# Assessment of Free -β Human Chorionic Gonadotropin for Early Screening of Gestational Diabetes Mellitus

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**Conflict of interest:** None declared

**Funding:** No funding sources

**Abstract**

**Background**: Regardless of the level of hyperglycemia, gestational diabetes mellitus is known as any grade of glucose intolerance identified during pregnancy. Factor influencing short and long-term hazards to moms and babies is hyperglycemia.

**Aim of the study:** to assess free-β human chorionic gonadotropin correlation with gestational diabetes mellitus and its usefulness in predicting gestational diabetes mellitus at 11–14th week of pregnancy.

**Material & Methods:** The study was prospective, observational cohort study, 80 participants allocated into the gestational diabetes mellitus group (n = 34) and control group (n = 46). Free-β human chorionic gonadotropin were assessed between 11–14 gestational weeks. Screening for using 75-g oral glucose tolerance test following 8-hour overnight fasting, with plasma glucose assessment in fasting and 2 hours at 24-28 weeks' gestation.

**Results**: There was variance between groups concerning fasting blood glucose & post prandial, where both are elevated in the gestational diabetes mellitus group. A significant difference was observed in the prevalence of a family history of diabetes between the GDM and control groups. The GDM group exhibited a markedly higher proportion of individuals with a family history of diabetes.

**Conclusion:** Since there was no variance between the groups under study, using free-β human chorionic gonadotropin as early gestational diabetes mellitus screening test is not suggested.

**Keywords:** Gestational Diabetes Mellitus, Fasting blood glucose, Free -β Human Chorionic Gonadotropin

*Tob Regul Sci.™ 2023 ;9(1): 7326 - 7334*

*DOI: doi.org/10.18001/TRS.9.1.519*

**Introduction:**

Any degree of glucose intolerance that is first recognized or develops during pregnancy is known as gestational diabetes mellitus (GDM). There are two types of GDM: A1GDM and A2GDM. Diet-controlled GDM, also known as A1GDM, is GDM that is responsive to nutritional therapy. Conversely, A2GDM is GDM that has been treated with drugs to achieve adequate glycemic control. Testing all pregnant women for gestational diabetes mellitus (GDM) at 24 weeks of gestation was recommended in 2014 by the US Preventive Services Task Force [1]. Within the first five years following pregnancy, type 2 diabetes mellitus (T2DM) will strike about 50% of women with GDM [2].

One of the most frequent medical issues associated with pregnancy is GDM, a condition whose prevalence has sharply increased globally. However, due to population, race, and ethnic variety, as well as different screening methods and diagnostic approaches, the incidence of GDM is also very variable [3].

During pregnancy, the placenta produces human chorionic gonadotropin (HCG). To preserve pregnancy, HCG encourages progesterone secretion by the corpus luteum. Additionally, the liver, colon, and pituitary glands produce smaller amounts of free-β hCG [4-6]. Combining the negative correlations between HCG and Oral glucose tolerance tests (OGTT) values measured in 2$^{nd}$ trimester and the absence of relationships between HCG and fasting blood glucose (FPG) or HbA1c levels tested during early pregnancy may indicate that the effects of HCG on glucose homeostasis may accumulate. HCG levels increase after implantation and reach their peak at approximately 10 weeks of pregnancy [7].

This investigation aimed to evaluate free beta-human chorionic gonadotropin (free-β hCG) levels in the first trimester (11-14 weeks) with gestational diabetes mellitus (GDM). The study design considered the conflicting results reported in prior research and addressed the gap in current knowledge regarding the predictive utility of these markers for GDM in the Egyptian population.

**Material & Methods:**

**Operational design**

A prospective, observational cohort study was performed at Endocrinology and gynecology outpatient clinics at Zagazig University Hospital. The assessment includes one year of data. Verbal and written informed consent were obtained from all participants after an explanation of the procedure and medical research. The research was conducted under the World Medical Association's Code of Ethics (Helsinki Declaration) for human research. This study was carried

out after the approval of the Institutional Review Board (IRB). The participants included in this investigation were allocated into the GDM group (n = 34) and Control Group (n = 46). Using a one-step approach, a 75-g OGTT is used for GDM screening at 24–28 weeks of gestation. Plasma glucose is evaluated during the fasting period and at 2 hours after the patient has fasted for 8 hours the previous night. When any of the subsequent plasma glucose levels were elevated, GDM is diagnosed: fasting ≥ 92 mg/dl and then two hours later, 153 mg/dL.

