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Evaluation of Maternal Serum Levels of Adiponectin and Tumor Necrosis Factor-Alpha in Iraqi Women with Gestational Diabetes Mellitus

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ABSTRACT

Gestational diabetes mellitus (GDM) is a complication of gestation that is characterized by impaired glucose tolerance with first recognition during gestation. It develops when β-cell of pancreas fail to compensate the diminished insulin sensitivity during gestation. This study aims to investigate the relationship between adiponectin and TNF-a levels with development gestational diabetes mellitus (GDM). This study included (150) pregnant women in first trimester (9-13 weeks) of pregnancy, during follow up in [late second trimester and early third trimester (24-28 weeks) of pregnancy], 75 of them diagnosed of GDM, and 75 of them without GDM (Control group). The adiponectin levels are significantly lesser in females who develop GDM than the control group ($P \leq 0.05$), while the TNF-a, insulin and OGTT concentrations were significantly higher in females with GDM than control group ($P \leq 0.05$). The results showed negative correlation between adiponectin and (FBG, Insulin, HOMA-IR, TNF-a) in GDM-2 group. Conclusions: Maternal late second trimester and early third trimester adiponectin concentrations were decreased in women with GDM compared with healthy pregnant. Therefore, adiponectin as an early biomarker may help to predict the development of GDM later in pregnancy. TNF-a levels were the most important pro inflammatory cytokines associated with increased risk of GDM. females, who develop gestational diabetes mellitus, have higher levels of insulin resistance from normal females.

Keywords: Gestational Diabetes Mellitus (GDM), Adiponectin, Tumor Necrosis Factor (TNF-a), Insulin, Oral Glucose Tolerance Test (OGTT), HOMA-IR.

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INTRODUCTION

The most recent definition of GDM is by the American Diabetes Association, defining GDM as diabetes diagnosed in second or third trimester (24-28 weeks) of pregnancy that was not clearly overt diabetes prior to gestation (ADA, 2020). GDM is a complication of pregnancy that is characterized by impaired glucose tolerance, it develops when pancreatic β-cells reserve is not sufficient to compensate for the increased IR during pregnancy. As a consequence, there are increased hepatic gluconeogenesis, severe insulin resistance and subsequently hyperglycemia (Mohammed, 2015). GDM appears to result from the same broad spectrum of physiological and genetic abnormalities that characterize diabetes outside of pregnancy. Indeed, women with GDM are at high risk for having or developing diabetes when they are not pregnant. Thus, GDM provides a unique opportunity to study the early pathogenesis of diabetes and to develop interventions to prevent the disease (Eades *et al.,* 2017 and Lee *et al.,* 2018). GDM has been associated with numerous adverse effects influencing the maternal and neonatal health status both at short and long term (Shriraam *et al.,* 2013 and Sajani *et al.,* 2014).

Adiponectin was originally discovered nearly simultaneously by four independent research groups in the mid-1990's and named it adipocyte complement-related protein of 30kDa (Acrp30), adipose complement related protein (AdipoQ), adipose most abundant gene transcript 1 (apM1), and gelatine binding protein 28kDa (GBP-28). The name adiponectin was proposed by Arita *et al.*, in 1998 (Mohammed, 2015). The adiponectin molecule (MW 30kD) is a 244-amino-acid long adipokine secreted from adipocytes (Kadowaki and Yamaushi, 2005 and Jalil,2020). Physiological Functions of Adiponectin: 1) Anti-atherosclerotic agent: it inhibits the formation of lipid-laden foam cell, as well as the inflammatory adipocytokine, TNF-α. 2) Anti-inflammatory agent: it inhibits the phagocytic activity of macrophages and the production of TNF-α by these macrophages. 3) Anti-oxidant: it stimulate the endothelial cells of blood vessels to produce nitric oxide. 4) Specific anti-diabetic functions: adiponectin stimulate fatty-acid oxidation and therefore decreases tissue triglyceride (TG) content in the liver and muscle. Tissue TG interfere with insulin-stimulated phosphatidylinositol 3-kinase activation and subsequent glucose transporter 4 translocations and glucose uptake, leading to insulin resistance. Thus, decreased tissue TG content in muscle may contribute to the improved insulin signal transduction. Adiponectin also decreases glucose levels by inhibiting both the

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expression of hepatic gluconeogenic enzymes and the rate of endogenous glucose production (Mohammed, 2015).

