Percentage of pregnant women with gestational diabetes in Telafer district and its relationship to some biomarkers

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Abstract

Gestational diabetes is a kind of diabetes that is initially identified throughout the duration of gestation. Like other kinds of diabetes, pregnancy-related diabetes impacts how cells utilize sugar (glucose). High blood sugar levels brought on by gestational diabetes can have a negative effect on gestation and the fetus's health. Research has not yet determined why some women develop gestational diabetes and not others. Excess weight before pregnancy often plays a role. The research was designed to verify the percentage of pregnant women with gestational diabetes residing in the Tal Afar district in northwestern Iraq (with a Turkmen majority) and compare it with the percentage mentioned by other researchers for Iraqi pregnant women (of Arab nationality). As well as confirming the possible link between insulin resistance and high blood sugar, cholesterol and cortisol levels.This study included two groups of women from Tal Afar district, their ages ranged between (16-40 years), the first group included 85 pregnant women, while the second group included 40 non-pregnant married women. The results of our study indicated that there was a significant ($p < 0.05$) increase in random glucose, total cholesterol, and cortisol levels, from the first phase until the third phase. Finally, the study indicated that there was a significant increase ($p < 0.05$) in the level of total cholesterol in obese pregnant women compared with women of normal weight. Our study concluded that the percentage of women suffering from gestational diabetes in Tal Afar district does not differ significantly from the percentage indicated in Iraqi cities with an Arab majority. It was also found that the vast majority of healthy pregnant women suffer from a physiological increase in glucose, cholesterol and cortisol levels. It has also been shown that obesity has a clear effect on cholesterol levels in the blood, and there is a direct relationship between fatigue, chronic stress, and lack of sleep with cortisol levels in the blood of healthy pregnant women.

Keywords: Gestational diabetes, Glucose, pregnant women, Cortisol, Cholesterol

Introduction:

Diabetes mellitus is a widely prevalent hormonal disorder, impacting over one hundred million individuals globally. It is brought on by the pancreas' inability or failure to produce enough insulin, which causes blood glucose levels to rise. It has been shown that it harms a number of bodily functions, including the blood vessels, kidneys, heart, and nerves (Ismail, 2009). There are actually two main types of the illness: insulin-dependent diabetes mellitus (IDDM, type 1) and non-insulin-dependent diabetic mellitus (NIDDM, type 2) (Arora et al. 2009). Pregnancy diabetes, in its simplest form, is diabetes that manifests during pregnancy. Some speculate that proinsulin may contribute to beta-cell stress regardless of the mechanism is still unclear. Others believe that excessive quantities of hormones such as progesterone, cortisol, human placental lactogen, and others might affect the functioning of beta cells and peripheral insulin sensitivity. (Kühl, 1998).

According to Amirian et al. (2020), GDM is a silent disease that can harm both the mother and the fetus and result in unfavorable outcomes such polyhydramnios, pre-eclampsia, , fetal macrosomia, hyperbilirubinemia, hypocalcemia, and polycythemia on the mother and fetus. On the other hand, as reported by Sudasinghe et al. (2018), Type 2 diabetes and the metabolic syndrome are more likely to take place in a kid of a mother with GDM., and cardiac problems in the coming years.

Although the majority of research indicated that GDM and normal pregnancy had different circulating lipid patterns, the findings have been mixed (Li et al., 2021). Patients with GDM reportedly exhibited decreased quantities of HDL--C and higher concentrations of TG, TC, and LDL-C (Li et al., 2015). However, according to other studies, there was no difference in the first , second, and third phases of gestation between patients with GDM and healthy pregnant women in terms of raised blood TC and LDL-C levels (Li et al., 2021).Based on a study by Amirian et al. (2020), women with GDM had greater levels of TC, LDL-C, VLDL-C, and TG than women who were not diabetic during pregnancy, while their levels of HDL-C were lower. TC, LDL-C, and TG decrease in the first phase and rise in the second and third, according to a different study by Mankuta et al. (2010). The researchers' findings suggest that stress hormones were strongly connected with insulin resistance , which could represent a significant factor in the onset of gestational diabetes (Feng et al., 2020).

