ORIGINAL ARTICLE

The Hormonal Levels of Progesterone in Second and Third Trimesters of Gestational Diabetes Mellitus Patients with or without Family History

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ABSTRACT

Background: Gestational diabetes mellitus (GDM) is intolerance of glucose with varying degrees of severity which isinitially recognized during pregnancy. GDM generally has few symptoms and it is commonly diagnosed during pregnancy by screening.
Aim: In the present study the responses of the pertinent hormone of the pregnancy progesterone have been investigated in gestational diabetes and non-GDM subjects especially in context of positive and negative family history in second and third trimester.
Methods: The present cross sectional 2 stage study with non-probability convenient sampling was done in Arif Memorial Teaching Hospital 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. After taking their consent on consent Performa general data of the pregnancy and blood sampleswere taken.
Results: Serum progesterone was estimated by ELISA with specific monoclonal antibodies. The results were analyzed in relation to GDM, non-GDM, positive and negative family history in second and thirdtrimesters. In second trimester Progesterone exhibited 4 times and 3 times increases in positive and negative family history subjects respectively compared to respective non-GDM groups In third trimester also the responses of these hormones were numerous times increases in GDM than non-GDM.
Conclusion: The analysis of results of the hormones within GDM and non-GDM category and between the trimesters has shownsome statistically noticeable results.The excessive increase of progesterone do support that it may be one of the cause of insulin resistance in GDM.
Keywords: Progesterone, Gestational Diabetes Mellitus, Family History

INTRODUCTION

Pregnancy is a physiological phenomenon including changes in body to develop an embryo and later into fetus. It usuallylasts for 39- 40 weeks, starting from the first day of the woman's last menstrual cycle and is divided into three trimesters, each lasting three months[1]. Each trimester has its own developmental landmarks and significance.

Thecommonest metabolic abnormality i.e., diabetes mellitus in which defective secretion of insulin occurs leads to increased concentration of glucose in blood[2]. This increased level of glucose effectthe 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].

In first trimester of a non-diabetic pregnancy, insulin action is boosted by estrogens and progesterone and glucose levels tend to decline[4]. Later with increasing weeks of gestation insulin action resists and leads to increased levels of blood glucose levels.

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The stress of pregnancy is the important factor to develop gestational diabetes mellitus as it uncovers the susceptibility of genes to develop noninsulin dependent diabetes mellitus. Chronic resistance of insulin anddefective pancreatic beta cells functions result in development of gestational diabetes. Usually this resistance begins in 2nd trimester of pregnancy and continues till end of gestation

Generally diagnostic criteria forgestational diabetes is 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 pregnanciesareeffected by gestational diabetes[5]. Gestational diabetes occurs when insulin receptors do not perform function in its physiological limits in second and third trimesters of pregnancy and remits following delivery[6]. This is considered due to pregnancy-related factors that interfere with susceptible insulin receptors. This in turn causes inappropriately raised blood sugar levels.GDM results in the presence of increasing peripheral resistance due to delayed or insufficient insulin secretion[7].

During the middle phase of the gestation the hormonal changes and changes in metabolism result in glucose intolerance[8]. In the early phase of a non-diabetic pregnancy, insulin action is enhanced by estrogens and

progesterone and glucose concentration tend to decline[4]. Later with increasing weeks of gestation insulin action resists and leads to increased levels of blood sugar levels. No known cause other than hormones which are released during gestation are responsible for increasing insulin resistance which results in abnormalities in metabolism of glucose[9].

Family history is often associated with diabetic risk factors[10] which mainlyeffect the incidence of gestational diabetes mellitus along with trimesters of pregnancy which is characterized by progressive insulin resistance that begins in near second trimester of pregnancy[10]. It is noted that trimesters along with positive family history in pregnancy influences the gestational diabetes mellitus.

Steroid hormones in pregnancy support fetal growth and development by controlling metabolic processes of embryogenesis and organogenesis. It also playsessential role in regulation of parturition timing. Different steroid hormones are expressed at different stages of intrauterine life which are critical for fetal growth and development, suggesting the temporal and spatial expression of steroid hormones[11].Among these steroids progesterone is important andprominent.

Progesterone is produced continuously throughout pregnancy because as pregnancy can be maintained at low estrogen concentrations, but not with low progesterone thus being the most important steroid hormone of pregnancy. The corpus luteum is stimulated to sustain progesterone secretion by rising concentrations of human chorionic gonadotrophin (hCG) following implantation[12]. After first trimester of pregnancy, hCG concentrations decline and progesterone synthesis is relocated to placental trophoblast cells[13].