Tumour Necrosis Factor-alpha (TNF-α), formerly known as cachectin, is synthesized and secreted from macrophages as a non-glycosylated protein of 25 kDa. Its orientation is reversed (its amino terminus is intracellular, and its carboxy terminus is extracellular). After synthesis, latent pro-TNF-α (25kDa) is deposited on the cell surface of monocytes and other cells. TNF-α converting enzyme (TACE) cleaves pro-TNF-α, releasing an active soluble 17kDa form. The 17kDa membrane fragment, including the carboxy terminus, is proteolytically cleaved by a matrix metalloproteinase from the plasma membrane of the mononuclear phagocytes and assembled in to a stable 51kDa to 52kDa biologically active homotrimer (secreted form) (Laham *et al.*, 1994 and Mohammed, 2015 and Turki Jalil et al.,2019).

MATERIALS AND METHODS

This study included (150) pregnant women in first trimester (9-13 weeks) of pregnancy, during follow up in [late second trimester and early third trimester (24-28 weeks)], 75 of them diagnosed of GDM, and 75 of them without GDM (Control group). It was determined sample size according to the equation Stephen Thompson (Thompson, 2012 and Sallam *et al.*, 2019). Serum adiponectin, TNF-a and insulin were measured using ELISA Kits (Demeditec Diagnostics GmbH, Germany). Serum blood glucose estimated by assay kit (Randox, England). Biochemical parameters measured in the [first trimester (9-13 weeks)] and [second and third trimester (24-28 weeks)].

OGTT Protocol

1- The subjects should have a normal mixed diet for the previous three days prior to the test.

2- The subjects is fasted overnight and blood is drawn for glucose measurement before 75 grams is administered orally in 200 mL of water.

3- Blood glucose is measured every sixty minutes for two hours (Lehmann *et al.*, 2015).

Calculate Homeostatic Model Assessment-Insulin Resistance (HOMA-IR) :

Serum blood glucose and serum insulin estimated from fasting morning blood samples were utilised to calculate IR by the homeostatic model assessment-insulin resistance

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(HOMA-IR), which is approximated using the equation below (Matthews *et al*., 1985 and Zhu *et al*., 2015).

HOMA-IR= Glucose (mmol/L) × Insulin (µIU/mL) / 22.5

Collection of Blood Sample

After an overnight fasting for about 8 hr, (5 mL) of venous blood was collected from pregnant women with and without GDM, were transferred to plain tube and allowed to clot at room temperature to get serum by putting it in empty disposable tubes and centrifuged to separate it at 3000 rotor per minute (rpm) for 10 min, the serum samples were separated and stored at (-20˚C) for later measurement biochemical parameters, unless used immediately.

Statistical Analysis

The statistical analysis was done using spss v 23 the results were expressed as mean ± standard deviation (mean ± SD). It was used T test to compare study groups, Pearson's correlation was applied to determine the relationship among the present study parameters. P-values ($P \leq 0.05$) were considered statistically significant.

RESULT AND DISCUSSION

The characteristics of the studied groups are presented in table (1).

Table (1): General characteristics data of the patients and control groups

Parameters	GDM Mean ± SD	Control Mean ± SD	P- value
No.	75	75	------
Age of gestational 1st (weeks) at sampling	10.88±1.41	10.72±1.40	0.911
Age of gestational 2nd & 3rd (weeks) at sampling	25.91±1.39	26.13±1.40	0.890
Age of maternal (Yrs)	31.42±3.67	30.67±3.75	0.951

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BMI 1st trimester	28.78±2.21	28.10±2.09	0.561
BMI 2nd & 3rd trimester	30.08±2.81	29.37±2.75	0.515

GDM: Gestational diabetes mellitus / **BMI**: Body mass index

Table (1) shows no significant difference in the age of gestational, age of maternal and BMI in GDM group in comparison with the Control group ($p \leq 0.05$).