Subjects & Methods:

1- Volunteers and study protocol:

Blood samples were drawn from 125 women (ages between 16-40 years) from the city of Tal Afar (northwestern Iraq), which has a Turkmen majority. 85 of them were pregnant, while the remaining 40 were non-pregnant women (control group). The sampling period was in October and November of the 2022.

2- Sample collection:

Each participant gave 5 ml of blood, which was permitted to coagulate for twenty minutes at room temp prior being spun in a centrifuge for around ten minutes at 4000 revolutions per minute to obtain serum samples, which were subsequently kept at - 20.

3- Estimation of glucose and total cholesterol in the blood:

The level of glucose and total cholesterol in blood serum was estimated using the enzymatic method in which the reactant is oxidized according to the Trinder reaction (Burtis and Ashwood, 1999), according to the following equations:

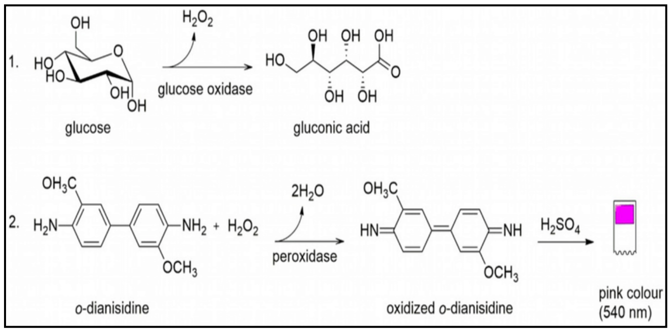

Figure (2-1): The principle of the enzymatic method for determining blood glucose

$$\text{Cholesteryl acetate} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{Acetic acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \Delta\text{-Cholestenone} + H_2O_2$$

$$2H_2O + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

Figure (2-2): The principle of the enzymatic method for determining total cholesterol in the blood

Test Procedure:

We take three test tubes, and follow the following steps:

Table (2-1) Summary of additives for determination of glucose and cholesterol

Material used	Glucose or Cholesterol in the blood	Standard tube	Blank tube
Reagent	1 ml	1 ml	1 ml
Serum	10 µl		
Standard solution		10 µl	
Distilled water			10 µl

4- Determination of cortisol hormone:

Human cortisol hormone ichroma Kit Catalog No. INS-CO-EN (Rev. 10) was used to quantify the levels of cortisol hormone in serum (Appendix C), which uses a competitive immunodetection method.

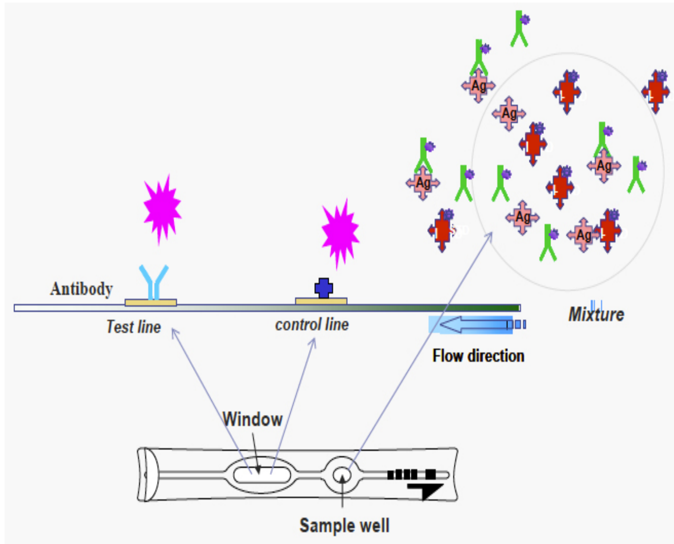

Figure (2-3): A graphic showing the immune reaction to cortisol determination

Test procedure:

1) 30 µl of serum was added using a micropipette to a tube containing detection buffer.

2) The detection buffer cover was closed and the sample was mixed thoroughly by shaking it several times.

