ORIGINAL ARTICLE

The Hormonal Levels of Cortisol in Second and Third Trimesters of Gestational Diabetes Mellitus patients with or without family history

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ABSTRACT

Gestational diabetes mellitus (GDM) is a condition which is recognized initially in nondiabetic pregnant women who develop raised blood sugar levels which goes normal after delivering fetus.In physiological adaptations ofpregnancythe level of numerous hormones generally are increased. In the present study the responses of thecortisol in pregnancy have been investigated in gestational diabetes and non-GDM subjects specially in context of positive and negative family history in second and third trimester.The present cross sectional 2 stage study with non-probability convenient sampling was done in Arif Memorial Teaching Hospital, Lahore and Hameed Latif Hospital, Lahore. 110 pregnant females from rural and urban areas of Lahore were the study population, out of which 55 had GDM and 55 were controls/non-GDM. The results were analyzed in relation to GDM, non-GDM, positive family history in second and third semester.In second trimester cortisol were found increased several times in GDM than non GDM subjects Cortisol showed 51% and 100% increases in positive and negative family history. In third trimester also the responses of this hormone were numerous times increases in GDM than non-GDM. The pattern of these increases was varied in positive and negative family history subjects. The analysis of results of the hormones within GDM and non-GDM category and between the semesters has shown some noticeable and statistically noticeable results.The excessive increases of cortisol do support the general opinion that this hormone is one of thecause of insulin resistance in GDM. The significantly varied responses of this hormone in different family history of the subjects and in different trimester clearly demonstrate that other mechanisms are certainly involved in the initiation of insulin resistance in GDM.

Keywords: Cortisol, Gestational Diabetes Mellitus, Family History

INTRODUCTION

Pregnancy which is divided into three trimesters[1], is the period which starts from conception and ends with the fetal birth. It is a diabetogenic condition which leads to hyperinsulinemiain which insulin resistance develops with a compensatory raised pancreatic β-cell response .

During pregnancy, the woman undergoes many physiological changes to maintain homeostasis. As a consequence of hyperphagia during first trimester of pregnancy, body weight and fat mass increases remarkably[2]. In the first few weeks of pregnancy an estimated 3.3kg of fat is stored[2].These storage fats become essential to maternal tissues in later stages of pregnancy, since most of the circulating glucose is used in the third trimester by the placenta and fetus[2].

The metabolic abnormalities which results in diabetes mellitus is due to defective secretion of insulin[3].This increased level of glucose effect the microvessels and patient suffering from abnormalities like neuropathy, nephropathy and retinopathy. Defect in carbohydrate metabolism is the main cause of diabetes mellitus which is the heterogeneous disease which deteriorate the actions of insulin as a result of which hyperglycemia occur which is the prominent feature of this disorder[3].

Gestational diabetes occurs when insulin receptors were not perform theirrole in physiological limits in second and third trimesters of pregnancy and remits following delivery[4].This may be due to the presence of human placental lactogen which may interferes with these insulin receptors which causes inappropriately increase sugar levels.

GDM is reported in second and third trimester and disappears after the delivery of fetus[4].It refers to the appearance of increased blood sugar levels in a pregnant lady previously not suffering from diabetes mellitus. The majority of such females progress to overt non insulin dependent diabetes mellitus with time. 30000 to 90000 individuals suffering from gestational diabetes per year reported in USA[5].In 7–8% of all pregnancies it is observed that beta cells of pancreas is not producing sufficient insulin to counteract the insulin resistance which begins and progress in second to third trimester of pregnancy[6]. Noknown cause other than hormones which are released during gestation are responsible for increasing insulin resistance which results in abnormalities in metabolism of glucose[6]. Generallydiagnostic criteria forgestational diabetesis blood screening during second and third trimesters of pregnancy which showshigh levels of glucose in blood samples. Depending on the population studied.it is noted that 3-10% of pregnanciesis effected by gestational diabetes[7].