Fasting Blood Glucose Concentration

Table (2) shows a significant increase in the concentration of serum FBG in GDM-2 group in comparison with the Cont-2 group ($p \leq 0.05$). The results obtained in this study show a significant increase in the concentration of serum FBG in the GDM-2 group in comparison with the GDM-1 group ($p \leq 0.05$).

It is recommended that all pregnant women should have FPG test to exclude pre-pregnancy diabetes mellitus by the International Association of Diabetes and Pregnancy Study Groups (IADPSG). FPG is a well predictive index for GDM diagnosis, but it has been reported that it is inappropriate to use FPG as the diagnostic basis of GDM at the early stage of pregnancy as FPG decreases with increasing gestational age. Pre-pregnancy obesity or overweight is an independent risk factor for GDM. In women pre-pregnancy with overweight and obesity, the predictive power of FPG was even higher. (Wei *et al.*, 2019). Riskin-Mashiah *et al.* found that FPG may be used as a screening test to assess risk, but not as a diagnostic test in early pregnancy: A higher FPG in the first trimester, even though in the normal range, constituted a risk for GDM in later pregnancy (Riskin-Mashiah *et al.*, 2010). Alunni *et al.* found that implementing FPG (and HBA_1c) screening in early pregnancy, nearly doubled the incidence of GDM and predicted the need for more pharmacotherapy (Alunni *et al.*, 2015).

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Table (2): FBG concentrations for studied groups

FBG concentrations (mg/dL)						
Control Groups	**Mean±SD**	**No.**	**GDM Groups**	**Mean±SD**	**No.**	**p-value**
Cont-1	75.27±6.09	75	**GDM-1**	78.16±6.12	75	0.837
Cont-2	80.52±6.43		**GDM-2**	97.30±10.76		0.000
p-value	0.288			0.000		

Cont-1: Healthy Pregnants at (9-13 weeks) / **Cont-2**: Healthy Pregnants at (24-28 weeks)
GDM-1: Pregnants with GDM at (9-13 weeks) / **GDM-2**: Pregnants with GDM at (24-28 weeks)

Figure (1) shows no significant negative correlation between serum adiponectin and serum FBG in GDM-1 group with correlation coefficient (r= - 0.11) and significant negative correlation in GDM-2 group with correlation coefficient (r= - 0.36).