While progesterone concentrations are initially low during the phase of luteal production, they rise exponentially once the placenta takes over as the main site of steroid synthesis and continue to increase until the end of pregnancyeight times that of at 14th week rising up to 150ng/ml**[13]**. For the maintenance of pregnancy,the most important hormoneis progesterone as it promotes uterine quiescence and suppresses maternal immune response to prevent rejection of the fetus[14].

Progesterone is the main naturally occurring human progestogen which is involved in the female menstrual cycle and support pregnancy.Actions of estradiol are required before the exposure of progesterone in the luteal phase[15].Progesterone in pregnancy is of great importance for the reason it is produced continuously throughout pregnancy, first by the corpus luteum and later by the placenta. Following implantation, the corpus luteum is stimulated to sustain progesterone secretion by rising concentrations of hCG[13]. After six to ten weeks of pregnancy, hCG concentrations decline and progesterone synthesis is relocated to placental trophoblast cells[16]. It is also argued that Insulin resistance is maintained by TNF-α and leptin. The placenta is the chief source of TNF-α in human pregnancy, with the highest production rates evident in late gestation[17]. It is likely that insulin resistance inducing factors modulate progesterone to different degrees in varied states of GDM, non-GDM positive and negative family history and different trimesters.

The present study has been carried out with the objectives of determining the circulatory levels of progesterone, GDM in relation to family history of the disorder in second and third trimester,also to compare the levels of the hormonesin various groups of GDM and the normal pregnancies to find any hormonal adaptations in the studied specific conditions.

MATERIALS AND METHODS

It was cross-sectional 2 stage study.From September, 2013 to February, 2014 total 110 pregnant females were selected from Arif Memorial Teaching Hospital and Hameed Latif Hospital, Lahore for sampling after following inclusive criteria which include gestation week more than 12 weeks (2nd and 3rd trimesters only). Among 110 samples 55 pregnant females of 2nd and 3rd trimesterwereselected and classified as pregnant females with gestation diabetes mellitus (GDM) and 55 pregnant females without GDM, (Control).Personal, obstetric history, family history for diabetes mellitus, last menstrual period (LMP), gestational diabetes time period, predisposing factors with previous pregnancies, life style, educational status and general physical examination were recorded on questionnaire.

Methods & Biochemical Analysis: 5 ml of blood sample was collected in disposable syringes from the pregnantfemales. After coagulation and centrifugation, serum was separated and stored at a temperature of -20 ^{0}C for assessment of Serum Progesterone level.which 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 $p \leq 0.05$.

RESULTS

The hormones levels of progesterone were assayed in gestational diabetic (GDM) subjects in relation to the trimester and family history. Similar study was done in normalwomenas 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: A marked difference in the progesterone levels had been observed in GDM that is almost 04 times greater than the control subjects and It was highly significant statistically ($p<0.001$), (Table 1).

GDM with Negative family history: A marked difference in the progesterone levels had been observed in GDM that was almost 03 times greater than the control subjects. It was significant different statistically ($p<0.001$), (Table 1).

GDM with different family history: No difference in the progesterone levels had been observed in these two diabetics groups. It was insignificant statistically (p value 0.999), (Table 1).

Non-GDM with different family history: A slight increase in the progesterone levels had been observed in subjects with negative family history that was almost 15%. It was highly insignificant statistically (p value 0.547) (Table 1).

Table 1: Comparison ofProgesterone ng/ml according to 2nd trimestergestational diabetics and family history

2nd trimester Group	N	Mean	SEM	t-test	p-value
Diabetic family history positive.	15	41.719	1.458	12.42	<0.001*
Non -diabetic family history positive	18	11.121	1.889		
Diabetic family history negative	10	41.723	4.35	5.779	<0.001*
Non-diabetic family history negative	13	13.09	2.777		
Diabetics family history positive	15	41.719	1.46	.001	0.999
Diabetics family history negative	10	41.72	4.36		
Non-diabetics family history positive	18	11.12	1.89	0.609	0.547
Non-diabetics family history negative	13	13.09	2.78		

* Difference inProgesterone (pg/ml) is statistically significant at 0.05

THIRD TRIMESTER

GDM with positive family history: A marked difference in the progesterone levels had been observed in GDM that was almost 37 % greater than the control subjects. It was highly significant different statistically (p <0.001), (Table 2).