As family history effects the trimester factor also influences the metabolic disorder. It is oftenreported that females with positive family history of diabetes have more chance to developmetabolic disorder. So, family history is often related with diabetic risk factors[8].Family History mainly influences the incidence of gestational diabetes mellitus along with trimesters of pregnancy which is

characterized by progressive insulin resistance that startsin near second trimester of pregnancy [9]. 9The important demographic factors which are responsible to develop gestational diabetes mellitus are advancing age, increase body fat deposition and overweight along with positive family history[10].

Cortisol, a steroid hormone produced by the adrenal cortex.Its main function is to raise theblood sugar level through gluconeogenesis, conquer the immune system, and augment metabolisms[11]. In the 11th week of gestation free cortisol levels start to rise and higher levels are noted in the second and third trimester of pregnancy[12].30 to 32 weeks of gestation excessive production of cortisol has important role in fetal lung surfactant production which is necessary for maturation of the fetal lungs[13]. It is also noted that with increasing trimesters free cortisol levels increases

The present study has been carried out with the objectives of determining the circulatory levels of cortisol in GDM in relation to family history of the disorder in 2nd and 3rd trimester. Also to compare the levels of the hormonesin various groups of GDM and the normalpregnancies .

MATERIALS AND METHODS

Study subjects: It was cross-sectional 2 stage study. From September, 2013 to February, 2014 total 110 subjects for sampling was selected from Arif Memorial Teaching Hospital, Lahore and Hameed Latif Hospital, Lahore. Among 110 samples 55 samples were pregnant with gestation diabetes mellitus and other 55 samples belong to normal pregnant females. 55pregnant females without GDM and labeled as (Control). Remaining 55 were pregnant with GDM and labeled as (GDM). Selections of samples were made on inclusive and exclusive criteria. whichinclude; Gestation week more than 12 weeks (2nd and 3rd trimesters only)Patients of Cushing syndrome, pre-eclampsia, liver disease, renal disease, cardiac disease, sepsis, recent surgery or history of trauma, on exogenous corticosteroid therapyand endocrine disorders were excluded from study. Pregnant females of 2nd and 3rd trimester were selected and classified as pregnant females with GDM and pregnant females without GDM, Personal, obstetric history, family history for diabetes mellitus, last menstrual period (LMP), gestational diabetes time period, predisposing factors with previous pregnancies, in case of multigravida, life style, educational status and general physical examination were recorded on questionnaire.

Methods & Biochemical Analysis: Afteraseptic measures five milliliter (ml) of blood sample was taken in disposable syringes from the pregnant females by venipuncture. After clotting and centrifugation serum was stored in serum cups at a temperature of -20 ^{0}C for assessment of Serum Cortisol level. All the tests were done in duplicate by ELISA technique using Access Bechman Coulter (USA).

Statistical Analysis: In the comparisons of various groups mean, standard deviation and standard error were calculated and the significance of the difference between the groups was determined with 2 sample t- test. The significance of the difference was taken at $p \leq 0.05$.

The correlation between the different parameters was analyzed by SPSS for further elucidation of the results. The significance of correlation was taken atp ≤ 0.05

RESULTS

The hormones levels of cortisol, were assayed in gestational diabetic (GDM) subjects in relation to the trimester and family history. The observations were made in second and third trimester. Similar study was done in normal or non-diabetic women as the control group study. The categories of family history were distinguished as positive family history and negative family history. The division of trimester was based on second and third trimester of gestation.

Second trimester

GDM with positive family history: The mean circulatory level of cortisol was 33.38 ± 2.21 µg/dl in the gestational diabetics and 22.649 ± 1.77 µg/dl in the non-diabetics normal control pregnant subjects. An increase in the cortisol levels had been observed in GDM that was almost 51 % greater than the control subjects. It was significant statistically ($p<0.001$),(Table 1).