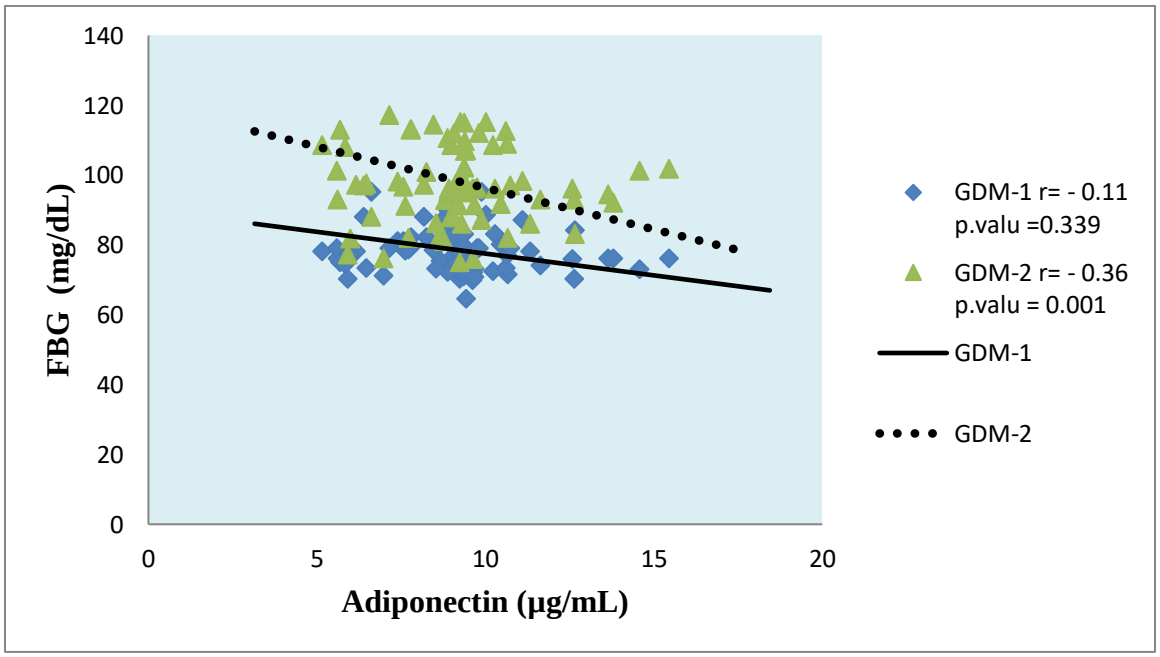

Figure (1): Correlation between serum adiponectin and serum FBG Oral Glucose Tolerance Test

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Table (3) shows a significant increase in the OGTT/1-h in GDM-2 group in comparison with the Cont-2 group ($p \leq 0.05$). The present study shows a significant increase in the OGTT/2-h in the GDM-2 group in comparison with the Cont-2 group ($p \leq 0.05$).

Oral glucose tolerance test (OGTT) used to diagnosis of GDM during pregnancy (Mert *et al.*, 2015). It was consider the "gold standard" in the diagnosis of GDM (García-Claver *et al.*, 2020). The method and the thresholds of the diagnostic screening tests in GDM have changed according to the results of some recent studies (Mert *et al.*, 2015). The Hyperglycemia and Adverse Pregnancy Outcome study showed continuous relationships between carrying out a 75-g oral glucose tolerance test (OGTT) at 24-32 weeks' gestation and perinatal outcomes. At <24 weeks' gestation, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) does not recommend carrying out a 75-g OGTT due to insufficient data on the association between 75-g OGTTs and perinatal outcomes. Alternatively, fasting plasma glucose (PG) is used to diagnose GDM at <24 weeks' gestation. At 24-28 weeks' gestation, GDM is diagnosed based on a 75-g OGTT (Iwama *et al.*, 2019).

Table (3): OGTT for studied groups

OGTT (mg/dL)						
Control Groups	**Mean±SD**	**No.**	**GDM Groups**	**Mean±SD**	**No.**	**p-value**
Cont-2 1-h	129.72±6.15	75	**GDM-2 1-h**	182.06±7.79	75	0.013
Cont-2 2-h	103.25±8.52		**GDM-2 2-h**	158.13±6.31		0.005

- Legend as in table (2)

Serum Insulin Concentration

The results in table (4) illustrating a significant increase in the concentration of serum insulin in GDM-2 group in comparison with the Cont-2 group ($p \leq 0.05$). The results obtained in this study show a significant increase in the concentration of serum insulin in the GDM-2 group in comparison with the GDM-1 group ($p \leq 0.05$).

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Table (4): Insulin concentrations for studied groups

Insulin concentrations (µIU /mL)						
Control Groups	**Mean±SD**	**No.**	**GDM Groups**	**Mean±SD**	**No.**	**p-value**
Cont-1	7.53±1.02	75	**GDM-1**	7.82±1.15	75	0.118
Cont-2	7.95±0.85		**GDM-2**	12.07±2.53		0.000
p-value	0.772			0.000		

- Legend as in table (2)

Figure (2) shows no significant negative correlation between serum adiponectin and serum insulin in GDM-1 group with correlation coefficient (r= - 0.06) and significant negative correlation in GDM-2 group with correlation coefficient (r= - 0.38).

