plain vial for lipid profile measurement.

1.5 Laboratory Methods

Determination of HbA1c was done using an RX Daytona automated analyzer. A lot

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specific values were entered as given in the HA 3444 calibrator insert. The chemistry parameters are predefined on the hard drive of the analyzer PC. Samples were then loaded and analyzed automatically. The assay gives accurate and precise results for a range of total haemoglobin varying between 7 g/dl and 23 g/dl.

1.6 Data management and statistical analysis

All data was processed using the Statistical Package for Social Sciences (SPSS) version 20.0S. Data were expressed as mean ± standard deviation. Comparison of the parameters between the cases and controls was done using "student t test". Correlation between parameters was done using Pearson's correlation test. The "p value" of < 0.05 was considered statistically significant. A receiver operator characteristic curve was plotted for HbA1c for determination of its sensitivity and specificity in the detection of cases of gestational diabetes.

II. RESULTS

2.1 Comparison of clinical and demographic data in cases and controls.

The clinical and demographic characteristics of cases and control populations are presented in Table 1a and Table 1b. There was no statistically significant difference ($p > 0.05$) between the two study groups in terms of maternal age.

Table 1a. Clinical and demographic characteristics of cases and controls

Parameters	Cases (Gestational diabetes) N=30	Controls(normal pregnant women) N=30	p-value
Age[a] (years)	27.06±4.89	26.64±3.18	0.701
Per-pregnancy BMI (kg/m^2)	23.49±2.16	20.95±1.15	0.001
GCT (mg/dl)	152.6±13.58	98.88±33.98	0.001
Serum Cholesterol (mg/dl)	196.36±41.78	161.83±24.97	0.003
Serum Triglyceride (mg/dl)	173.73±68.15	123.06±26.55	0.004
HbA1c (%)	5.89±0.79	4.63±0.38	0.001

Abbreviations: N- number of subjects, BMI – Body Mass Index, GCT- Glucose Challenge Test
Data are presented as mean ± SD and t-test is used for comparison

2.2 Distribution of blood glucose levels in cases of GDM at OGTT

Using the ADA criteria[6], two or more values meeting or exceeding the cut-off points were diagnosed as a case of gestational diabetes mellitus.

Table 1b. Distribution of OGTT in cases (N=30)

OGTT	Mean	Std.Deviation
0 hour (fasting)	107.124	18.2836
1 hour	189.38	7.0479
2 hour	166.4	7.9767
3 hour	137.33	17.8041

2.3 Correlation of HbA1c with other parameters

A statistically significant correlation between pre-pregnancy BMI, serum cholesterol level and blood sugar level at GCT with HbA1c was seen on taking the overall cases and controls. Table 2a.

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Table 2a.

PARAMETERS		OVERALL	CASE	CONTROL
Pre-pregnancy BMI	Pearson Correlation 'p'value	0.531 < 0.05	0.147 0.437	0.343 0.063
S.Cholesterol	Pearson Correlation 'p'value	0.279 0.031	0.026 0.893	0.108 0.569
S.Triglyceride	Pearson Correlation 'p'value	0.240 0.650	0.079 0.679	0.112 0.569
GCT	Pearson Correlation 'p'value	0.556 < 0.05	0.138 0.466	0.039 0.840

2.4 Correlation of HbA1c with OGTT values in cases of gestational diabetes

In the present study, the HbA1c value of cases positively correlated with levels of OGTT at 0,1,2 and 3 hours, however not statistically significant. Table 2b.

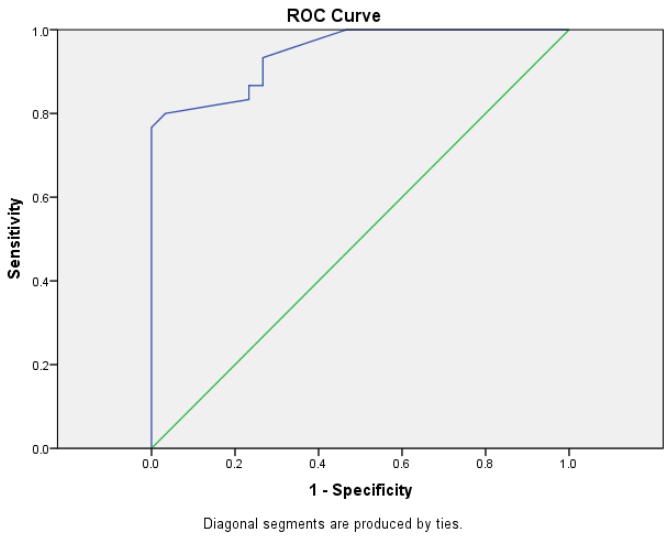
Table 2b. Correlation of HbA1c with OGTT values

