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The Value of Pregnancy Associated Plasma Protein -A as an Early Screening Marker for Gestational Diabetes Mellitus

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Abstract:Introduction: Regardless of the level of hyperglycemia, gestational diabetes mellitus (GDM) is defined as any grade of glucose intolerance that is identified during pregnancy. Hyperglycemia is a factor that influences the short- and long-term hazards to mothers and babies.

Background: To assess the correlation of pregnancy-associated plasma protein A (PAPP-A) with gestational diabetes mellitus and its usefulness in predicting gestational diabetes mellitus at 11–14th week of pregnancy in Egypt.

Material & Methods: This was a prospective, observational cohort study with 80 pregnant participants. PAPP-A levels were assessed between 11 and 14 gestational weeks. Screening for GDM 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 of gestation.

Results: After Screening for GDM at 24-28 weeks of gestation, the gestational diabetes mellitus (GDM) group (n = 34) and those without GDM group (n = 46). There was variance between the groups concerning fasting blood glucose and postprandial blood glucose levels, both of which were elevated in the gestational diabetes mellitus group. There was variation between both groups with respect to PAPP-A, which was reduced in the gestational diabetes mellitus group, and substantial variance between both groups regarding family history of diabetes, which increased in the gestational diabetes mellitus group.

Conclusion: PAPP-A could be used as an early gestational diabetes mellitus screening procedure at 11–14th week of pregnancy.

Keywords: Pregnancy-associated plasma protein A, Gestational Diabetes Mellitus, Fasting blood glucose, postprandial blood glucose

Introduction

Any degree of glucose intolerance that is first recognized or that 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 responsive to nutritional therapy. Conversely, A2GDM is GDM that has been treated with drugs to achieve adequate glycemic control. Testing all pregnant women with 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) occurs in approximately 50% of women with GDM **[2]**.

One of the most frequent medical issues associated with pregnancy is GDM, a condition with a sharp global increase in prevalence. However, due to population, race, and ethnic variety, as well as different screening methods and diagnostic approaches, the incidence of GDM is also highly variable **[3]**.

Insulin-like growth factor-1 (IGF-1) is raised when pregnancy-associated plasma protein A (PAPP-A) is formed by trophoblasts during pregnancy and is observed as early as the 28th day after fertilization. Insulin resistance is caused by a decrease in IGF-1 levels following low blood levels of PAPP-A **[4]**. As low PAPP-A levels are an emerging risk factor for GDM, taking this into account can aid in the early identification of at-risk individuals. Additionally, pregnant women should request this test for aneuploidie screening between weeks 11 and 14 of gestation **[5]**.

Pregnancy-associated plasma protein-A (PAPP-A) is a protein originate in the maternal circulation and is generated by the placenta. The widespread tradition is to employ the unit multiple of median (MoM) as a gestational age-reliant representation of PAPP-A concentration. At a low level maternal serum PAPP-A in the first trimester have been concomitant with a widespread variety of fetal pathology and pregnancy-related complications:-

- aneuploidy (including Down syndrome)
- miscarriage
- pregnancy-induced hypertension
- intrauterine growth restriction
- gestational diabetes mellitus[6].

Owing to their prospective role in placental pathology and carbohydrate homeostasis, PAPP-A and free β-hCG measurements may possibly be of importance in screening for GDM along with screening for chromosomal abnormalities **[7]**.

This study was designed to assess the correlation of PAPP-A levels with GDM and its usefulness in predicting GDM at 11–14th week of pregnancy.