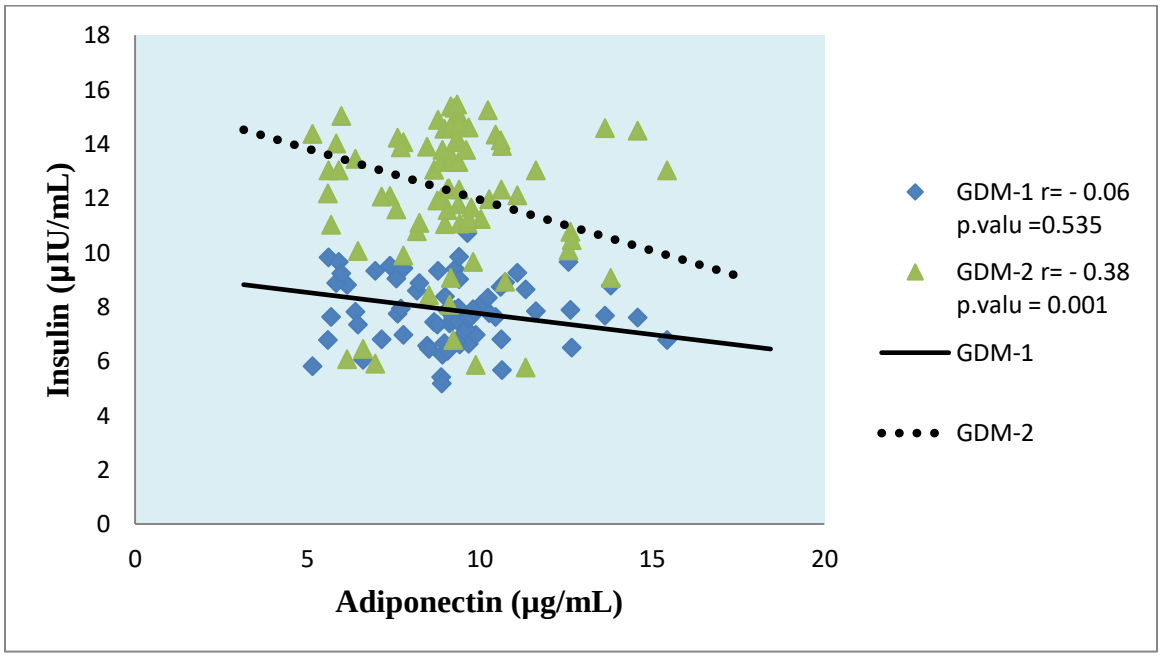

Figure (2): Correlation between serum adiponectin and serum insulin

Hemostatic Model Assessment-Insulin Resistance (HOMA-IR)

Table (5) illustrating a significant increase in the HOMA-IR in GDM-2 group in comparison with the Cont-2 group ($p \leq 0.05$). The results obtained in this study show a

significant increase in the HOMA-IR in the GDM-2 group in comparison with the GDM-1 group ($p \leq 0.05$).

HOMA-IR method requires measuring single fasting serum glucose and the corresponding fasting plasma insulin level (Wallace *et al.*, 2004 and Stolzenberg-Solomon *et al.,* 2005), that have been used HOMA-IR to estimate insulin resistance. Although widely used HOMA-IR is only a surrogate measure and thus our findings require confirmation using a more accurate assessment of insulin resistance.

Gestational diabetic mellitus is associated with both insulin resistance and an impaired insulin secretion .There is a lack of insulin during a period of time with high insulin needs, to compensate the insulin resistance that develops during the third trimester of pregnancy. In the maternal tissues where glucose uptake is insulin-dependent, the uptake is decreased because of the lack of insulin and postprandial hyperglycemia develops. Since the maternal-placental fetal transfer of glucose is concentration dependent, the hyperglycemia of the mother leads to an increased placental transfer of glucose to the fetus (Salafia *et al.,* 2015).

The mechanisms behind insulin resistance induced by the pregnancy present are still not fully understood. In pregnant rats (are believed to be similar to humans) the degradation of insulin by the placenta is increased, which leads to accelerated insulin removal. There are also different hormonal and metabolic changes during the second half of pregnancy which facilitate insulin resistance. One is the high plasma level of progesterone during the second part of pregnancy (Ladyman *et al.,* 2010).