GDM with Negative family history: A marked difference in the progesterone levels had been observed in GDM that was almost 2 times greater than the control subjects. It was highly significant statistically (p <0.001), (Table 2).

GDM with different family history: A slight increase in the progesterone levels had been observed in subjects with negative family history that was almost 08 % greater than the family history positive. It was highly insignificant statistically (p value 0.105), (Table 2).

Non-GDM with different family history: An increase in the progesterone levels had been observed in subjects with positive family history that was almost 40 %. It was highly significant statistically (p value 0.003), (Table 2).

Table 2: Comparison of Progesterone ng/mlaccording to 3rd trimester gestational diabetics and family history.

3rdtrimester Group	N	Mean	SEM	t-test	p-value
Diabetic family history positive	18	44.58	1.498	14.58	<0.001*
Non-diabetic family history positive	10	32.81	3.428		
Diabetic family history negative	12	48.22	1.416	10.887	<0.001*
Non-diabetic family history negative	14	20.38	2.02		
Diabetics family history positive	18	44.58	1.50	1.675	0.105
Diabeticsfamily history negative	12	48.22	1.42		
Non-diabetics family history positive	10	32.81	3.43	3.313	0.003
Non-diabetics family history negative	14	20.38	2.03		

* Difference inProgesterone is statistically significant at 0.05

Table 3: Comparison ofProgesterone ng/ml according to 2nd trimester & third trimester gestational diabetics and family history.

Group	N	Mean	SEM	t-test	p-value
Diabetics 2nd trimester family history positive.	15	41.72	1.46	1.354	0.185
Diabetics 3rdtrimester family history positive	18	44.58	1.50		
Diabetics 2nd trimester family history negative	10	41.72	4.36	1.528	0.142
Diabetics 3rd trimester family history negative	12	48.22	1.42		
Non-diabetics 2nd trimester family history positive	18	11.12	1.89	6.048	<0.001*
Non-diabetics 3rdtrimester family history positive	10	32.81	3.43		
Non-diabetics 2nd trimester family history negative	13	13.09	2.777	2.14	0.042
Non-diabetics 3rdtrimester family history negative	14	20.38	2.02		

* Difference in Progesterone is statistically significant at 0.05

COMPARISON OF 2ND AND 3RD TRIMESTER

GDM with positive family history: A slight increase in the progesterone levels had been observed in subjects of third trimester with positive family history that was almost 07 % greater than the gestational diabetic subjects of second trimester. It was not significant statistically (p value 0.185), (Table 3).

GDM with Negative family history: A slight increase in the progesterone levels had been observed in subjects of third trimester that was almost 14 % greater than the gestational diabetic subjects of second trimester with family history negative. It was not significant statistically (p value 0.142), (Table 3).

Non GDM with Positive family history: A marked rise in the progesterone levels had been observed in subjects of third trimester with positive family history that is almost 03 times greater than the subjects of second trimester. It is highly significant statistically (p value < 0.001), (Table 3).

Non GDM with negative family history: A marked rise in the progesterone levels had been observed in subjects of third trimester that was almost 54% greater than the second trimester. It was significant statistically (p value .042), (Table 3).

DISCUSSION

The present study elaborates the adaptation and influenceof pregnancy on the responsesofprogesterone 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.The responses have been assessed while comparing a particular characteristic of the number of trimester and the family history with their respective controls. The available information seems to provide some information of the responses of the hormones in the variable states of GDM and non-GDM subjects. Pregnancy is a physiological phenomenon which starts with conception and completed with delivery, it includes physiological changes in body for the development of fetus. It usually lasts for 40 weeks, and is divided into three trimesters, each lasting three months[1]. Each trimester marks its own significance and developmental landmarks. Maternal body faces metabolic changes during pregnancy which can be divided into an anabolic and a catabolic phase[18]. The first and second trimester of pregnancy corresponds with anabolic phase of pregnancy and is directed at nutrient storage and the buildup of reserves, which are then mobilized in the catabolic phase of third trimester when they are required for fetal growth and to prepare the mother for lactation[19].

It is observed thatwith increasing trimesters the requirements of nutrients also increase to balance the changes of pregnancy. Homeostatic mechanisms work actively in pregnancy to ensure the wellbeing of fetus. 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[20].These changes are brought about by hormones secreted by the corpus luteum, placenta, and maternal organs to maintain the balance between metabolic changes. The catabolic state which is characteristic of late gestationis attained through changes in insulin production and sensitivity combined with a continuing increase in maternal food uptake[19].