Material & Methods:

Operational design

This prospective, observational cohort study was performed at the endocrinology and gynecology outpatient clinics at Zagazig University Hospital, Egypt. The assessment includes one year of data collection. Verbal and written informed consent were obtained from all participants after an explanation of the procedure and medical research. This research was conducted in accordance with the World Medical Association's Code of Ethics (Helsinki Declaration) for human research. This study was approved by the Institutional Review Board (IRB). The participants included in this investigation were the GDM group (n = 34) and without GDM group (n = 46) groups. The serum levels of PAPP-A were assessed between 11 and 14 weeks of gestation. Using a one-step approach, a 75-g OGTT was performed for GDM screening at 24–28 weeks of gestation. Plasma glucose was evaluated during the fasting period and 2 h after the patient had fasted for 8 h the previous night. When any of the subsequent plasma glucose levels were elevated, GDM was diagnosed as fasting ≥ 92 mg/dl and then two hours later, 153 mg/dL.

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

Patients 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 babies and last menstruation. Complete physical and clinical examination. The investigations included routine laboratory investigations, including complete blood count, liver function, kidney function, and fasting blood glucose. Radiological investigations: obstetric pelvi-abdominal ultrasonography Other special investigations included PAPP-A levels and 75-g glucose OGTT in women at 24–28 weeks of gestation

Assay procedure for (PAPP-A)

After adding 100μL of the standard or sample to each well, cells were incubated at 37 °C for 2 h. Next, 100 μL of Detection Reagent A was added after aspiration. Waited for one hour at 37 °C. I aspirated the sample three times and then washed it. Detection reagent B (100 μL) was then added. Left at 37 °C for 1 h; no further handling. Floating five times after aspiration. The substrate Solution (90 μL) was added. The mixture was then warmed to 37 °C for 15–25 min. The 50μL stopped solution was added. The absorbance was measured at 450 nm as soon as possible.

The IRB has reviewed and assessed the above-named study regarding the potential risks and benefits based on the Declaration of Helsinki. The "ratio" of risk to benefit is reasonable, given the goals of the study. The variables assessed, including the proposed subject populations, proposed procedures and scientific background are supporting the study. The IRB approved that it is within the ethical guidelines as outlined in the Declaration of Helsinki having met the requirements set forth by the Institutional

Review Board by an expedited review process.

IRB#:9395-27-3-2022

Approval DATE: 27-3-2022

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation with the Helsinki Declaration of 2013 and later versions. Informed consent was obtained from all patients who were included in the study.

Statistical-analysis:

Statistical analysis was performed using IIBM SPSS version 23.0. (IBM Corporation, Armonk, New, USA). When describing quantitative data, we used the mean standard deviation, and when expressing qualitative data, we used the number and percentage. All percentages of categorical variables were compared using the chi-squared 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 U test is a useful statistical tool. The Pearson correlation coefficient (r) is used to determine the degree and direction of a linear association between two variables. Spearman's correlation coefficient test (r-test) was used to investigate the relationship between two or more nonparametric quantitative variables. All tests were two sided. Statistical significance was set at $P < 0.05$.

Results:

This study included 80 pregnant women; there was no substantial variance in age and BMI between the groups.

There was a marked variation between both groups respecting (FBG and 2hPP) blood glucose levels, which were markedly increased in the GDM group.

There was substantial variance between the two groups with respect to PAPP-A, which was significantly lower in the GDM group. There was a remarkable variation between both groups regarding family history of diabetes, with marked variance in the GDM group.

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
PAPP-A MoM	0.88 (0.61 – 1.28)	0.97 (0.67 – 1.37)	<0.001	HS

NS: Non-significant; S: Significant; HS: Highly significant
This study included 80 pregnant women, 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
PAPP-A	0.019	0.007*	1.019	0.999	1.041

*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).

