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Original Research Article

Cord Blood C Peptide Levels in Gestational Diabetes Mellitus

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Abstract

Objective of The Study: To assess C-peptide levels in Cord Blood of Infants born to Diabetic Mothers (GDM and type 2 DM mothers) and to find it's correlation with the anthropometric measures of the new born. ***Materials and Methods:*** The study sample comprised 40 babies born to GDM mothers and 20 babies born to type 2 DM mothers. Controls consist of 30 babies born to non-diabetic mothers .Instituitional ethics committee approval was obtained. Anthropometric measures of the newborn including gestational age, birth weight, ponderal index, length of the babies and head circumference were measured. 10 ml of Cord blood was collected and cord serum was separated and stored at -20^{0}C until further analysis. C-peptide levels in the cord serum was measured by ELISA. ***Result:*** Significant increase was observed in the serum C-peptide levels in infants born to GDM and type 2 DM mothers. Birthweight, head circumference and other anthropometric measures were also found to be significantly increased in the infants of diabetic mothers and they showed a strong correlation with C-peptide levels. ***Conclusion:*** When the mother has type 2 diabetes / gestational diabetes mellitus (GDM), fetal hyperinsulinemia is common despite treatment of disease. This effect is more pronounced in those women with Type 2 DM. This maternal diabetic environment could be a contributing factor for the infants to develop obesity and abnormal glucose tolerance later in life. Therapeutic targets should be aimed at controlling the fetal hyperinsulinemia of GDM which could prevent the development of diabetes in the future.

Keywords: GDM, Type 2DM, Cord serum C peptide, Anthropometric measures, Fetal hyperinsulinemia.

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INTRODUCTION

Diabetes during pregnancy is associated with increase in maternal and perinatal morbidity. The hallmark of this condition is increased insulin resistance. Maternal hormones are thought to interfere with the action of insulin as it binds to the insulin receptor. Since insulin promotes the entry of glucose into most cells, insulin resistance prevents glucose from entering the cells. As a result, glucose remains in the bloodstream, where glucose levels rise. More insulin is needed to overcome this resistance; more insulin is produced than in a normal pregnancy. Macrosomia [1], congenital cardiac and central nervous system anomalies, skeletal malformations and respiratory distress syndrome are some of the well known complications occurring in infants of diabetic mothers. Moreover, human epidemiological and animal studies suggest that the intrauterine diabetic environment increases the risk of hypertension, obesity, and type II diabetes in adulthood in the offspring of diabetic mothers. Fetal hyperinsulinemia at birth acts as a marker of this risk and it may also have potential prognostic implications. Thus, higher insulin levels in utero might be a cause of later metabolic complications [2-4]. We have measured the concentrations of C-peptide in cord blood together with birthweight and other anthropometric measures at birth. The correlation between C-peptide and the anthropometric measures like birthweight, ponderal index and head circumference, is also assessed in this study.

C-peptide was first described in 1967 in connection with the discovery of the insulin biosynthesis [5]. It serves as an important linker between the A and the B chains of insulin and facilitates the efficient assembly, folding, and processing of insulin in the endoplasmic reticulum. Equimolar amounts of C-peptide and insulin are then stored in secretory granules of the pancreatic beta cells and both are eventually released into the portal circulation. Initially, the sole interest in C-peptide was as a marker of insulin secretion and it has been of great value in the further understanding of the pathophysiology of type 1 and type 2 diabetes. The first

documented use of the C peptide test was done in 1972. During the past decade, however, C- peptide has been found to be a bioactive peptide in its own right, with effects on microvascular blood flow and tissue health. C-peptide has been shown to bind to the surface of a number of cell types such as neuronal, endothelial, fibroblast and renal tubular cells, at nanomolar concentrations to a receptor that is likely G-protein coupled. The signal activates Ca^{2+} dependent intracellular signaling pathways such as MAPK, PLCγ and PKC, leading to upregulation of a range of transcription factors as well as eNOS and Na+K+ATPase activities [6]. The latter two enzymes are known to have reduced activities in patients with type I diabetes and have been implicated in the development of long-term complications of type I diabetes such as peripheral and autonomic neuropathy. In vivo studies in animal models of type 1 diabetes have established that C-peptide administration results in significant improvements in nerve and kidney function. Cpeptide has also been reported to have anti-inflammatory effects and it aids in repair of smooth muscle cells [7].