3) Then 75 µl of the sample mixture was withdrawn and loaded into the groove of the sample well on the cartridge.

4) The cartridge loaded with the sample was inserted into the incubator chamber (at 25 °C) for 10 minutes.

5) The loaded sample cartridge has been inserted into the ichroma™ device.

6) Finally, the test result was read on the display of the ichroma™ test tool.

Statistical analysis

The data was presented as mean values with standard error. ANOVA and a student's t-test were employed to contrast the results between groups. $P \leq 0.05$ values were considered significant. All statistics were analyzed using SPSS (SPSS 2021, version 25.0) and Microsoft Excel 2016.

Results:

1- Physiological analysis

The ages volunteers were divided into three groups as shown in Table (3-1), while the distribution of age groups between the two groups (pregnant and non-pregnant) did not show a significant difference between them as shown in Table (3-2) ($P = 0.782$).

Table (3-1): Distribution of sample according to age groups

Age group	Pregnant (85)	Non pregnant (40)
<18	8 (9.41 %)	3 (7.5 %)
18 - 40	70 (82.35 %)	34 (85 %)
> 40	7 (8.23 %)	3 (7.5 %)

Table (3-2): Comparison of the mean ages between the two main groups

Age group	Healthy pregnant	Non pregnant
Mean ± SD	25.5 ± 6.2	25.6 ± 6.7
***p*-value**	**0.782**	

Results interpreted as mean ± SD and p-value corresponding to the paired sample t-test.
Statistical test was done after considering the *p*-value = **0.05**

2- Percentage of pregnant women with gestational diabetes and its relationship to some physical and biochemical variables

According to Figure (3-1), the number of women with gestational diabetes was 11 out of 85 pregnant women from Iraqi Tal Afar City), at a percentage of (12.9%).

As shown in the same Figure (1-3), it was found that among the women with gestational diabetes, 5 of them (45.4%) were in their third phase. We also found that the same percentage of women were overweight or obese (BMI >25), and the same number reported poor sleep or chronic fatigue. Also, it was found that among the women with gestational diabetes, 7 (63.6%), 4 (36.3%), and 3 (27.2%) were suffering from having a family history of the disease, increased cholesterol, and high cortisol, respectively.

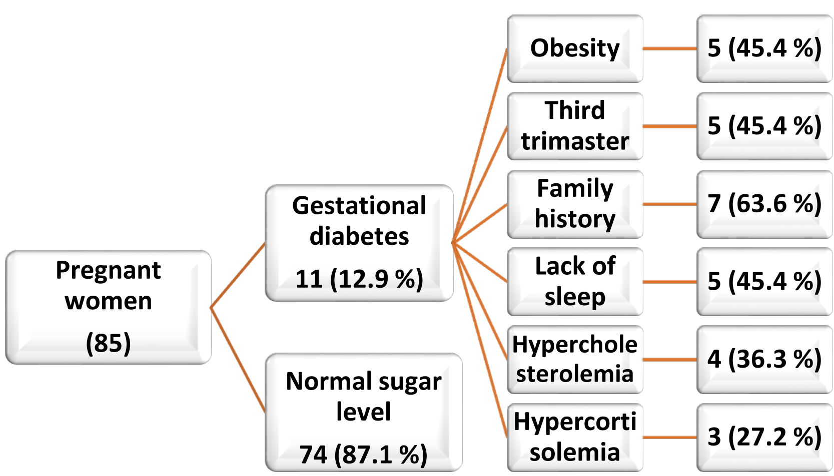

Figure (3-1): Percentage of pregnant women with gestational diabete

3- The difference in the levels of parameters between the two groups studied (healthy pregnant women and non-pregnant women)

As shown in Table (3-3) and Figure (3-2), our research revealed a statistically significant rise (p 0.05) in the amount of random sugar and total cholesterol but a statistically unimportant rise ($p > 0.05$) in cortisol levels in the healthy pregnant group contrasted to the non-pregnant group.