Cases with the following criteria were included: pregnant women aged more than 18 years old with a singleton pregnancy had their 1st antenatal visit prior to 14 weeks of gestation and had periodic follow-up visits.

Cases with the following criteria were excluded: women with pre-GDM, multiple gestations, congenital or genetically malformed fetuses, and women with delivery < 24 gestational weeks.

**Patients and Methods:**

Full history of diabetes mellitus, multiple pregnancies, macrosomic baby and last menstruation. Complete physical and clinical examination. Investigations include Routine lab investigations: complete blood count, liver functions, kidney function, and fasting blood glucose. Radiologic investigations: obstetric pelvi-abdominal ultrasound. Other special investigations: free-β HCG 75-g glucose OGTT in women at 24–28 gestation weeks.

**Assay procedure for (HCG)**

Each specimen was mixed gently and kept at room temperature (18–26 °C) along with the kit reagents. Pipette 50 µL of sera, control, and hCG standards. Filled each well with 100 µL of enzyme conjugate. Place a cover on the plate and let it sit at room temperature (18–26 °C) for 60 minutes. Liquid was taken out of every well. Three thorough washings using 300 µL of 1x wash buffer. Spread out over absorbing paper towels. To every well, 100 µL of TMB substrate was added. Ten minutes of incubation was conducted at room temperature. Every well was further supplemented with 50 µL of the stop solution. To blend the solution, the plate was gently shaken. Within fifteen minutes of adding the stopping solution, the absorbance on the ELISA Reader read 450 nm.

**Statistical analysis:**

A statistical analysis was performed on the data using IIBM SPSS, version 23.0. (IBM Corporation, Armonk, New York). When describing quantitative data, we used the mean standard deviation; when expressing qualitative data, we used the number and percentage. All percentages for categorical variables were compared using the Chi-square test. When comparing two sets of data that follow quantitative normal distributions, the student's t-test is the statistical tool of choice. When comparing two non-normally distributed groups with quantitative data, the Mann-Whitney test is a useful statistical tool. The Pearson correlation coefficient (r) is a method of determining the degree and direction of a linear association between two variables. The Spearman correlation coefficient test (r-test) is used to investigate the relationship between two or more nonparametric quantitative variables. All the tests were two-sided. A p-value < 0.05 is considered significant.

## Result

**Baseline Characteristics:**

- **Age and BMI:** No statistically significant differences were observed in age or body mass index (BMI) between the study groups.

**Blood Glucose Levels:**

- **Fasting Blood Glucose (FBG) and 2-hour Postprandial Glucose (2hPP):** Both FBG and 2hPP levels exhibited significant differences between the groups. The GDM group displayed markedly elevated blood glucose levels compared to the control group.

**Markers:**

- **Free β-hCG:** Median and interquartile range (IQR) values for free β-hCG were similar in both groups (0.7 [0.2 - 1.4] vs 0.7 [0.2 - 1.4]; p: 0.964). This indicates no statistically significant difference in free β-hCG levels between the groups.

**Family History of Diabetes:**

- A significant difference was observed in the prevalence of a family history of diabetes between the GDM and control groups. The GDM group exhibited a markedly higher proportion of individuals with a family history of diabetes.

**Correlations:**

- Free β-hCG levels did not show a statistically significant association with either age or BMI in the studied population.
- There was also no significant correlation between free β-hCG and FBG or 2hPP blood glucose levels.

- Table (1) : Demographic Data of both groups according to age & BMI :-

| | (GDM group) No. = 34 | | (Control group) No. = 46 | | P - Value | Sig |
|---|---|---|---|---|---|---|
| | Range | Mean ±SD | Range | Mean ±SD | | |
| Age(Years) | 18 – 32 | 23.62 ± 3.55 | 18 – 29 | 22.82 ±4.31 | 0.063 | NS |
| BMI (Kg/m2) | 20.7 - 24.9 | 21.05 ± 1.12 | 19.7 - 23.4 | 20.15 ± 1.02 | 0.23 | NS |

> 0.05 NS: Non significant; <0.05 S: Significant; < 0.01 HS: Highly significant

*:Chi-square test; •: Independent t-test

- This study included 80 pregnant women, there is statistically **non-significant difference** between both groups regarding age or BMI.