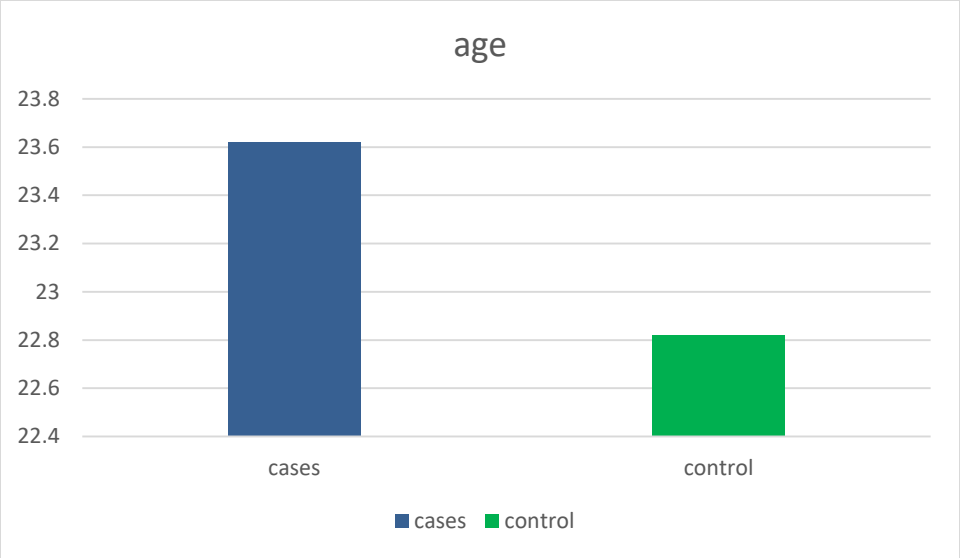

Fig 1: Mean ±SD of age in the studied groups
GDM group mean age was 23.62 ± 3.55
Control group mean age was 22.82 ±4.31

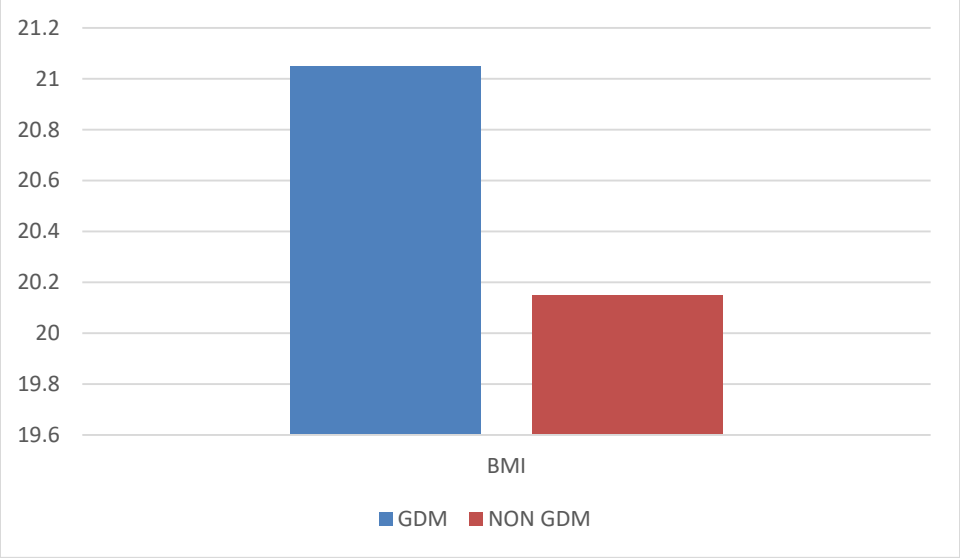

Fig 2: Mean ±SD of BMI in the studied groups
GDM group mean age was 21.05 ± 1.12
Control group mean age was 20.15 ± 1.02

