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A study on association between serum Pentraxin-3 and Gestational diabetes mellitus– A case control study.

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Abstract
Background: *In pregnancy there is heightened oxidative and inflammatory stress. The stress level is higher in case of Gestational diabetes mellitus(GDM). Pentraxin-3 (PTX3) is an acute phase protein that belongs to the Pentraxin super family.Our aim is to study the serum levels of Pentraxin-3 in pregnant women without any comorbidities and in Gestational Diabetes Mellitus(GDM).*
Material and methods: *Our study was case control study conducted among pregnant women of gestational age between 24 to 28 weeks attending the department of Obstetrics and gynaecology, Rajah Muthiah Medical College and Hospital, Chidambaram. 32 pregnant women diagnosed to have GDM were included as cases and the pregnant women without any comorbidities were taken as control. Informed and Written consent was obtained from all the study participants. 5 ml of random venous blood were collected following strict aseptic precautions and the serum Pentraxin-3 levels were estimated. Independent student "t" test was applied to find out the difference in mean between the study groups.*
Results: *The mean Pentraxin-3 value among the participants with GDM was 0.13 ± 0.05 pg/ml and that of the control group was 0.15*
± 0.07 pg/ml.The mean PTX-3 was mildly lower in GDM group than in control group(P value > 0.05).
Conclusion: *Though Pentraxin-3 is an inflammatory marker,our study has shown mildly lower levels of Pentraxin-3 among gestational diabetes mellitus group in comparison to the control group. More elaborate studies need to be done to assess the implication of PTX-3 as a biomarker of inflammation.*
Key words: *Pentraxin-3, Gestational diabetes mellitus, Inflammation, Pregnancy, Marker.*

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I. Introduction

Of all the conditions a woman encounter during her pregnancy, the most common one would be 'hyperglycaemia'. Increased blood glucose in pregnancy is found to be associated with many maternal and foetal morbidities. Both World Health Organisation and The International Federation of Gynecology and Obstetrics(FIGO) have classified 'hyperglycaemia in pregnancy (HIP)' into three different categories namely pre-gestational diabetes, Gestational diabetes mellitus and Diabetes in pregnancy. Of all the three states of hyperglycaemia, Gestational diabetes mellitus is found to be the most common contributing to around 8 to 9 of every 10 hyperglycaemia in pregnancy cases(2). Gestational diabetes mellitus (GDM) is defined as 'any degree of glucose intolerance with onset or first recognition during pregnancy'(20).The prevalence of GDM between 2005-2015 was reported to be 11.7% in the South East Asian region only next to Middle East and North African region with prevalence of 12.9%(3). The prevalence of GDM in India was reportedbetween 3.8% to 17.9%(4).

Pregnancy is a state with various oxidative and inflammatory stress. This stress plays an important role in placental development and its function, foetal development and labour. In GDM the levels of oxidative stress are heightened either due to over production of free radicals or defect in antioxidant defences(5,6).The above heightened oxidative stress would get reflected in the form of inflammation and it was found that women with hyperglycaemia had an acute phase inflammatory response(7). Tracking the inflammatory markers would aid in the diagnosis of GDM(8).

Pentraxin- 3 (PTX3),an acute phase proteins of the pentraxin family,found to be raised in GDM(7). Pentraxin-3 forms a super family of multifunctional proteins. The Pentraxin family in common have a C(Carboxy)-terminal region of a 200 amino acid domain containing a highly concentrated motif of 8

amino acid sequence which is called as the Pentraxin signature. The Pentraxin family can be divided into two groups, the short constituents and the long counterparts(9). PTX3 belongs to long chain Pentraxin group and synthesised locally by stromal and myeloid cells in response to proinflammatory signals and microbial moieties. It plays a regulatory role in inflammation and could be functional ancestor to antibodies(10). PTX3 was found to dampen inappropriate immune response, neutrophil recruitment and oxidative burst(9).Studies have reported a pathologic role for inflammation in the development of GDM alongside some studies had shown that the levels of PTX-3 were increased among the pregnant women with gestational diabetes mellitus than those without gestational diabetes mellitus(7).