Pregnancy must be monitored although occasionally because it may lead to a variety of complications that results in the maternal and fetal death. In our population where there is dearth of awareness for the importance of antenatal monitoring, the chances to develop complications like glucose intolerance or insulin sensitivity leading to gestational diabetes mellitus also increase[5]. The monitoring for GDM is on rise thus it not only provides the safety to fetus and mother but also provide data to understand the nature and mechanisms in the development of GDM in the screened population. This also provides information to global data for better understanding of the disorder. GDM is defined as any degree of glucose intolerance with onset or first recognized during pregnancy[21]. GDM usually becomes apparent during the late phase of pregnancy. It is related with both less secretion of insulin and the blocking effects of other hormones on the insulin that is produced, a condition called as insulin resistance [22].Diabetic symptoms usually disappear after delivery[21].This combination places women at risk of developing diabetes during pregnancy.

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. Progesterone in this case expressed greater in positive than the negative in the ratio of 4 to 3 times increased than their respective controls. GDM as prominently accompanied insulin resistance therefore sometimesthe causes of insulin resistance have been investigated under various aspects. Pregnancy with gestational diabetes mellitus is characterized by insulin resistance which usually begins in the late phase of pregnancy and progresses till the end of the pregnancy. The hormones profile in the third semester is in contrast to second semester. Unlike second trimester Progesterone showed greater response in negative (100% increase) compared to positive (37% increase) family history subjects. In the normal pregnancies subjects were categorized in those with positive and negative family history of diabetes/GDM. In these comparisons there are significantly varied responses of the hormones in the comparing categories. The progesterone did not show any variation in the comparing groups in second semester. In thirdtrimester compared to negative family history progesterone demonstrated lower expression in the positive family history.

The responses of the progesterone 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 subject progesterone demonstrated high expression.

The fact that insulin resistance quickly decreases after delivery which shows that the major contributors may are placental hormones. The present study has revealed that insulin resistance mechanism is not plainly due to the effect of placental hormones collectively. It points out that the mechanisms in GDM insulin resistance may be due to the placental hormones however their expressions are very complex and it provides strong evidence for further investigating the complexity of GDM in different populations.

Human chorionic somatomammotropin (HCS) stimulates insulin secretion in fetus and inhibits peripheral glucose uptake in mothers[23]. As the placental size increases due to progression of pregnancy so does the production of the mentioned hormones, leads to a more insulin-resistant state. In non-diabetic pregnant females, the 1sttrimester and 2ndtrimester insulin responses compensate for this reduction in insulin sensitivity, and this is related with pancreatic β-cell hypertrophy and hyperplasia[23]. However, women who have a deficit in this additional insulin secretory capacity develop GDM. Polymorphisms of susceptible genes of type 2 diabetes have been shown to relate to development of GDM[24].

Certain studies explained the direct role of TNF-α in the pathophysiology of insulin resistance. Raised TNF-α is related with insulin resistance in a wide range of conditions including obesity[25]**,** aging and muscle damage. TNF-α initiate a pathway that risessphingomyelinase and ceramides whichseems to delay insulin receptor autophosphorylation.

Gestational diabetes mellitus had been studied in 2nd and 3rd trimesters in relation to the family historyas the insulin sensitivity is predominantly influenced in the late

stages of pregnancy. As family history effects the trimester factor also influences the 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[26]. It is likely that the mechanisms that are influenced due to positive and negative family history interact and influence the specific pregnancy hormone i.e. progesterone which is discussed in present study.

Progesteroneinpregnancyis ofgreatest importance for thereason it is produced continuously throughout pregnancy, first by the corpus luteum and later by the placenta. Following implantation, the corpus luteum is stimulated to sustain progesterone secretion by rising concentrations of hCG[13]. After six to ten weeks of pregnancy, hCG concentrations decline and progesterone synthesis is relocated to placental trophoblast cells[19]. It is also argued that Insulin resistance is maintained by TNF-α and leptin. The placenta is a vital source of TNF-α in human pregnancy, with thehighest production rates evident in late gestation[21]. It is likely that insulin resistance inducing factors modulate progesterone to different degrees in varied states of GDM, non-GDM positive and negative family history and different trimesters.

In conclusion the analysis of the progesterone in present study reveals that its levels in pregnancy are affected variedly in different states of GDM rather that this hormone is directly responsible for the induction of insulin resistance.

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