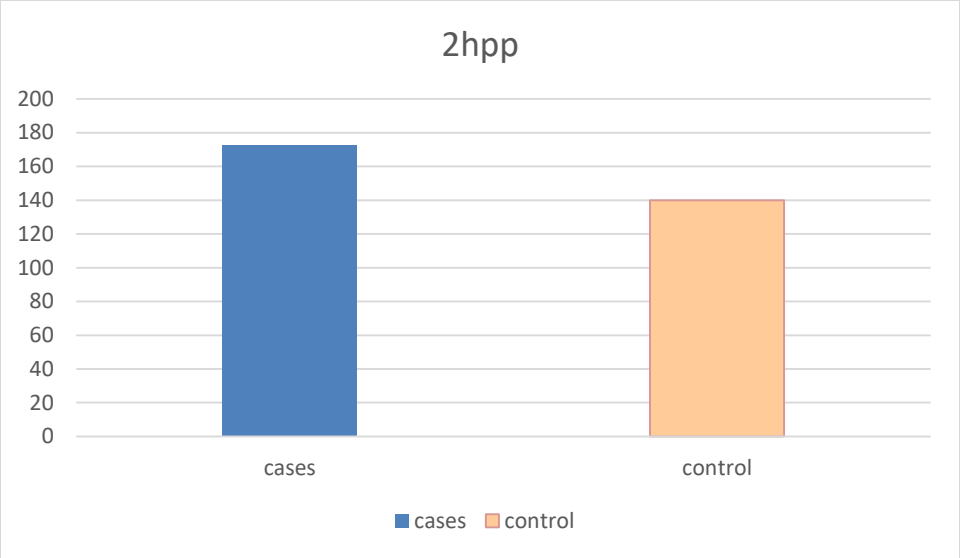

Fig 3: Mean ±SD of 2hPP in the studied groups
GDM group mean age was 172.58 ± 8.85
Control group mean age was 139.95 ± 3.91

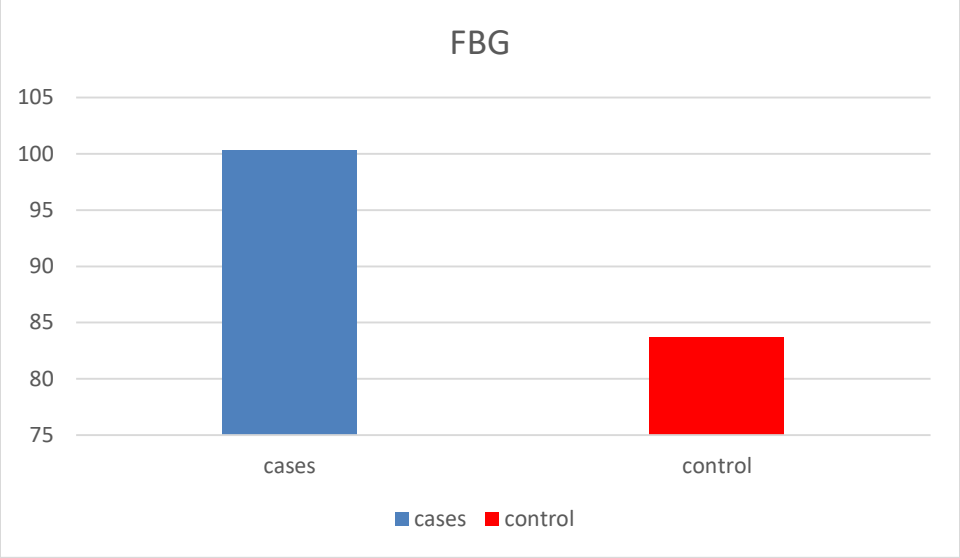

Fig 4: Mean ±SD of FBG in the studied groups
GDM group mean age was 99.93 ± 3.20
Control group mean age was 83.65 ± 3.04