Our present study aims to assess the serum levels of pentraxin -3 in pregnant women without any comorbidities and in GDM. Studies with similar objectives were not undertaken so far in the present setting. Our study would aid in better understanding of the role of Pentraxin 3 in the pathogenesis of GDM and role of PTX3 for diagnosing GDM.

II. Material and methods

Our present study was case control study carried out in department of Biochemistry along with The Obstetrics and gynaecology(OG) department of Rajah Muthiah medical college and Hospital, Chidambaram, Cuddalore district, Tamilnadu for a period of 1 year between May 2021 to May 2022. Ethical clearance for the study was obtained from our Institutional Human ethics committee (IHEC/758/202).

- **Study population** - Pregnant women who attended OPD and admitted in OG wards of RMMCH during the study period.
- **Sample size** - 32 pregnant women diagnosed with GDM according to criteria based American DiabeticAssociation(ADA) as cases.
- -32 pregnant women with normal glucose tolerance without any comorbidities were controls.
- **Inclusion criteria** - Pregnant women with age more than 20 years at 24 to 28 weeks of gestation.
- **Exclusion criteria**-Women with Type1&Type2 diabetes mellitus, Pregnancy induced hypertension, other chronicinflammatory Conditions, Liver,Renal and endocrine disorders.

Procedure methodology

Informed & written consent was obtained in the vernacular language from all the study participants. The baseline characteristics include age, sex, parity and gestational age were recorded.Anthropometric measurements like height and weight were measured. Blood pressure was recorded using a sphygmomanometer. Standard procedures were followed while taking all the above measurements.

5 ml of random venous blood were collected in proper vacutainer tubes. Haematological parameters, Total count and Differential count were done. Baseline biochemical investigations were estimated using Auto analyser (ERBA EM 200).Oral Glucose Tolerance Test(OGTT) was done and values were measured by semiauto analyser (ERBA CHEM-5 Plus).Special parameter, serum Pentraxin-3 levels were measured using kits manufactured by Elab science. The kit works on the principle of Sandwich-ELIZA technique. The concentration of the parameters were measured by ELISA reader(MINDRAY MR-96).

Statistical analysis

The data collected were entered into Microsoft excel 360 and master chart was created. The master chart was then loaded onto SPSS version 26 (Statistical Package for Social Sciences). Compilation of data and analysis was done using SPSS version 26. The data contained both qualitative and quantitative variables. Both descriptive and inferential statistics were applied during the analysis. The quantitative data were described using mean and standard deviation while the qualitative data were described using percentages. Bar diagram was used in places appropriate to represent the data. In order to find out the mean difference between the groups, independent student "t" test was applied. To find out the difference in the distribution of qualitative variable between the groups, Chi square test was applied. A "P" value of less than 0.05 was considered to be statistically significant.

III. Results

- Among the participants in the GDM group, 40.6% belonged to age group 21 to 25 years and 26 to 30 years.Among the participants in the control group, 34.4% belonged to age group 21 to 25 years and 46.9% belonged to age group 26 to 30 years. Among the participants in the GDM group, 50% belonged to Primi followed by 37.5% with G2P1L1. Among the participants in the control group, 62.5% belonged to Primi and 28.1% with G2P1L1.
- The mean weight among the GDM group was 67.88 ± 12.11 Kgs and that of the control group was

61.25 ± 12.72 Kgs. The mean weight was found to be higher in GDM than in controls and statistically significant (P value 0.037)(Table 1).The meanheight among the GDM group was 152.38 ± 7.32 CMs and that of the control group was 153.69 ± 5.47 CMs. The mean BMIamong the GDM group was 29.25 ± 4.88 Kg/m^2 and that of the control group was 25.88 ± 5.11 Kg/m^2. The mean BMI was found to be high in GDM than in control group with" P" value, 0.009 (Table 1).

- The mean OGTT values among those with GDM at 0 hour was 85.96 ± 4.77 mg/dL and that of the controls were 84.21 ±

6.63 mg/dL. The mean OGTT values among those with GDM at 2nd hour was 172.78 ± 12.88 mg/dL and that of the controls were 124.96 ± 12.85 mg/dL. The mean OGTT value at 2^{nd} hour was found to be high among the GDM group than the controlgroup with " P" value of 0.001(Table no 1).