MATERIALS AND METHODS

Study Population

After approval from Institutional Ethics Committee of GGH, Chennai, the study was carried out at the Institute of Obstetrics and Gynaecology & Hospital for women and children, Egmore, Chennai.

Cases

The study sample comprised 40 babies (20M,20 F) born to GDM mothers and 20 babies (9M,11F) born to type 2 DM mothers. GDM mothers were diagnosed with 100gm glucose tolerance test (ADA Criteria). Both GDM and Type2 DM mothers were on treatment with diet modifications, exercise and insulin.

Controls

Consists of 30 babies (15M,15F) born to non-diabetic mothers.

Inclusion Criteria

Mothers aged between 25-35 years and without any other medical complications of pregnancy.

Exclusion Criteria

Mothers with complications like preeclampsia, preterm deliveries, twin pregnancies and other complications during labour were excluded.

Other Parameters Considered:

- Gestational age : Calculated with LMP and USG findings in first trimester
- Birth weight and placental weight measured
- Ponderal index of babies measured using birth weight and length of the babies [Weight (g) *100 / Length $(Cm)^3$]
- Head circumference of babies measured

SAMPLE COLLECTION AND PROCESSING

- 10 mL of Cord blood was collected in plain tubes from umbilical vein with precautions to prevent hemolysis
- Sample collection was completed in 1 month
- Serum was separated and stored at -20^0C until further analysis
- Cord serum C-peptide was measured by ELISA

ESTIMATION OF SERUM C PEPTIDE METHODOLOGY

Direct solid phase enzyme immunoassay from Diametra for quantitative determination of C-peptide in human serum.

Principle

In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin (S.Avidin) coated on the well and exogenously added biotinylated monoclonal anti-C peptide antibody (Ab). Upon mixing monoclonal biotinylated antibody, the enzyme-labelled antibody and serum containing the native antigen (Ag), reaction results between the native antigen and the antibodies to form a soluble sandwich complex. The interaction is illustrated by the following equation:

BtAb(m) = Biotinylated Monoclonal Antibody (Excess Quantity)
AgN = Native Antigen (Variable Quantity)
EAb = Enzyme labelled Antibody (Excess Quantity)
EAb –AgN – BtAb(m) = Antigen-Antibodies Sandwich Complex
ka = Rate Constant of Association
k-a = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody.

Ka EAb + AgN + BtAb(m) <->EAb – AgN – BtAb(m) K-a

This interaction is illustrated below: Streptavidin C. W. = Streptavidin immobilized on well Immobilized complex = sandwich complex bound to the well.

The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce colour. By utilizing several different calibrators of known antigen values, a

dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

Test Components

- C-peptide Standards 6x (1 vial = 2 mL)
- Conjugate (Anti C-pep-HRP conjugate + Mouse-Anti-C-pep conjugate biotinylated) (1 bottle – 13 ml)
- Coated Microplate (Microplate coated with streptavidin)
- TMB-substrate (1 bottle – 12 ml)
- Stop solution (Sulphuric acid 0.15 mol/L) (1 bottle – 12 ml)
- Conc. Wash Solution 50x (1 vial) 20 ml (NaCl 9 gr/l; Tween20 1gr/l)

Detection limit

This method allows the quantitative determination of C-Peptide from 0.2 to 10.0 ng/ml. EAb-AgN-BtAb(m) + StreptavidinC.W. => Immobilized Complex

Preparation of the Standard

(S0,S1,S2,S3,S4,S5) :The standard has approx. the following concentration: S0 S1 S2 S3 S4 S5 ng/ml 0 0.2 1.0 2.0 5.0 10.0

Each standard was reconstituted with 2.0 mL of distilled water.

Preparation of Wash Solution

We diluted the contents of concentrated wash solution 50x to 1000 mL with distilled water in a suitable storage container.

Preparation of the Sample

The samples were stored at temperatures of –and 20°C .Frozen samples were thawed under tap water.

Assay Procedure

- All reagents were brought to room temperature
- 50 µL of each standard prepared (0, 0.2, 1, 2, 5, 10 ng/ml) was added into appropriate wells in the microtiter plate.
- 50 µL of serum samples were added in the other wells
- 100 µL of the conjugate was then added to the wells containing standards and serum samples
- Incubated at room temperature for 2 hours
- Wells washed with 300 ml of diluted wash solution
- Wash repeated twice
- TMB substrate 100 µL is added to the wells
- Incubated at room temperature for 15 minutes
- 100 µL stop solution added to the wells
- The absorbance was read at 450 nm

Reference Value

Adult (Normal) 0.7 – 1.9 ng/mL

Sensitivity

The lowest detectable concentration of C-peptide that can be distinguished from the zero standard is 0.025 ng/mL at the 95 % confidence limit.