The women with GDM appear to have β-cell dysfunction that occurs on a background of chronic insulin resistance. Insulin secretion in women with GDM can increase considerably over weeks or months in association with the acquired insulin resistance of pregnancy. However, the increase occurs along an insulin sensitivity–secretion curve that is approximately 50% lower than that of normal pregnancy. Therefore, insulin secretion in women with GDM can increase by advanced gestation, but the insulin secretion rate in women with GDM is lower than that in women with NGT (Lim *et al.,* 2011).

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GDM develops in women who cannot compensate for the normal metabolic changes of pregnancy. Normal pregnancy is associated with an approximate 60% decrease in insulin sensitivity in late pregnancy (Haeger, 2008). In order to ensure an adequate energy supply to the fetus, there is an increase in the levels of several hormones that oppose the effects of insulin, creating a state of insulin resistance. These hormones include human placental lactogen, placental growth hormone, estrogen, progesterone, cortisol, human chorionic gonadotrophin and prolactin (Saltiel, 2000). Norma glycaemia in pregnancy can only be maintained with a near doubling of basal insulin levels, as well as enhanced nutrient stimulated insulin secretion In humans, it appears that enhanced function of existing beta cells accounts for the majority of the increase in insulin secretion, rather than hyperplasia The increased insulin resistance reverts with delivery of the placenta (Antosh and Meduri, 2006). Most GDM patients are associated with insulin resistance as noticed previously (Naseem *et al.,* 2012). Previous study reported a 67% decrease in pancreatic β cell functions in GDM (Qader *et al.,* 2008).

Table (5): HOMA-IR for studied groups

HOMA-IR						
Control Groups	**Mean±SD**	**No.**	**GDM Groups**	**Mean±SD**	**No.**	**p-value**
Cont-1	1.39±0.20	75	**GDM-1**	1.50±0.23	75	0.059
Cont-2	1.57±0.18		**GDM-2**	2.91±0.72		0.000
p-value	0.915			0.000		

- Legend as in table (2)

Figure (3) shows no significant negative correlation between serum adiponectin and HOMA-IR in GDM-1 group with correlation coefficient (r= - 0.10) and significant negative correlation in GDM-2 group with correlation coefficient (r= - 0.45).

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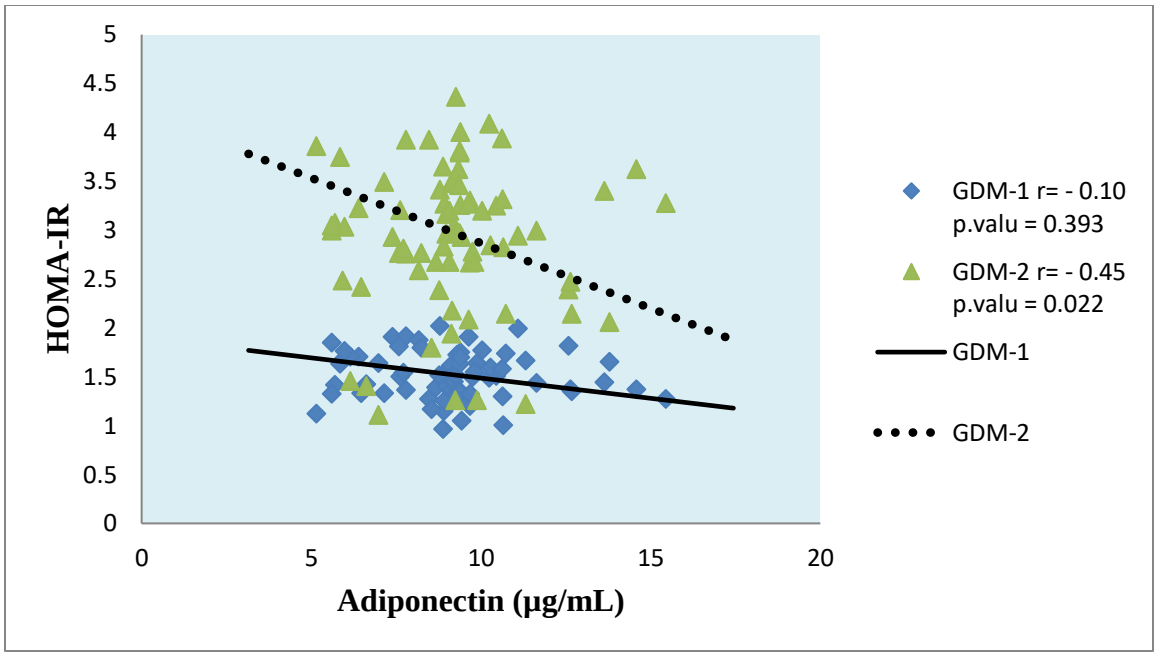