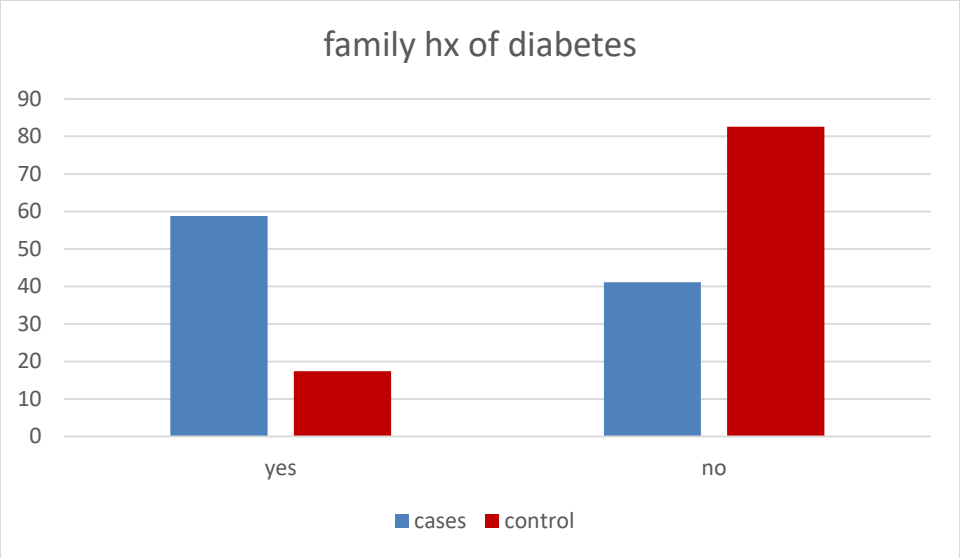

Fig 5: Family history of diabetes in the studied groups

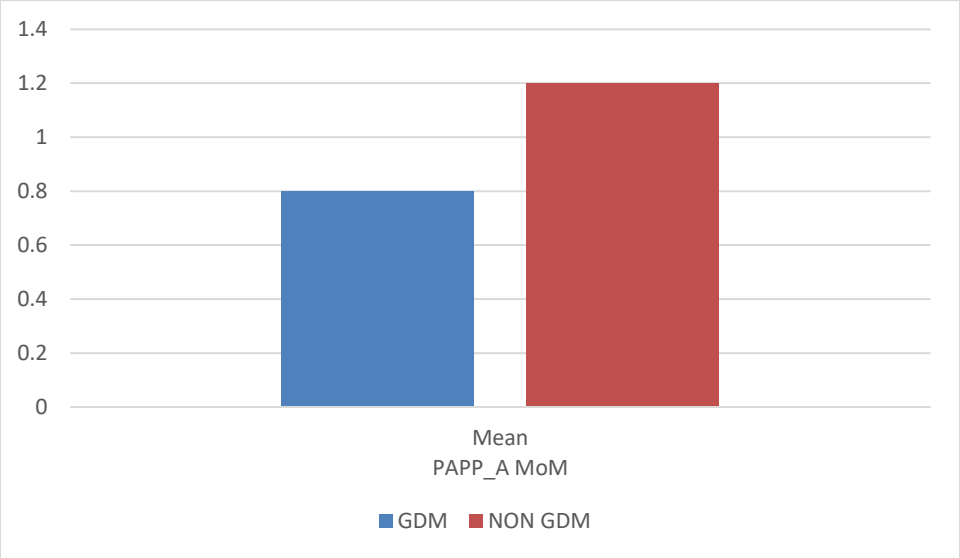

Fig 6: Mean **±SD** PAPP_A of the studied groups
GDM group mean age was 0.8 ± 0.24
Control group mean age was 1.2 ± 0.26

DISCUSSION

Approximately 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. The rates of the condition were dependent on the criteria of the diagnosis and study participants. The hallmark of GDM is decreased glucose tolerance due to dysfunctional maternal pancreatic β-cells, which leave insufficient insulin to maintain glucose homeostasis during pregnancy [8].

Many investigations have reported links between low PAPP-A levels in early pregnancy and GDM progression. PAPP-A appears to play a role in IGF bioavailability during pregnancy. This is significant because the IGF axis is essential for fetal growth and placental development [9].

In the first trimester, circulating PAPP-A values are measured, to screen for fetal chromosomal mutations such as Patau syndrome, Down syndrome, and Edward syndrome [10]. Numerous pregnancy or maternal variables, including maternal weight, gestational age, ethnicity, and smoking, are known to affect free hCG and PAPP-A [11].

Decreased concentrations of PAPP-A in the 1st trimester are correlated with an elevated risk of preeclampsia, preterm delivery, and spontaneous pregnancy [12].