Table (2): Demographic Data of both groups according to FBG & OGTT ( 2hPP ) :-

| | (GDM group) No. = 34 | | (Control group) No. = 46 | | P - Value | Sig |
|---|---|---|---|---|---|---|
| | Range | Mean ±SD | Range | Mean ±SD | | |
| FBG (mg/dL) | 94 – 109 | 99.93 ± 3.20 | 71 – 91 | 83.65 ± 3.04 | 0.001 | HS |
| OGTT( 2hPP ) | 138 – 191 | 172.58 ± 8.85 | 110 – 152 | 139.95 ± 3.91 | 0.001 | HS |

- This study included 80 pregnant woman, there is **statistically significant difference** between both groups regarding (FBG & 2hPP ) blood glucose where both are significantly higher in GDM group.

Table (3) Biochemical Markers, PAPP-A and HCG of the studied women in both groups

| Marker | (GDM group) No. = 34 | (Control group) No. = 46 | P - Value | Sig |
|---|---|---|---|---|
| Fβ-HCG MoM | 0.7 (0.2 - 1.4) | 0.7 (0.2 - 1.4) | 0.964 | NS |

NS: Non-significant; S: Significant; HS: Highly significant This study include 80 pregnant women, there is statistically **non- significant difference** between both groups regarding free β-HCG. There is **statistically significant difference** between both groups regarding PAPP-A where it was significantly lower in GDM group.

Table (4) : Comparison of family history of diabetes in both groups:-

| | | (GDM group) No.=34 | | (Control group) No.=46 | | P -Value | Sig |
|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | | |
| Family history of diabetes | yes | 20 | 58.8% | 8 | 17.4% | 0.0001* | HS |
| | no | 14 | 41.1 % | 38 | 82.6% | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | | | |

NS: Non-significant; S: Significant; HS : Highly significant

Chi-square test

This study included 80 pregnant women, there is **high statistically significant difference** between both groups regarding Family history of diabetes where it was significantly higher in GDM group.

Table (5): **Correlation between PAPP- A and different parameters in the studied patients:-**

| Parameter | PAPP- A | |
|---|---|---|
| | R | P |
| FBG ( mg/dL) | -1.579 | 0.001* |
| OGTT ( 2hPP ) | -1.495 | 0.001* |

There was **significant correlation** between PAPP_A and FBG & 2hPP in the studied patients where increased FBG & 2hPP blood glucose associated with lower levels of PAPP_A.

Table (6): **Correlation between HCG and different parameters in the studied patients:-**

| Parameter | β-HCG | |
|---|---|---|
| | R | P |
| Age (years) | 0.124 | 0.582 |
| BMI ($Kg/m^2$) | 0.221 | 0.324 |
| FBG (mg/dL) | 0.294 | 0.185 |
| OGTT ( 2hpp ) | –0.362 | 0.062 |

There was **no significant correlation** between β-HCG and age & BMI in the studied patients

There was **no significant correlation** between β-HCG and FBG & 2hPP blood glucose in the studied patients

Table (7): Binary logistic regression analysis for relevant predictors of patients with Gestational Diabetes Mellitus

| Predictors | B | p-value | OR | 95% CI | |
|---|---|---|---|---|---|
| | | | | Lower limit | Upper limit |
| BMI | 0.161 | 0.026* | 0.851 | 0.739 | 0.981 |
| Age | 0.029 | 0.269 | 1.029 | 0.978 | 1.084 |
| Free β-HCG | 0.341 | 0.092 | 1.407 | 0.946 | 2.093 |

*p<0.05 is statistically significant, (OR) adjusted odds ratio

and (CI) confidence interval

On doing multivariate analysis of Gestational Diabetes predictors among 80 pregnant women , low level of PAPP_A , increased BMI, independently increase risk of Gestational Diabetes Mellitus ( OR were **1.019. 0.851**, respectively).