OGTT	Pearsons Correlation	P value
0 hour	0.183	0.334
1 hour	0.210	0.265
2 hour	0.297	0.111
3hour	0.039	0.837

2.5 Predictive value of HbA1c for detection of cases of gestational diabetes

An receiver operating characteristic curve (ROC curve) was plotted for HbA1c and its sensitivity and specificity for detection of cases of gestational diabetes was calculated, Figure 1. The area under the ROC curve for HbA1c is 0.945 (p value < 0.001). The critical value of HbA1c ≥ 5.15 gives a sensitivity of 80% and a specificity of 96.7%.

Fig.1. ROC curve of HbA1c



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III. DISCUSSION

Pregnancy is characterized by a series of metabolic changes that promote adipose tissue accretion in early gestation, followed by insulin resistance and facilitated lipolysis in late pregnancy[7]. Development of insulin resistance during pregnancy is multi-factorial, including raised levels of maternal and placental hormones as well as increased caloric intake[8]. To preferentially direct maternal nutrients toward the feto-placental unit for adequate growth of fetus, a physiological state of insulin resistance is required. And the robust plasticity of β-cell function in the face of progressive insulin resistance as the pregnancy progresses is the hallmark of normal glucose regulation during pregnancy. Gestational Diabetes occur in women who fail to sufficiently increase insulin secretion to compensate for the decreased insulin sensitivity during pregnancy leading to hyperglycemia that is detected by routine screening during pregnancy[9].

Glycosylated haemoglobin (HbA1c) has been proposed as an initial investigation in the first trimester of pregnancy for diagnosis of previously unknown pre-pregnancy diabetes mellitus[10]. However, after the first trimester of pregnancy it remains controversial as to whether its value can be a good predictor of gestational diabetes or not.

The present study was conducted on 30 cases of women with gestational diabetes diagnosed at 24th to 28th weeks of pregnancy against an age-matched 30 normoglycemic pregnant women visiting the ante-natal clinic and fulfilling the inclusion criteria. The age ranges from 18 years to 37 years, most of the patients were of the age group between 26 to 30 years with a mean age distribution 26.85 ± 4.370 years. A systemic review and meta-analysis by Yueyi Li, et al[11] demonstrated a linear increase in the risk of GDM with successive age-groups, with a strong positive association between maternal age and GDM risk. However, since age-matched controls were taken for the present study, no significant difference was observed.

Pre-pregnancy BMI is known to be the primary factor in determining BMI during pregnancy, with high pre-pregnancy BMI being a risk factor for development of gestational diabetes and an independent risk factor for post-partum glucose intolerance. In the present study, the pre-pregnancy BMI was significantly higher in cases with a mean of 23.49 kg/m^2 (SD 2.161) as compared to controls, mean = 20.953 kg/m^2 (SD 1.159), p = < 0.05. Torloni MR, et al [12] and Farid N, et al[13] in their systemic review with meta-analysis also that found that the risk of development of GDM is positively associated with pre-pregnancy BMI. The present study result also showed a significant positive correlation between the overall pre- pregnancy BMI and HbA1c level, p= < 0.05. Similar finding was also seen by C. Capula, et al[14].

In the present study, a cut-off value of ≥ 140 mg/dl was taken for screening cases of gestational diabetes. Women having GCT ≥ 140 mg/dl were further taken up for confirmation of GDM by OGTT. The mean value of GCT in cases was 152.6 mg/dl (SD 13.584) whereas in controls it was 98.884 mg/dl (SD 33.988), p < 0.05. Weerakiet S, et al [15] calculated the area under the reciever-operator characteristic curve of a 50 gms GCT [0.63(95% CI] at a cut-off value of 140 mg/dl, and found that the sensitivity and specificity of the test were 90% and 61% respectively, therefore a 50 gm GCT could identify GDM in 54 out of 60 (90%) in their study. A 100 gm oral glucose tolerance test was done on all patients having a GCT ≥ 140 mg/dl for diagnosis of GDM. Patients meeting the ADA criteria for diagnosis of gestational diabetes were taken up as cases. In the present study, the mean blood sugar level at fasting, 1 hour, 2 hour and 3 hour were 107.124 mg/dl, 189.38 mg/dl, 166.4 mg/dl and 137.33 mg/dl respectively. A significant positive correlation was seen between the overall GCT and HbA1c level , however correlation between HbA1c and OGTT values in cases was not statistically significant.