Summary of Results

There are various parameters evaluated namely birthweight, head circumference, length of babies, ponderal index, placental weight, gestational age & C-peptide levels in babies born to GDM, Type 2 DM and Non diabetic mothers. The Mean and Standard deviation for the various parameters evaluated are presented in Table-1.

STATISTICAL ANALYSIS

- Statistical Analysis was done by Oneway ANOVA F-test for p-value ($P<0.05$ is considered to be significant)
- Bonferroni t-test was used for comparison of the levels of each parameter in various groups
- Karl pearson's correlation coefficient was used to analyse the correlation between various parameters

Pearson Correlation Coefficient

$$r = \frac{N(\Sigma XY) - (\Sigma X)(\Sigma Y)}{\sqrt{[N\Sigma X^2 - (\Sigma X)^2][N\Sigma Y^2 - (\Sigma Y)^2]}}$$

Interpretation for r-value Pearson correlation coefficient is denoted by —r and —r always lies between -1 to +1

0.0 – 0.2 Poor correlation 0.2 - 0.4 Fair correlation 0.4 - 0.6 Moderate correlation 0.6 – 0.8 Substantial correlation 0.8 - 1.0 Strong correlation

Table-1 shows comparison of C-peptide levels, birthweight, placental weight, ponderal index and head circumference among the three groups. Oneway ANOVA is used for the comparison. C-peptide levels are higher in cases than in controls. The birthweight, placental weight, ponderal index and head circumference are also higher in cases than in controls.

Table-2 shows a strong correlation of C-peptide with birthweight, ponderal index and head circumference in the various groups.

RESULTS

Table-1: Comparison of C Peptide Levels and Anthropometric Measures among various groups---

Parameters	Group						One way ANOVA	Bonferroni t-test
	GDM		Type 2 DM		Control			
	Mean	SD	Mean	SD	Mean	SD		
C peptide	2.00	0.88	2.17	0.76	1.12	0.34	F=17.89 P=0.001***	Control Vs GDM, type2
Birth Weight	3.21	.64	3.28	.71	2.82	.52	F=4.47 P=0.01**	Control Vs GDM, type2
Length	48.80	1.40	48.80	1.61	47.60	1.40	F=6.85 P=0.002**	Control Vs GDM, type2
Ponderal Index	2.738	0.357	2.785	0.363	2.592	0.269	F=2.51 P=0.09	Control Vs GDM, type2
Head Circumference	34.80	0.84	35.12	0.71	34.41	0.69	F=5.40 P=0.006**	Control Vs GDM, type2
Placental Weight	513.67	38.47	516.95	41.08	485.80	40.42	F=5.36 P=0.006***	Control Vs GDM, type2
Gestational Age	38.08	0.53	37.40	2.23	39.10	0.96	F=12.29 P=0.001***	Control Vs GDM, type2
Mother's Age	30.83 +	3.08	31.60	1.43	30.60	2.69	F=0.89 P=0.41	Control Vs GDM, type2

*significant at P<0.05 **Highly significant at P<0.01 ***Very High significant at P<0.001

A comparison of C Peptide Levels among the three groups is represented in Fig-1

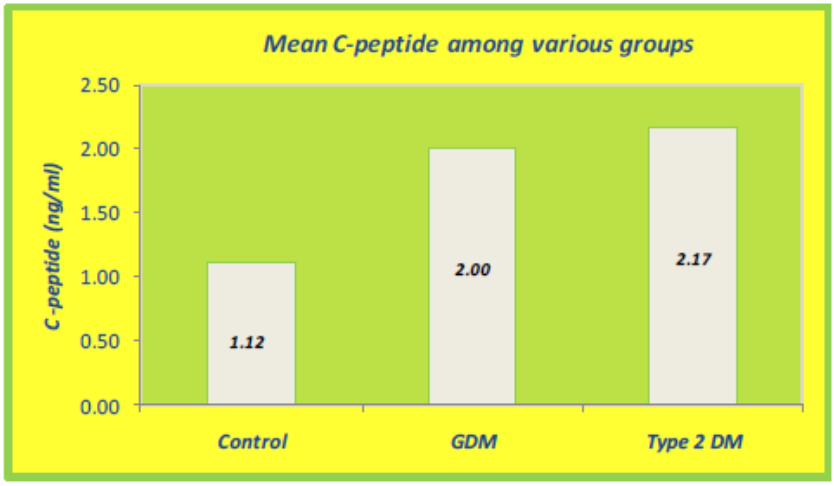


Fig-1: Mean C-Peptide among various groups

A Comparison of anthropometric measures of the newborn in all the three groups is represented in Fig 2, 3, & 4.