GDM with Negative family history: The mean circulatory level of cortisol was 37.025±3.335 µg/dl in the gestational diabetics and 18.57±2.194 µg/dl in the non-diabetics normal control pregnant subjects. An increase in the cortisol levels had been observed in GDM that was almost 02 times greater than the control subjects. It was significant statistically ($p<0.001$) (Table 1).

GDM with different family history: The mean circulatory level of cortisol was 33.38±2.21 µg/dl in the 2nd trimester gestational diabetics with family history positive and 37.025 ± 3.34 µg/dl in the diabetics 2nd trimester with family history negative. An increase in the cortisol levels had been observed in the diabetics 2ndtrimester with family history negative but it was not significant statistically (p value 0.352) (Table 1).

Table 1: Comparison of Cortisol µg/dl accordingto 2nd trimester gestational diabetics and family history

2nd Trimester Group	N	Mean	SEM	t-test	p-value
Diabetic family history positive.	15	33.38	2.21	3.83	<0.001*
Non -diabetic family history positive	18	22.649	1.77		
Diabetic family history negative	10	37.025	3.335	4.80	<0.001*
Non-diabetic family history negative	13	18.57	2.194		
Diabetics family history positive	15	33.38	2.21	0.950	0.352
Diabetics family history negative	10	37.025	3.34		
Non-diabetics family history positive	18	22.65	1.77	1.456	0.156
Non-diabetics family history negative	13	18.58	2.19		

* Difference in Cortisol is statistically significant at 0.05

Non-GDM with different family history: The mean circulatory level of cortisol was 22.65±1.77 µg/dl in the second trimester non-diabeticswith family history positive and 18.58±2.19 µg/dl in the non-diabetics second trimester

with family history negative. Slightly increase in the cortisol levels had been observed in the non-diabetics second trimester with family history positive that was almost 22% but it was not significant statistically (p value 0.156), (Table 1).

Third trimester

GDM with positive family history: The mean circulatory level of cortisol was 37.39±2.06 µg/dl in third trimester gestational diabetics with family history positive and 37.35± 2.70µg/dl in the non-diabetics normal control pregnant subjects. No difference in the cortisol levels had been observed in GDM than the control subjects. Thus it was not significant statistically (p value 0.992) (Table 2).

GDM with negative family history: The mean circulatory level of cortisol was 41.04 ± 2.67 µg/dl in the third trimester gestational diabetics with family history negative and 22.23 ± 1.115 µg/dl in the non-diabetics normal control pregnant subjects. A marked difference in the cortisol levels had been observed in GDM that was almost 86% greater than the control subjects. It was significant statistically (p value 0.001) (Table 2).

GDM with different family history: The mean circulatory level of cortisol was 37.39 ± 2.06 µg/dl in the 3rdtrimester gestational diabetics with family history positive and 41.04 ± 2.67 µg/dl in the diabetics 3rdtrimester with family history negative. An increase in the cortisol levels had been observed in the diabetics 3rdtrimester with family history negative that was almost 10 % but it was not significant statistically (p value 0.283)(Table 2).

Non-GDM with different family history: The mean value of cortisol was 37.36 ± 2.71 µg/dl in the third trimester non-diabetics with family history positive and 22.24 ± 1.12 µg/dl in the non-diabetics third trimester with family history negative. A marked increase in the cortisol levels had been observed in the non-diabetics third trimester with family history positive that is almost 68 %. It is highly significant statistically (p value 0.001),(Table 2).

Table 2: Comparison of Cortisol µg/dl according to 3rd trimester gestational diabetics and family history.