Table (3-3): Comparison of the studied parameters among the two groups (healthy pregnant women and non-pregnant women)

Parameters	Groups	Mean ± SE	P- Value
Random blood sugar (mg/dl)	Non-pregnant	85.72 ± 3.03	**0.001**
	Healthy pregnant	102.66 ± 5.40	
Blood cholesterol (mg/dl)	Non-pregnant	156.2 ± 4.27	**0.0001**
	Healthy pregnant	212.22 ± 9.50	
Serum cortisol (nmol/L)	Non-pregnant	607.3 ± 28.7	**0.123**
	Healthy pregnant	669.9 ± 25.11	

Results interpreted as mean ± SE and p-value corresponding to the paired sample t-test.
Statistical test was done after considering the p-value = 0.05

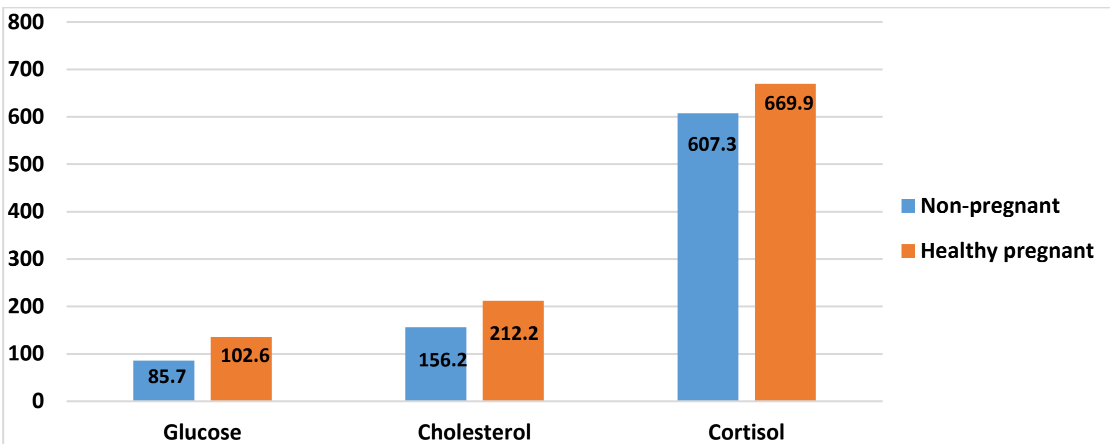

Figure (3-2): Representation of the difference in parameter values between the two groups studied (healthy pregnant and non-pregnant women)

4- Association between the three stages of pregnancy and biomarkers levels in the healthy pregnant group

The results of our study indicated, as shown in Table (3-4) and Figure (3-3), that there is a significant difference ($p < 0.05$) in the levels of random glucose, total cholesterol, and cortisol levels between the three stages of pregnancy (for the group of healthy pregnant women), where a gradual and significant increase in the levels of the three biomarkers was observed from the first phase to the third phase.

Table (3-4): The difference in the studied biomarker values among the three stages of pregnancy

Parameters	First phase Mean ± SE	Second phase Mean ± SE	Third phase Mean ± SE	*p*- Value
Random blood sugar (mg/dl)	77.2 ± 5.4	102.9 ± 8.4	144.05 ± 10.3	**0.0001**
Blood cholesterol (mg/dl)	153.07 ± 13.8	223.4 ± 9.4	290.4 ± 15.4	**0.0001**
Serum cortisol (nmol/L)	569.7 ± 41.6	667.8 ± 33.4	838.6 ± 39.6	**0.0001**

Results interpreted as mean ± SE and *p*-value corresponding to the ANOVA test.
Statistical test was done after considering the p-value = 0.05