Figure (3): Correlation between serum adiponectin and HOMA-IR

Serum Adiponectin Concentration

Table (6) illustrating a significant decrease in the concentration of serum adiponectin in GDM-2 group in comparison with the Cont-2 group ($p \leq 0.05$). The present study shows a significant decrease in the concentration of serum adiponectin in the GDM-2 group in comparison with the GDM-1 group ($p \leq 0.05$).

Numerous clinical studies have shown that circulating levels of adiponectin are lower in pregnant women with GDM, when compared to women with normal pregnancies (Soheilykhah *et al.*, 2009 ; Horosz *et al.*, 2011 and Lacroix *et al.*, 2013). There are also other studies showing no association between adiponectin and GDM (McLachlan *et al.*, 2006). Some studies have shown that there is an inverse relationship between adiponectin and fasting insulin or insulin resistance in GDM (Retnakaran *et al.*, 2004 and Retnakaran *et al.*, 2005).

Generally, low circulating adiponectin level has been clearly linked with IR, obesity, and type 2 DM. Administration of adiponectin in animal models has been shown to improve β-cell function, ameliorate IR, lower blood glucose level and prevent the development of type 2 DM (Briana and Malamitsi-Puchner,

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2009 ; Paz-Filho *et al.,* 2011). These suggest that adiponectin deficiency might play a role early in the pathogenesis of DM. Therefore, it is not surprising that hypoadiponctinaemia has been strongly associated with GDM, since women with GDM are at risk of developing type 2 DM in future (Mohammed, 2015). Lower adiponectin level in the first trimester is a significant predictor for the subsequent development of GDM later in pregnancy (Briana and Malamitsi-Puchner, 2009). Thus, Lain *et al.*, 2008 suggest that females with first three months adiponectin levels under the 25th percentile are ten times more likely to be diagnosed with gestational diabetes mellitus as compared to females with elevated adiponectin concentrations. Gestational diabetes mellitus commonly diagnosed in the second or third trimester of gestation**.**

Most importantly, low circulating adiponectin level is an independent predictor of β-cell failure in the years following a pregnancy complicated by GDM, thereby implicating it as a possible factor in the progression to type 2 DM in these women (Sulistyoningrum *et al.,* 2013). Therefore, low circulating adiponectin level is an established component of the GDM phenotype before, during and after pregnancy (Doshani and Konje, 2009).

Table (6): Adiponectin concentrations for studied groups

Adiponectin concentrations (ng/mL)						
Control Groups	**Mean±SD**	**No.**	**GDM Groups**	**Mean±SD**	**No.**	**p-value**
Cont-1	9.62±1.99	75	**GDM-1**	9.15±2.07	75	0.258
Cont-2	8.76±2.52		**GDM-2**	5.11±1.33		0.022
p-value	0.261			0.008		

- Legend as in table (2)

Serum TNF-a Concentration

The results in table (7) illustrating a significant increase in the concentration of serum TNF-a in GDM-2 group in comparison with the Cont-2 group ($p \leq 0.05$). The results

obtained in this study show a significant increase in the concentration of serum TNF-a in the GDM-2 group in comparison with the GDM-1 group ($p \leq 0.05$).