The range of HbA1c value was significantly higher in cases was 4.8% to 6.78% (mean = 5.89%, SD=0.798) whereas in controls it was 3.9% to 5.2% (mean = 4.64%, SD 0.383) , p<0.05. Saleh A, et al [16] and Rajesh R, et al [17] also found that the mean HbA1c of patients with GDM was significantly higher than those without GDM. Baxi L, et al[18] used HbA1c as a screening tool for macrosomia in GDM and suggested that an increase in level of HbA1c signals a potentially enhanced average blood glucose level which demands intensive management of GDM.

In normal pregnancy, lipid metabolism undergoes an adaptive changes. Physiological increase in 2 to 3 fold rise in plasma triglyceride is seen during second and third trimester of pregnancy with a lesser increase in levels of total cholesterol, high-density lipoprotein (HDL-cholesterol), and low-density

lipoprotein (LDL-cholesterol) [19-21]. In the present study, the level of serum total cholesterol and triglyceride levels were significantly higher in cases, mean = 196.3667 (SD41.780) and mean = 173.734 mg/dl (SD 68.154) as compared to controls, mean = 161.834 (SD24.970) and mean =123.067 (SD26.551) respectively , p<0.05. Our findings were consistent with findings of J.Wang, et al [22] who also found an increased lipid profile in patients with gestational diabetes mellitus. Mc Growder, et al[23] also found that total cholesterol and triglyceride levels were significantly higher in GDM (p=0.39 and p=0.33), respectively. They also found higher concentration of HbA1c in women with GDM, p=0.001. In the present study, a significant positive correlation was seen in the overall cholesterol level with HbA1c, p = 0.031. However, the correlation between serum triglyceride and HbA1c was statistically not significant, p=0.654.

A receiver operator characteristic (ROC) curve plotted for HbA1c for prediction of GDM, as diagnosed by the ADA criteria using a two-step approach demonstrated a sensitivity and specificity of 80% and 96% respectively at a critical value of ≥ 5.15. Rajesh R, et al[17] in their study found that at a cut-off HbA1c value of ≥ 5.45%, the sensitivity and specificity was 85.7% and 61.1% for diagnosis of GDM. Lai Y, et al[24] in their study showed that the optimal cut-off point of HbA1c for GDM diagnosis at 5.0% 31 mmol/mol), with a sensitivity of 60.1%, a specificity of 65.3%, with a positive predictive value of 28.1% and a negative predictive value of 87.9%. However, they concluded that HbA1c test is weekly correlated with OGTT during pregnancy and offers limited value in diagnosing GDM among Chinese pregnant individuals. Agarwal MM, et al [25] used a cut-off value of < 5.5% to rule out GDM and achieved a sensitivity of 82.1% and at ≥ 7.5% the specificity was 95.8%. They concluded that despite all progress in methodology, HbA1c remains a poor screening test to detect cases of gestational diabetes. Also Renz PB, et al[26] in their meta-analysis concluded that HbA1c presented high specificity but low sensitivity with an optimal cut-off point at 5.4%.

The early diagnosis of gestational diabetes is vital to minimize adverse pregnancy outcome to both the mother and their offspring. HbA1c is indispensable for managing long-term glycemic index with high prognostic value in diabetic patients. Many studies also confirmed the value of HbA1c in diagnosing cases of GDM with different cut-offs. However, many inconclusive results regarding the diagnostic utility of HbA1c in gestational diabetes are also found. In our study, the level of HbA1c was significantly higher in GDM as compared to non-GDM controls, however, HbA1c was only weakly correlated with blood glucose levels of OGTT. The discrepancies in these studies may be attributed to multiple factors like ethnic differences of the study population and different laboratory methods although standardized.

The limitations of the study included a small sample size, larger number of participants will provide a more comprehensive result. Additionally, the study was not extended throughout the pregnancy, follow up of GDM patients through each trimester of pregnancy may provide a better correlation of parameters and associated adverse pregnancy outcome.

In conclusion, an HbA1c cut-off value 5.15% has a high specificity and sensitivity and maybe used in with OGTT for diagnosis of gestational diabetes mellitus. However, it lacked adequate sensitivity and specificity as to replace the two-step approach in the diagnosis of GDM and further investigations are needed.

Abbrevations:
GDM : Gestational Diabetes Mellitus
HbA1c : Glycosylated Haemoglobin
ADA : American Diabetes Association
IGT: Impaired Glucose Tolerance
NGT : Normal Glucose Tolerance
GCT : Glucose Tolerance Test
OGTT : Oral glucose Tolerance Test
ROC CURVE : Receiver Operator Characteristic Curve

Data Availability :
The data used to support the study findings are available from the corresponding author upon request.

Conflicts of Interest:
The authors have no conflicts of interest to declare.

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