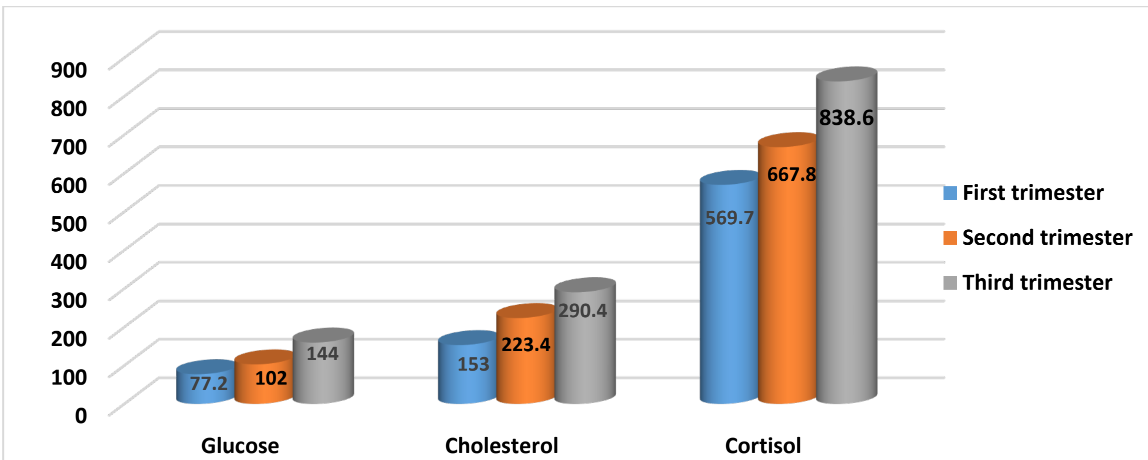

Figure (3-3): Representation of the difference in parameter values between the three stages of pregnancy within (the group of healthy pregnant)

5- Associations between weight and biomarker levels in the healthy pregnant group

Our study indicated a significant increase ($p < 0.05$) in total cholesterol levels and a non-significant increase in blood glucose ($p > 0.05$) in the group of obese pregnant women compared to their counterparts of normal weight, as shown in Table (3-5) and Figure (3-4) while we will not notice any difference in cortisol levels between the two groups mentioned.

Table (3-5): Effect of weight on biomarkers studied in healthy pregnant women

Parameter	Variants	Mean ± SE	*p*- Value
Random blood sugar (mg/dl)	Normal weight	89.38 ± 7.39	**0.107**
	Obesity	113.95 ± 7.40	
Blood cholesterol (mg/dl)	Normal weight	179.35 ± 12.56	**0.003**
	Obesity	240.17 ± 12.48	
Serum cortisol (nmol/L)	Normal weight	669.91 ± 34.43	**0.454**
	Obesity	670.05 ± 36.49	

Results interpreted as mean ± SE and p-value corresponding to the paired sample t-test.
Statistical test was done after considering the *p*-value = 0.05

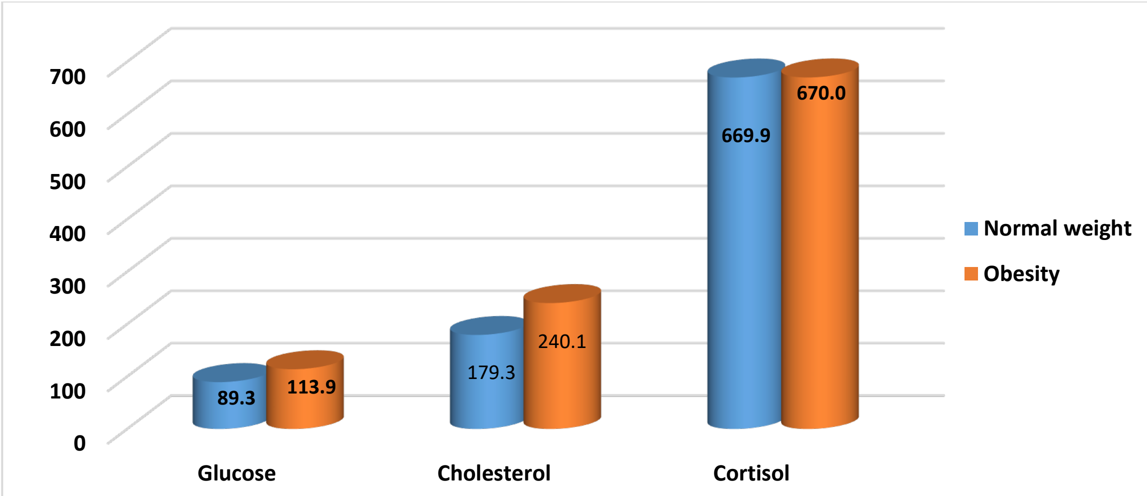

Figure (3-4): Representation of the difference in parameter values between obese and non-obese pregnant women