An inverted association between glycated hemoglobin (a marker applied to evaluate glucose levels throughout three months) and PAPP-A indicates that PAPP-A could reflect the grade of glycemic control[13].

In our study, the patient's ages were between 18 and 32 years in (the GDM) group and between 18 and 29 years in the without GDM 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 without GDM 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).

In our study, there was a remarkable variation in PAPP-A between the groups, which was significantly lower in the GDM group. Mean PAPP-A MoM 0.4 - 1.3 in (GDM group) with Mean ± SD 0.8 ± 0.24 and in (without GDM group) 0.8 -1.8 with mean ±SD 1.2 ± 0.26.

Although numerous studies have assessed the link between PAPP-A in the first trimester and the occurence of GDM, the outcomes still controversial.

A meta-analysis by Talasaz et al [17] of 17 publications proposed that a low PAPP-A level in early gestation was concomitant with GDM; the sensitivity and specificity for predicting GDM were 55% (53–58%) and 90% (89–90%), correspondingly, with an AUC (0.7) indicating low precision.

The current results are comparable to those of Talasaz et al in that the PAPP-A level in the GDM group was much lower than in the without GDM group. This study state that a low serum PAPP-A in the first trimester was related to the incidence of GDM

In the meta-analysis of 13 studies (9 done in Europe, 2 in Australia, and 2 in China) by Donovan et al, [18] women with GDM had lower levels of PAPP-A in the first trimester compared with those who were without GDM.

Other studies advocated no differences in PAPP-A levels in women with or without GDM in the first trimester. Husslein et al [19], Reasonably, the differences may be correlated to ethnicity, research design, diagnostic criteria for GDM, or the study's statistical power.

Beneventi et al. [16] revealed that both the first trimester median and adjusted MOM PAPP-A levels were substantially lower in the GDM group than in the normal group. PAPP-A (adjusted MoM) 1.2 (0.8-1.6) among the control group and 0.7 (0.5-1.2) among the GDM group (p< 0.001).

Furthermore, **Yanachkova et al**. [14] reported that PAPP-A (adjusted MoM) was substantially decreased in the GDM group compared to that in normal participants (p < 0.0001).

Xiao et al. [5] found that serum PAPP-A levels in 1st trimester were remarkably reduced in women with GDM compared to the control (P < 0.001), with a median PAPP-A MoM of 0.88 (0.60–1.28) among the GDM group and 0.97 (0.67–1.37) in the control group.

Additionally, Cheuk et al. [15] demonstrated no statistically significant difference in PAPP-A MoM between the control group and women whose GDM was diagnosed at an earlier stage (P = 0.11).

In our study on the Correlation between PAPP-A and age, there was no significant association between PAPP-A and age (P < 0.478). **Cheuk et al.** [15] revealed that the association between maternal age and PAPP-A level was not significant (P < 0.89).

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) and median maternal age of 32 (29–34) in the GDM group and 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.

In contrast, **Cheuk et al.** [15] showed that women in the GDM group had a 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% in the GDM group and 17.4% in the without GDM group (P< 0.0001).

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

Also,Yanachkova et al. [14]reported that marked variations were detected respect to family history of DM (p < 0.001); it was 57.8% in the GDM group and 14.8 % in 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 a marked variation between both groups regarding (FBG and 2hPP) blood glucose levels between the groups. FBG 94 – 109 with a mean of 99.93 ± 3.20 in 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 without GDM group, where both are significantly higher in GDM group.

Yanachkova et al. [14] reported statistically significant differences in fasting plasma glucose [mg/dL]; the mean (SD) was 98 among the GDM group and 86 in the GDM and control group, respectively (p < 0.001).

CONCLUSION:

PAPP-A could be used as an early gestational diabetes mellitus screening procedure at 11–14th week of pregnancy. Early detection of GDM-related risks in women may facilitate plans to alter the risk factors that contribute to the advancement of the disease and, consequently, prevent overt DM and its consequences.

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