- The mean AST values were 22.59 ± 5.29 U/L and 20.50 ± 5.43 U/L among those in the GDM and control group, respectively. AST values were higher in GDM group than in controls though it was not statistically significant.The mean ALT values were 25.66 ± 7.22 U/L and 28.81 ± 7.01 U/L among those in the GDM and control group, respectively. ALT values were high in control group.The mean ALP values were 117.16 ± 67.21 U/L and 96.88 ± 34.43 U/L among those in the GDM and control group, respectively. The mean total bilirubin values were 0.72 ± 0.04 mg/dL and 0.74 ± 0.05 mg/dL among those in the GDM and control group, respectively. The mean direct bilirubin values were 0.18 ± 0.03 mg/dL and 0.17

± 0.04 mg/dL among those in the GDM and control group, respectively. The mean total protein values were 5.76 ± 0.28 g/dL and 5.84 ± 0.26 g/dL among those in the GDM and control group, respectively. The mean albumin values were 3.33 ± 0.28 g/dL and 3.38 ± 0.30 g/dL among those in the GDM and control group, respectively. The mean globulin values were 2.63 ±

0.28 g/dL and 2.59 ± 0.27 g/dL among those in the GDM and control group, respectively. Marginal increase in globulin mayreflect the mild to moderate inflammation associated with GDM.

- The mean Urea values were 21.59 ± 3.43 mg/dL and 20.75 ± 5.26 mg/dL among those in the GDM and control group, respectively. The mean creatinine values were 0.72 ± 0.08 mg/dL and 0.72 ± 0.09 mg/dL among those in the GDM andcontrol group, respectively.

- The mean total cholesterol values were 215.19 ± 44.78 mg/dL and 193.72 ± 28.19 mg/dL among those in the GDM and control group, respectively. Total cholesterol values were high in GDM group than in control group(P value 0.026). The mean triglyceride values were 142.25 ± 39.25 mg/dL and 137.84 ± 36.51 mg/dL among those in the GDM and control group, respectively. The mean HDL values were 43 ± 1.84 mg/dL and 43.44 ± 1.74 mg/dL among those in the GDM and control group, respectively. The mean LDL values were 133.44 ± 41.17 mg/dL and 108.31 ± 27.09 mg/dL among those in the GDM and control group, respectively (Table no 2). The LDL values were found to be more among GDM than the controls.(P value

0.00 6).The derangement of lipid profile is common in pregnant women as increased with blood glucose particularly LDLlevels. The increased LDL predispose to cardiovascular disease in GDM.

Table no 1: **Baseline characteristics among the participants between cases and controls.**

Variable		GDM (n=32)		Control(n=32)		P value
		N	%	N	%	
Age group(In years)	≤ 20	2	6.3	3	9.4	
	21-25	13	40.6	11	34.4	
	26-30	13	40.6	15	46.9	
	>30	4	12.5	3	9.4	
Parity	Primi	16	50	20	62.5	
	G2P1L1	12	37.5	9	28.1	
	G3P2L2	4	12.5	2	6.3	
	G4P3L3	0	0	1	3.1	
Weight (In kgs)		67.88	12.11	61.25	12.72	0.037*
Height (In CMS)		152.38	7.32	153.69	5.47	0.420

BMI (Kg/M^2)		29.25	4.88	25.88	5.11	0.009*
OGTT values	0 hour	85.96	4.77	84.21	6.63	0.231
	2^{nd} hour	172.78	12.88	124.96	12.85	0.001*

* P value of less than 0.05 is statistically significant.

Table no 2: Difference in biochemical parameters between cases and controls.