6- Associations between stress and biomarker levels in the healthy pregnant group

Finally, compared to pregnant women who get enough rest, our study found a significant (p 0.05) rise in cortisol the amount in the group of pregnant women who experience short sleep duration and chronic fatigue. However, we did not find any important differences ($p > 0.05$) in glucose or cholesterol quantities between the two groups, as shown in Table (3-6) and Figure (3-5).

Table (3-6): The effect of the sleep hours number on the studied biomarkers of healthy pregnant women

Parameter	Variants	Mean ± SE	*p*- Value
Random blood sugar (mg/dl)	Enough sleep	100.45 ± 5.93	**0.283**
	Lack of sleep	112.14 ± 13.17	
Blood cholesterol (mg/dl)	Enough sleep	218.13 ± 10.18	**0.153**
	Lack of sleep	201.98 ± 24.55	
Serum cortisol (nmol/L)	Enough sleep	655.00 ± 28.12	**0.04**
	Lack of sleep	734.21 ± 54.36	

Results interpreted as mean ± SE and p-value corresponding to the paired sample t-test.
Statistical test was done after considering the *p*-value = **0.05**

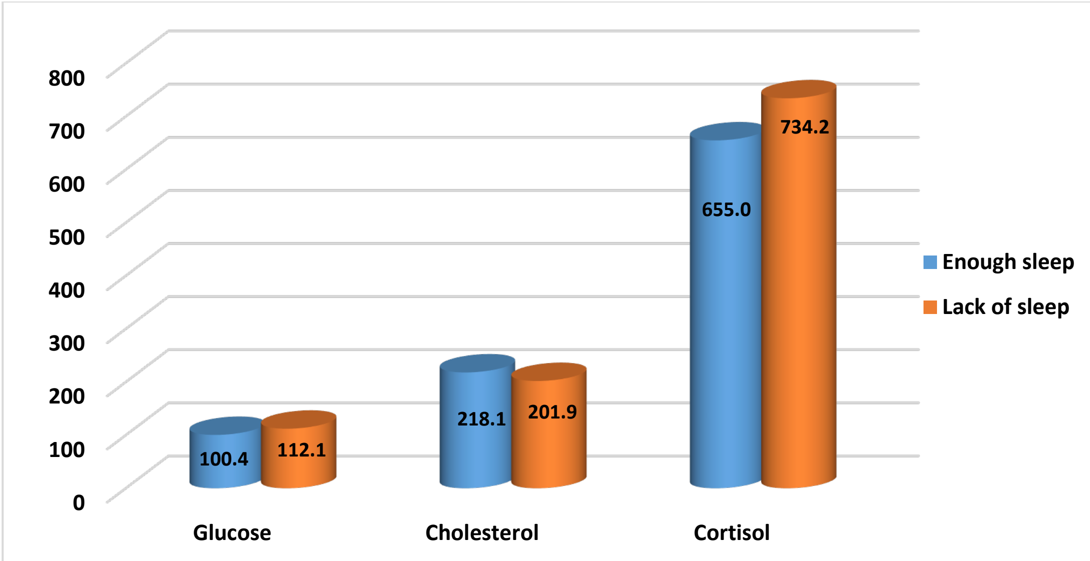

Figure (3-5): Representation of the difference in parameter values between pregnant women who sleep enough hours and those who do not take enough rest

Discussion:

1- Gestational diabetes prevalence and its association with several physical and biochemical parameters

The number of women with gestational diabetes was 11 out of 85 pregnant women from Iraqi Tal Afar City), at a percentage of (12.9%). Based on Al-Rifai et al. (2021) estimations of the prevalence of GDM internationally (ranging from 1% to 28%), there is a significant ethnic difference among various groups. Several genetic variations linked to GDM share shared susceptibility loci with T2DM, according to a study of 22 research. GDM is a polygenic illness that varies with the environment, just like T2DM (Agarwal, 2020).