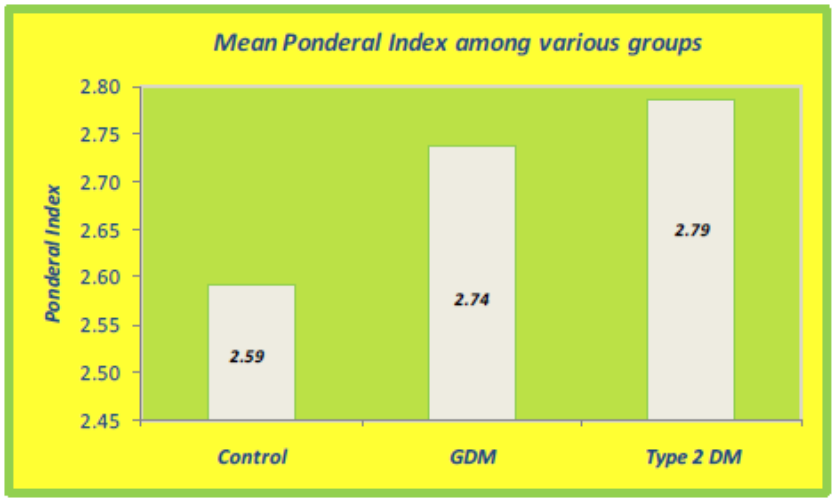


Fig-2: Mean Ponderal Index among various groups

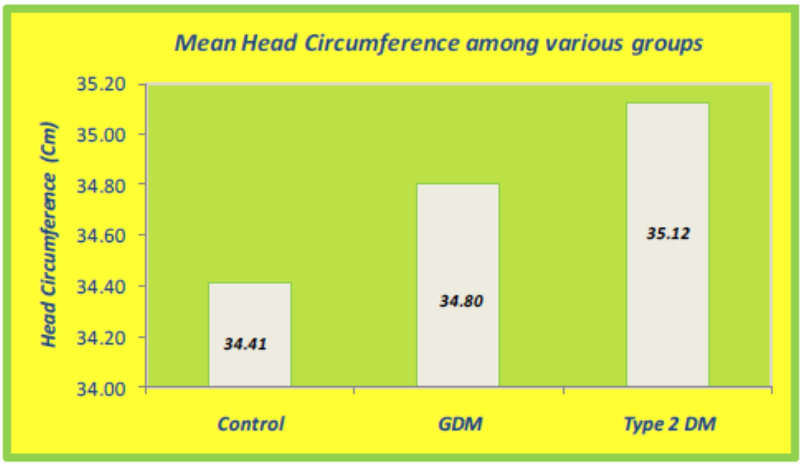


Fig-3: Mean Head Circumference among various groups

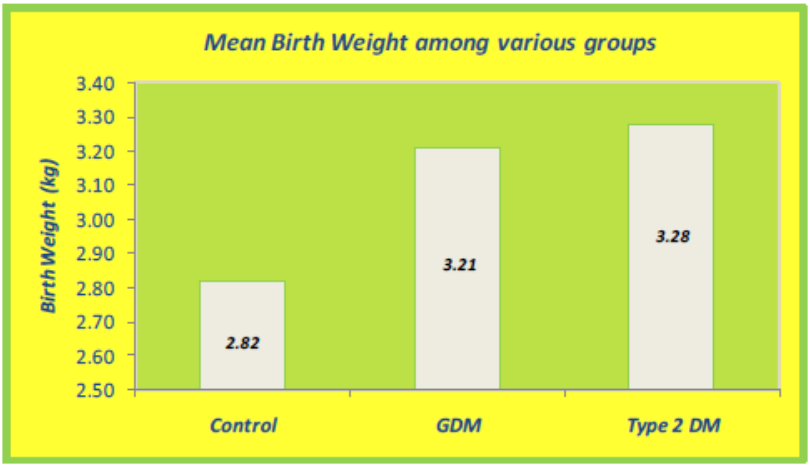


Fig-4: Mean Birth Weight among various groups

Table-2: Correlation of C-peptide with Anthropometric Measures

Group	Parameters	C Peptide	
		Correlation	
GDM	Birth Weight	Pearson Correlation	0.976(**)
		Sig. (2-tailed)	0.000
		N	40
	Length	Pearson Correlation	0.823(**)
		Sig. (2-tailed)	0.000
		N	40
	Ponderal Index	Pearson Correlation	0.961(**)
		Sig. (2-tailed)	0.000
		N	40
	Head Circumference	Pearson Correlation	0.871(**)
		Sig. (2-tailed)	0.000
		N	40
Type 2 DM	Birth Weight	Pearson Correlation	0.956(**)
		Sig. (2-tailed)	0.000
		N	20
	Length	Pearson Correlation	0.914(**)
		Sig. (2-tailed)	0.000
		N	20

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	Ponderal Index	Pearson Correlation	0.868(**)
		Sig. (2-tailed)	0.000
		N	20
	Head Circumference	Pearson Correlation	0.868(**)
		Sig. (2-tailed)	0.000
		N	20
Control	Birth Weight	Pearson Correlation	0.911(**)
		Sig. (2-tailed)	0.000
		N	30
	Length	Pearson Correlation	0.830(**)
		Sig. (2-tailed)	0.000
		N	30
	Ponderal Index	Pearson Correlation	0.906(**)
		Sig. (2-tailed)	0.000
		N	30
	Head Circumference	Pearson Correlation	0.793(**)
		Sig. (2-tailed)	0.000
		N	30

** Correlation is significant at the 0.01 level (2-tailed)

DISCUSSION

Exposure of the fetus to maternal diabetes results in characteristic changes in birth weight, adiposity and fetal insulin production. We have used cord blood insulin as an indicator of exposure to an abnormal intrauterine environment. Notably, insulin at birth is significantly increased in our population, not only in offspring of mothers with type 2 diabetes, but also in offspring of mothers with GDM .Maternal diabetes is clearly associated with an increase in the risk of type 2 diabetes and obesity in the offspring, in keeping with in utero programming of disease [8, 9] and forming a―vicious cycle of type 2 diabetes‖ [10]. More recently, studies [11, 12] in hepatocyte nuclear factor-1α mutation carriers (maturity-onset diabetes of the young) have supported a role of the intrauterine environment in modifying the risk of diabetes. Such studies are of interest, as