Variable	GDM (n=32)		Control(n=32)		P value
	Mean	SD	Mean	SD	
AST (U/L)	22.59	5.29	20.50	5.43	0.124
ALT (U/L)	25.66	7.22	28.81	7.01	0.081
ALP (U/L)	117.16	67.21	96.88	34.43	0.134
Total bilirubin (mg/dL)	0.72	0.04	0.74	0.05	0.199
Direct bilirubin (mg/dL)	0.18	0.03	0.17	0.04	0.553
Total protein (g/dL)	5.76	0.28	5.84	0.26	0.185
Albumin (g/dL)	3.33	0.28	3.38	0.30	0.528
Globulin (g/dL)	2.63	0.28	2.59	0.27	0.690
Urea (mg/dL)	21.59	3.43	20.75	5.26	0.451
Creatinine (mg/dL)	0.72	0.08	0.72	0.09	0.759
TC (mg/dL)	215.19	44.78	193.72	28.19	0.026*
TGL (mg/dL)	145.25	39.25	137.84	36.51	0.437
HDL (mg/dL)	43	1.84	43.44	1.74	0.334
LDL (mg/dL)	133.44	41.17	108.31	27.09	0.006*

* P value of less than 0.05 is statistically significant.

➢ The mean haemoglobin among the GDM group was 10.74 ± 0.92 mg/dl and that of the control group was 10.25 ± 1.98 mg/dl.

The haemoglobin was found to be marginally high in GDM group (P value 0.212).The mean total count among those with GDM was 9396.88 ± 2300.97 per microlitre of blood and that of control was 8996.88 ± 1716.42 per microlitre of blood. Though the mean total count was more in the GDM group than in the control group, the difference was not found to be statistically significant.The mean neutrophil percentage for the GDM group was 65.21 ± 11.38 and that of the control group was 66.49 ± 9.66. The mean lymphocyte percentage for the GDM group was 28.37 ± 11.24 and that of the control group was 28.43 ± 9.19. The mean monocyte percentage for the GDM group was 4.23 ± 1.47 and that of the control group was 4.71 ±

1.77. The mean platelet counts among those with GDM and controls was 2.35 ± 0.65 $*10^9$ L per litre and 2.48 ± 0.66 * 10^9 L per litre. There were no statistical difference in haematological parameters between the groups with P value of more than 0.05 (Table no 3). These above parameters were done as a part of routine investigations and does not alter the outcome of our study.

Table no 3: **Difference in haematological parameters between the cases and controls.**

Variable	GDM (n=32)		Control(n=32)		P value
	Mean	SD	Mean	SD	
Haemoglobin (mg/dl)	10.74	0.92	10.25	1.98	0.212
Total count (per microlitre of blood)	9396.88	2300.97	8996.88	1716.42	0.434
Neutrophil (%)	65.21	11.38	66.49	9.66	0.629
Lymphocyte (%)	28.37	11.24	28.43	9.19	0.982

Monocyte (%)	4.23	1.47	4.71	1.77	0.252
Platelets (*10^9L per litre)	2.35	0.65	2.48	0.66	0.454

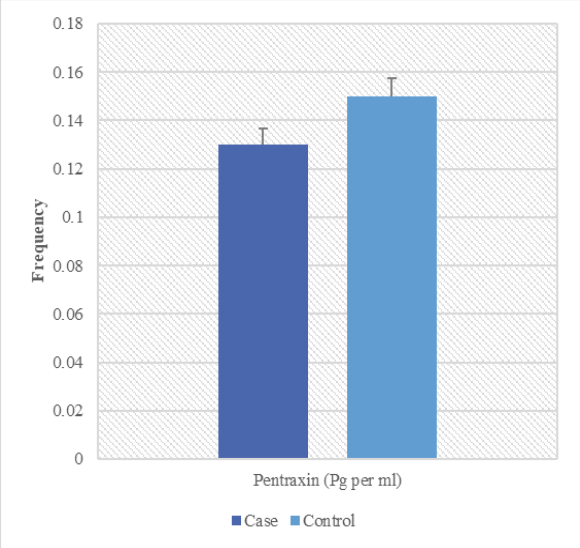
Table no 4: Comparison of mean Pentraxin-3 values between the cases and controls

Variable	GDM (n=32)		Control(n=32)		P value
	Mean	SD	Mean	SD	
Pentraxin-3 (Pg per ml)	0.13	0.05	0.15	0.07	0.114

* P value of less than 0.05 is statistically significant

➢ The mean pentraxin-3 values among the GDM group was 0.13 ± 0.08 Pg per ml and that of the control group was
0.15 ± 0.13 Pg per ml (Table no 4) .The PTX-3 values were found to be marginally low in GDM women(P value-0.114) may result in higher risk of Acute coronary syndrome in later period.