In the Iraqi city of Tikrit (north of Baghdad), in a recent study conducted two years ago (2020) on 120 women aged between 20-45 years, it was found that the prevalence of GDM was 13.3% (Mohammad, 2020). From this, it can be said that there are no statistically significant differences in the percentage of pregnant women with gestational diabetes in the city of Tal Afar compared to cities populated by Arab residents. However, this discussion is considered preliminary, because the number of pregnant women was small in the two studies.

Gestational diabetes mellitus (GDM) has been defined as diabetes that manifests itself with greater frequency throughout the second or third period of gestation. One of the main causes of GDM is insulin resistance (Vejrazkova et al., 2014). Hormonal changes cause insulin resistance in pregnant women. Insulin activity is hindered by maternal hormones. In order to conquer this resistance during a typical pregnancy, extra insulin produced by GDM is utilized to raise the blood glucose level (Hossain et al., 2020). Based on the above, the development of gestational diabetes in the third phase can be explained by the increased secretion of insulin-resistant hormones, which the placenta increases during the last stages of pregnancy.

Despite the fact that the cause of GDM is not entirely understood.Mother,, weight gain, greater mother's age, and women from certain ethnic groups have been reported as being at elevated risk (Zhang et al., 2016), . Although the majority of studies indicated that GDM and regular pregnancy had different plasma fatty patterns, the findings have been mixed (Huang et al., 2016). According to studies, people with GDM had higher levels of TG, TC, and LDL-C and a lesser quantities of HDL-C (Li et al., 2021).Yet, according to other research, there was no difference in the first, second, or third periods of gestation between patients with GDM and healthy pregnant women in terms of raised blood TC and LDL-C levels (Wang et al., 2019).

Several studies, such as (Rahnemaei et al., 2022), (Mankuta et al., 2010), and (Shen et al., 2016), indicated an increase in blood cholesterol in cases of gestational diabetes and also mentioned that cholesterol, LDL, and triglycerides rise more in the third phase of gestation. In addition to a difficult pregnancy, mother lipid disorder also has a negative impact on the birth outcomes (Nasioudis et al., 2019).

Researchers have shown that women with pregnancy-related diabetes typically have cortisol levels that are considerably greater than that of controls, and that cortisol correlates positively with the degree of resistance to insulin (Feng et al., 2020), and (da Costa et al., 2016).

2- The difference in the levels of parameters between the two groups studied (healthy pregnant women and non-pregnant women)

Due to the tremendous increase in workload that the female body faces during pregnancy, which may lead to a disturbance in glucose metabolism, this may lead to high blood glucose levels in pregnant women (Li et al., 2021). Despite the complexity and incomplete understanding of the processes underlying insulin resistance, it has been discovered that a variety of variables such as uterine hormonal substances, being overweight, nutrition, and genes, can affect insulin resistance whilst pregnant (Hunt &Schuller, 2007).

Regarding the relatively high levels of cholesterol during pregnancy, in addition to our study, many studies indicated that pregnancy is characterized by a rise (up to 40-50%) in the level of cholesterol in the mother's blood (Leiva et al., 2013). Zeljković et al. (2022) also indicated a statistically significant increase in the different types of fats in the blood of pregnant women, both those with gestational diabetes and even healthy pregnant women. The cause of the increase in fat (including cholesterol) may be the increased secretion of estrogen, which stimulates the liver to synthesize more fat. In addition, estrogen inhibits lipoprotein lipase (LPL) function by lowering LPL gene transcription throughout gestation, which decreases the elimination of fat (Hossain et al., 2020).