3rd Trimester Group	N	Mean	SEM	t-test	p-value
Diabetic family history positive		37.39	2.06	0.010	0.992
Non-diabetic family history positive	10	37.35	2.70		
Diabetic family history negative	12	41.04	2.67	6.852	<0.001*
Non-diabetic family history negative	14	22.23	1.115		
Diabetics family history positive	18	37.39	2.06	1.095	0.283
Diabetics family history negative	12	41.04	2.67		
Non-diabetics family history positive	10	37.36	2.71	5.75	< 0.001
Non-diabetics family history negative	14	22.24	1.12		

* Difference in Cortisol is statistically significant at 0.05

COMPARISON OF 2ND AND 3RD TRIMESTER

GDM with positive family history: The mean circulatory level of cortisol was 33.38 ± 2.21 µg/dl in the second trimester gestational diabetics with family history positive and 37.39 ± 2.06 µg/dl in the diabetics 3rdtrimester with family history positive. An increase in the cortisol levels had been observed in the diabetics 3rdtrimester with family history positive that is almost 11 % but it was not significant statistically (p value 0.195), (Table 3).

GDM with negative family history: The mean value of circulatory level of cortisol was 37.03±3.34µg/dl in the second trimester gestational diabetics with family history negative and 41.04±2.67µg/dl in the diabetics 3rdtrimester with family history negative. An increase in the cortisol levels had been observed in the diabetics 3rdtrimester with family history negative that was almost 09 % but it was not significant statistically (p value 0.353), (Table 3).

Non GDM with Positive family history: The mean circulatory level of cortisol was 22.65 ± 1.77 µg/dlin the second trimester of non-diabetics with family history positive and 37.36 ± 2.71 µg/dl in the non-diabetics third trimester with family history positive. An increase in the cortisol levels had been observed in the non-diabetics third trimester with family history positive that is almost 68 % so it was highly significant statistically (p value 0.001),(Table 3).

Table 3: Comparison ofCortisol µg/dl accordingto 2nd trimester &3rdtrimester gestational diabetics and family history.

Group	N	Mean	SEM	t-test	p-value
Diabetics 2nd trimester family history positive.	15	33.38	2.21	1.324	0.195
Diabetics 3rdtrimester family history positive	18	37.39	2.06		
Diabetics 2nd trimester family history negative	10	37.03	3.34	0.952	0.353
Diabetics 3rd trimester family history negative	12	41.04	2.67		
Non-diabetics 2nd trimester family history positive	18	22.65	1.77	4.723	<0.001*
Non-diabetics 3rdtrimester family history positive	10	37.36	2.71		
Non-diabetics 2nd trimester family history negative	13	18.57	2.194	1.52	0.141
Non-diabetics 3rdtrimester family history negative	14	22.23	1.115		

* Difference in Cortisol is statistically significant at 0.05

- **Non GDM with negative family history**

The mean circulatory level of cortisol was 18.57 ± 2.194 µg/dl in the second trimester of non-diabetics with family history negative and 22.23 ± 1.115 µg/dl in the non-diabetics third trimester with family history negative. A slightly increase in the cortisol levels had been observed in the non-diabetics third trimester with family history negative that was 18 % almost so it was not significant statistically (p value 0.141), (Table 3).

DISCUSSION

The present study elaborates the adaptation and influence of pregnancy on the responses ofcortisol in second and third trimesters with and without family history of GDM while comparing with non-GDM state. The state of GDM as understood from numerous studies to be the result of insulin resistance and other associated mechanisms causing significant hyperglycemia in the pregnancy. Responses of the hormones are varied in the same trimester with positive and negative family history. Even in the normal non-GDM pregnancy with positive and negative family history had been compared there were adaptation variations

It is observed thatwith increasing trimesters the requirements of nutrients alsoincreaseto balance the changes of pregnancy. GDM usually becomes apparent during the late phase of pregnancy. It isrelated with both reduced secretion of insulin and the delaying effects of other hormones on the insulin and this condition statedas insulin resistance.After delivery symptoms of diabetes usually disappear**[14]**.The behavior of the pertinent pregnancy hormones in the second trimester with or without family history of GDM has been found to be varied and statistically significant compare to the profiles of the hormones in non GDM subjects.Cortisol demonstrated lower expression in positive compared to negative family history.