our population is also believed to be at high genetic risk of obesity and type 2 diabetes and therefore likely to be prone to the same programming effects. How important is the finding of raised fetal insulin levels at birth? Importantly, the risk of later adverse metabolic consequences appears to relate to fetal insulin levels [13-15]. In this context, our finding of increased concentrations of insulin in cord blood of offspring of mothers with type 2 diabetes and GDM suggests that such a cycle is also potentially present in our population. We have also found a significant increase in the birthweight, head circumference and other anthropometric measures in the infants of diabetic mothers and they show a strong correlation with C-peptide levels as evident from Table-2.

The importance of GDM as a clinical entity has recently been demonstrated by an Australian randomized trial in which the identification and treatment of GDM significantly reduced the rate of fetal macrosomia and serious adverse perinatal outcome [16]. Thus, the finding that fetal hyperinsulinemia is a common outcome of pregnancy in women with GDM in this population would support the importance of detection and careful monitoring of this group and suggests that similar studies be carried out in other groups.

CONCLUSION

Statistically significant increase in C -peptide concentrations in newborns of both type 2 diabetes and GDM mothers is observed. Similar increase in head circumference, birthweight and other anthropometric measures in the newborns of both type 2 diabetes and GDM mothers is present. C-peptide levels correlate well with the neonatal anthropometric measurements (Birth weight and Head Circumference), When the mother has type 2 diabetes or gestational diabetes mellitus (GDM), fetal hyperinsulinism is common despite treatment of disease. This effect is more pronounced in those women with Type 2 DM. This maternal diabetic environment could be a contributing factor for the infants to develop obesity and abnormal glucose tolerance later in life.

SCOPE FOR FURTHER STUDY

Further studies examining the health of children of mothers with diabetes in this population and whether maternal glycemic control can impact upon this risk, are essential.

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