Several studies have indicated a link between cortisol and pregnancy, as cortisol rises from 2 to 4 times more than the normal level. Cortisol decreases the synthesis of GLP-1, and this in turn lowers the secretion of insulin. Major gluconeogenic enzymes are made to express themselves when cortisol is present, which raises glucose synthesis in the liver and glycogen breakdown. According to Scaroni et al. (2017), cortisol inhibits the muscle's ability to transfer the insulindependent sugar transporter 4 (GLUT4) to the membrane of the plasma, which impairs glucose absorption and activates the glycogen synthase kinase-3, which slows glycogen formation and induces breakdown of proteins.

3- Association between the three stages of pregnancy and biomarkers levels in the healthy pregnant group

A gradual and significant increase was observed in the levels of the three vital indicators (glucose, cholesterol, and cortisol) from the first phase to the third phase. Our study agreed with a number of studies that indicated an increase in fat levels in the third phase of pregnancy, such as (Rahnemaei et al., 2022), (Agarwal, 2020), and (Shen et al., 2016), However, TC, LDL-C, and TG have been shown to decrease in the first gestation and rise in the subsequent phases (Mankuta et al., 2010).

An elevated blood sugar level could result from increased insulin-resistance-releasing hormones as pregnancy goes on, which includes estrogen and cortisol. Free cortisol levels begin to climb around the eleventh week of gestation, and higher amounts appear in the later stages of gestation. The generation of fetal lung surfactant, which is crucial for the creation of fetal lungs, is significantly influenced by excessive cortisol production between 30 and 32 weeks of pregnancy (Bandoli et al., 2018).

Despite extensive studies on lipid levels during gestation, the findings are conflicting (Rahnemaei et al., 2022). It is known that metabolizing carbohydrates significantly impacts the way lipids are metabolized.

4- Associations between weight and biomarker levels in the healthy pregnant group

A study he did (Wang et al., 2022), indicated that women who were overweight before pregnancy had a worse lipid profile (higher TG, lower HDL) even after one year from birth, indicating that the lipid profile during pregnancy is subject not only to physiological changes but also to the patient's metabolic status.

Resistance to insulin caused by abdominal obesity is influenced in part via adipokines and free fatty acids (FFA). Resistin and retinol-binding protein 4 are two types of adipokines that reduce the response to insulin. Additionally, cytokines produced by fat tissue macrophages play a role in developing insulin resistance (Flock et al., 2011).

The hormone-sensitive lipase, the primary enzyme responsible for the breakdown of intracellular lipids, is effectively blocked by the subsequent rise in insulin (Evans et al., 2002). A crucial modulator of FFA migration from adipose tissue is insulin. As a result, an insulin resistance score significantly affects how TG-rich lipoproteins and FFA undergo metabolism (Karpe et al., 2011).

The studies showed that diverse factors, including liver excess output of VLDL, lowered circulating TG lipolysis, deficient peripheral FFA capturing, greater flow of FFA from fatty cells to the hepatocytes and other tissues, and building of lipoproteins like dense small portions (LDL), participate in the dyslipidemia seen in weight gain caused by resistance to insulin. (Klop et al., 2013).

5- Associations between stress and biomarker levels in the healthy pregnant group

The response to physiological stress leads to increased secretion of the hormone cortisol, during great effort or lack of sleep. Designed to increase short-term energy availability, cortisol acutely impairs insulin action and increases hepatic glucose production. Hypercortisolism associated with exposure to chronic stress may exacerbate insulin resistance and type 2 diabetes (Adam et al., 2010).

Fatigue and stress may over-activate the hypothalamus, pituitary, and adrenal glands, leading to increased cortisol secretion and insulin resistance, which increases the risk of GDM in pregnant women (Levine et al., 2007).

Conclusions

The percentage of pregnant women with gestational diabetes in the Tal Afar district (northwestern Iraq) is 12.9%. 36.3% of women with gestational diabetes were diagnosed with high cholesterol. 27.2% of women with gestational diabetes reported high cortisol concentrations. The blood sugar and cholesterol amounts in healthy pregnant women have been shown to be significantly greater compared to those of non-pregnant women. From the first to the third phases of gestation, there was a noticeable and progressive rise in the blood levels of cortisol, glucose, and cholesterol in pregnant women.

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