Pregnancy with gestational diabetes mellitus is characterized by insulin resistance with a compensatory riseof β-cell response and increased insulin level. Insulin resistance usually begins in the late phase of gestation and progresses, till the end of the pregnancy. It is observed that hormones of placentaare major contributor to the insulin-resistant state and this state likely plays a role in changing the maternal energy metabolism from carbohydrates to lipidsensuring that the fetus has an adequate supply of glucose for energy[15].

In the normal pregnancies subjects were categorized in those with positive and negative family history of diabetes/GDM. In these comparisons cortisol did not show any variation in the comparing groups in second semester. In third trimester compared to negative family history cortisol shows lower expression in the positive family history.It is noted that certain group of females with GDM who showssign of islet cell autoimmunity. Later in life these females may have more chances of developing autoimmune form of diabetes. Any defect in the β-cell for exampleany mutation in Glucokinase may leadβ-cell's inability. In late pregnancy, insulin sensitivity falls by nearly 50%[15].

The responses of the cortisol hormone have also been compared between second and third trimester. In GDM subjects the comparison of negative and positive family history did not show conspicuous results however in non-GDM subjects the family history of GDM factor have shown very significant results. In positive family history subjects cortisol demonstrated high expression.Most of the studies have suggested that increased maternal adiposity and the insulin desensitizing effect ofcertain placental hormonesare two major contributors 0f insulin resistance. After delivery insulinresistance decreases rapidly, which shows that placental hormones are major contributor. The present study has revealed that insulin resistance mechanism is not plainly due to the effect of cortisol. It points out that the mechanisms in GDM insulin resistance may be due to the all placental hormones including cortisol however their expressions are very complex and it provides strong evidence for further investigating the complexity of GDM in different populations.

In fetus human chorionic somatomammotropin(HCS) arouses pancreatic secretion of insulin and in mothers it inhibits peripheral glucose uptake[15]. As the pregnancy advances, the placental size increases which leads to increase production of the placental hormones, so more insulin-resistant state develops. In non-diabetic pregnant females, in the first and second trimester due toβ-cell hypertrophy and hyperplasia, insulin responses compensate for this reduction in insulin sensitivity[16]. However, women who have shortage of extra insulin secretory capacity may develop GDM.

Other factors are also highlighted by some studies like TNF-α andleptin which are secreted by fat cells and placenta [17]. It is evident that inlategestation increase production of TNF-α produced by placenta[18]. It is also noted that immediately after delivery increase levels of leptinandTNF-α fall rapidly[18].It means that quickreversal of insulin resistance after placental delivery is may due to decreasedmaternal levels ofleptin and TNF-α.Furthermore muscle damage, aging, insulin resistance and obesity all are related with raised TNF-α levels[19]. The mechanism of insulin resistance due to placental hormones has not been proposed regarding the intermediate mechanisms specifically in relation to those discussed above.

Gestational diabetes had been studied in 2nd and 3rd trimesters in relation to the family history[20],as the insulin sensitivity is predominantly influenced in the late stages of pregnancy. Family history effect the trimester factor aswellas influence onthe metabolic disorder. It is often reported that females with positive family history of diabetes are at more risk of developing the metabolic disorder. So, family history is often related with diabetic risk factors[10]. It is likely that the mechanisms that are influenced due to positive and negative family history interact and influence the specific hormonecortisol which were studied in the present investigation.

Cortisol has a blocking effect on insulin, which usually starts about 20 to 24 weeks ofgestation. As the placenta grows, the hormone is produced more, and insulin resistance becomes greater. Cortisol also has shown different responses in relation to the variables in the present study. The variability is understood to be linked with different states such as GDM, non-GDM and positive or negative family history.

In conclusion the analysis of the cortisol in present study reveals that its levels in pregnancy are affected variedly in different states of GDM rather that it is directly responsible for the induction ofinsulin resistance.

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