## Discussion

About 9–25% of pregnancies worldwide are affected by GDM, which can be identified as any grade of glucose intolerance that was initially detected during pregnancy. Rates of the condition are dependent on the criteria of diagnosis and study participants. The hallmark of GDM is decreased glucose tolerance due to maternal dysfunctional pancreatic β-cell, which leaves insufficient insulin to maintain glucose homeostasis during pregnancy [8,9].

Free β-hCG as well as the PAPP-A decreased concentrations in the 1st trimester are correlated with an elevated risk of pre-eclampsia, preterm delivery, and spontaneous fetal loss [11-13].

**In our study,** patients' ages were between 18 and 32 years in (the GDM) group and ages were between 18 to 29 years in the control group with no marked variance (p= 0.063). Moreover, the Patient's BMI ranged between 20.7 - 24.9 with a mean of 21.05 ± 1.12 in the GDM group, and BMI ranged between 19.7 - 23.4 with a mean of 20.15 ± 1.02 in the control group with no substantial variation ( p= 0.23 )

**Our study coped with** a study performed by Yanachkova et al. [14], who reported that cases with GDM and controls were not markedly varied concerning age and BMI, with maternal age of 33.3 years among the GDM group and 32.8 years among the control group (p= 0.251), mean BMI 26.08 among GDM group and 22.9 among the control group (p= 0.422).

**Also, Xiao et al.** [5], stated that compared to women in the control group, GDM women were older and had a higher BMI (P < 0.001), median maternal years 32 (29–34) in GDM group 29 (27–32) in the control group. Median maternal pre-pregnancy BMI, kg/m2 20.83 (19.23–23.03) among the GDM group and 19.72 (18.43–21.40) among the control group.

On the other hand, **Cheuk et al.** [15], showed that women in the GDM group had higher BMI and were noticeably older (34 vs. 32 years) than those in the non-GDM group.

**In our study, the** family history of DM was substantially varied between groups; the family history of DM was 58.8% among the GDM group and 17.4% among the control group (P< 0.0001).

**Besides, Xiao et al.** [5], stated that the number of individuals with a family history of DM was higher among the GDM group (7.5%) than among the control group (3.9%) (P< 0.01).

**Also, Yanachkova et al.** [14], stated that marked variations were detected respecting the family history of DM (p < 0.001); it was 57.8% among the GDM group and 14.8 % among the control group.

**Cheuk et al.** [15], showed that family history of diabetes there was a high substantial variance between both groups; there was a family history of diabetes in 30.2% of the GDM group and 20.5% in the control group (P= 0.01).

**Furthermore, in our study,** there was marked variation between both groups regarding (FBG & 2hPP) blood glucose. FBG range (94 – 109) with a mean of 99.93 ± 3.20 and 2hPP (138 – 191) with a mean of 172.58 ± 8.85 in the GDM group also FBG (71 – 91) with a mean 83.65 ± 3.04 and 2hPP (110 – 152) with mean 139.95 ± 3.91 in control group, where both are significantly higher in GDM group.

**Yanachkova et al.** [14], stated that there were statistically significant differences identified regarding fasting plasma glucose [mg/dL]; the mean (SD) was 98 among the GDM group and 86 among the control group (p < 0.001).

**In our study,** 80 pregnant women were included, and there was a non-remarkable variance between groups regarding HCG; freeβ-HCG MoM in the GDM group was 0.7 (0.2 - 1.4) and in the control group was 0.7 (0.2 - 1.4) (P: 0.964)

**Xiao et al**[5],. showed that freeβ-hCG MoM levels were lower among the GDM group than among the normal participants, but the variance between groups was not remarkable (P > 0.05).

**Yanachkova et al.** [14], showed that groups were not varied in terms of Free β-hCG adjusted MoM (p = 0.325). **Moreover, Cheuk et al.** [15], **showed** that groups were not varied in terms of Free β-hCG adjusted MoM, Free β-HCG (MoM) 1.05 (0.73-1.64) in the GDM group and 1.02 (0.71-1.55) in the control group (P= 0.29).

**Conclusion:**

Since there was no marked variance between the groups under study, using hCG as an early GDM screening test is not suggested. Early detection of GDM-related risks in women may facilitate plans to alter risk factors that contribute to the disease's advancement and, consequently, prevent overt DM and its consequences.

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