Figure 1: **Bar chart showing difference in mean Pentraxin-3 values between the cases and controls**



IV. Discussion

Gestational diabetes mellitus (GDM) is defined as 'any degree of glucose intolerance with onset or first recognition during pregnancy'. Pregnancy is a physiological state with various oxidative and inflammatory stress. This moderate stress plays an important role in placental development and its function, foetal development and labour. In GDM the levels of oxidative stress are heightened either due to over production of free radicals or defect in antioxidant defences(5,6). Pentraxin-3 (PTX3) is an acute phase protein of the Pentraxin family. It was found to be raised in case of GDM in earlier study(7).

Our case control study was carried out along with department of obstetrics and gynaecology, Rajah Muthiah Medical College and Hospital to find out the difference in mean Pentraxin -3 levels between pregnant women and in GDM patients.

In our study the mean weight and mean BMI were found to be more in the gestational diabetes mellitus group than in the control group. Consequently risk and incidence of GDM will proportionately increase with increase in BMI. Overweight and obesity with GDM are well-established risk factors for development of foetal complications, leading to Type 2 diabetes mellitus in GDM women, cardiovascular diseases at a later date.In our study Total cholesterol and LDL were increased significantly in GDM. Tortoni MR *et al* in their systematic review came out with similar evidence and reported the pooled odds ratio for overweight, moderately obese and morbidly obese to develop GDM to be 1.97, 3.01 and 5.55, respectively(11). Similar result was also reported by CHU SY *et al* in a meta-analysis. The later study reported the pooled odds ratio for overweight, obese and morbidly obese for the development of GDM as 2.14, 3.56 and 8.56, respectively(12). Therefore the above mentioned studies agree with our findings.
Kim SY *et al* reported a similar association where the proportion of GDM among the participant who were overweight, obesity and extremely obese were 15.4%, 9.7% and 21.1%, respectively(13). Interventions

aiming to decrease the mean weight and BMI among women will also decrease the incidence of GDM in them.

In our study mean Pentraxin-3 levels were found to be mildly lower in GDM group than in control group though it was not statistically significant and also revealed that GDM women were having high BMI and deranged lipid profile .The lower levels of PTX3 along with high BMI and high lipid profile may indicate higher risk of cardiovascular complications in these rural pregnant women.

PTX3 is also having anti-inflammatory,anti-microbial and cardioprotective action (18,19).However Studies done on Pentraxin -3 have so far produced a wide variety of results. Qu X *et al* (2019)(14), Yu N *et al* (2019)(15) and Yildirim M *et al* (2015)(16) had reported a higher mean Pentraxin-3 among the GDM than the control group with better prognosis in the postpartum period. while Lekva et al in 2016 (17) reported GDM cohort to have low circulating Pentraxin 3 levels than the controls. Lekva *et al* hypothesized that women with GDM may be presented with regulated Pentraxin-3 and would be associated with various metabolic risk factors and increased cardiovascular complications at five years follow up. So it is evident that lower levels of Pentraxin-3 may be associated with increased cardiovascular risk in these GDM women.A systematic review or metanalysis regarding levels of PTX3 among GDM women would provide a more definitive result. Interventions aiming to decrease the mean weight and BMI among women will also decrease the incidence of GDM and their complications in them.

V. Conclusion

Pentraxin-3 is an inflammatory marker, was found to be mildly low among GDM group in comparison with the control group.We hereby conclude that our understanding of action of PTX3 is still incomplete.More elaborate studies with large sample for longer follow up period need to be done to assess the implication of Pentraxin-3 as a biomarker of inflammation.In case of test group patients in our study we intend to follow up over period of time to assess the risk of cardiovascular diseases and other GDM related complications.

VI. Limitations

The study results cannot be generalised, since we have selected only 32 GDM women for our study.The temporality between the biochemical parameter and effect cannot be established. But the present understanding regarding the pathogenesis of GDM is that oxidative stress could play a pivotal role in development of insulin resistance.The present study is a single centre study while a multicentre study with larger sample would provide a more clear picture.

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