TNF-α is related to GDM, obesity, glucose intolerance and type 2 diabetes mellitus (Winkler *et al.,* 2002 and Altinova *et al.,* 2007). TNF-α was reported as a significant predictor of IR during pregnancy by exerting a significant effect on insulin-mediated glucose disposal. Plasma level of TNF-α showed a downward trend during early pregnancy and increased during the third trimester, thus mirroring insulin sensitivity changes during those periods. Plasma TNF-α level correlates inversely with insulin secretion in normal pregnancy and was significantly higher in GDM group (Catalano *et al.,* 1993).

In-vitro studies have described a direct role for TNF-α in the pathophysiology of insulin resistance (Catalano *et al.,* 1993; Laham *et al.,* 1994 ; Al-Noaemi and Shalayel, 2011). Increased TNF-α can exacerbate insulin resistance, which is normal in pregnancy; this favors the development of GDM (Coughlan *et al.,* 2001). TNF-α down regulates insulin receptor signalling in cultured adipocytes, hepatocytes, and skeletal muscle. TNF-α activates a pathway that increases ceramides and sphingomyelinase and appears to interfere with autophosphorylation of insulin receptor (Al-Noaemi and Shalayel, 2011). TNF-α also impairs the association between insulin receptor substrate IRS-1 with the insulin receptor, by promoting serine phosphorylation of the insulin receptor substrate IRS-1. It is evident in pregnancy that insulin receptor and IRS-1 tyrosine phosphorylation are impaired, and there is increased serine phosphorylation in skeletal muscle during late pregnancy. Therefore, elevated circulating levels of TNF-α in late pregnancy could attenuate insulin signalling, thus causing the increased IR observed in pregnancy (Barbour *et al.,* 2007). The increase in circulating TNF-α levels in pregnant women with GDM is also associated with an increased TNF-α in the skeletal muscle and the impaired insulin signalling persist in obese women with GDM up to one year postpartum (Barbour *et al.,* 2007; Al-Noaemi and Shalayel, 2011). Therefore, TNF-α regulation during pregnancy can prevent the deleterious effects of insulin resistance. According to some authors, such verification contradicts the classical concept that insulin resistance in pregnancy is exclusively induced by placental hormones such as progesterone, human chorionic gonadotropin, prolactin, and estradiol (Kirwan *et al.,* 2002).

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Also, it has been shown that placenta and subcutaneous adipose tissues from women with GDM release greater amount of TNF-α in response to high glucose compared with normal glucose. On the other hand, there was no stimulatory effect of high glucose on TNF-α release by tissues from normal pregnant women which suggests that TNF-α might be involved in the pathogenesis and/or progression of GDM (Coughlan *et al.*, 2001 and Kuźmicki *et al.*, 2006).These findings could highly explain the increase in the level of TNF-α throughout pregnancy. The increased TNF-α levels in pregnancy fall rapidly after delivery, (Catalano *et al.*, 1993) which is consistent with the idea that the increase in circulating TNF-α during late pregnancy is mainly due to placental secretion. These findings may also help to explain the rapid reversal of insulin resistance after delivery, since maternal levels of TNF-α decrease substantially after delivery of the placenta (Laham *et al.*, 1994). According to the authors, these observations suggest that the tissues of women with GDM increase the release of TNF-α in response to hyperglycemia. Because TNF-α is involved in the metabolic regulation of glucose, lipids, and insulin resistance, these data are consistent with the hypothesis that TNF-α is involved in GDM development (Coughlan *et al.*, 2001 and Kuźmicki *et al.*, 2006).

Table (7): TNF-a concentrations for studied groups

TNF-a concentrations (pg/mL)						
Control Groups	**Mean±SD**	**No.**	**GDM Groups**	**Mean±SD**	**No.**	**p-value**
Cont-1	2.95±0.45	75	**GDM-1**	3.00±0.65	75	0.104
Cont-2	2.81±0.43		**GDM-2**	6.30±1.57		0.000
p-value	0.570			0.000		

- Legend as in table (2)

Figure (4) shows no significant negative correlation between serum adiponectin and serum TNF-a in GDM-1 group with correlation coefficient (r= - 0.16) and significant negative correlation in GDM-2 group with correlation coefficient (r= - 0.40).

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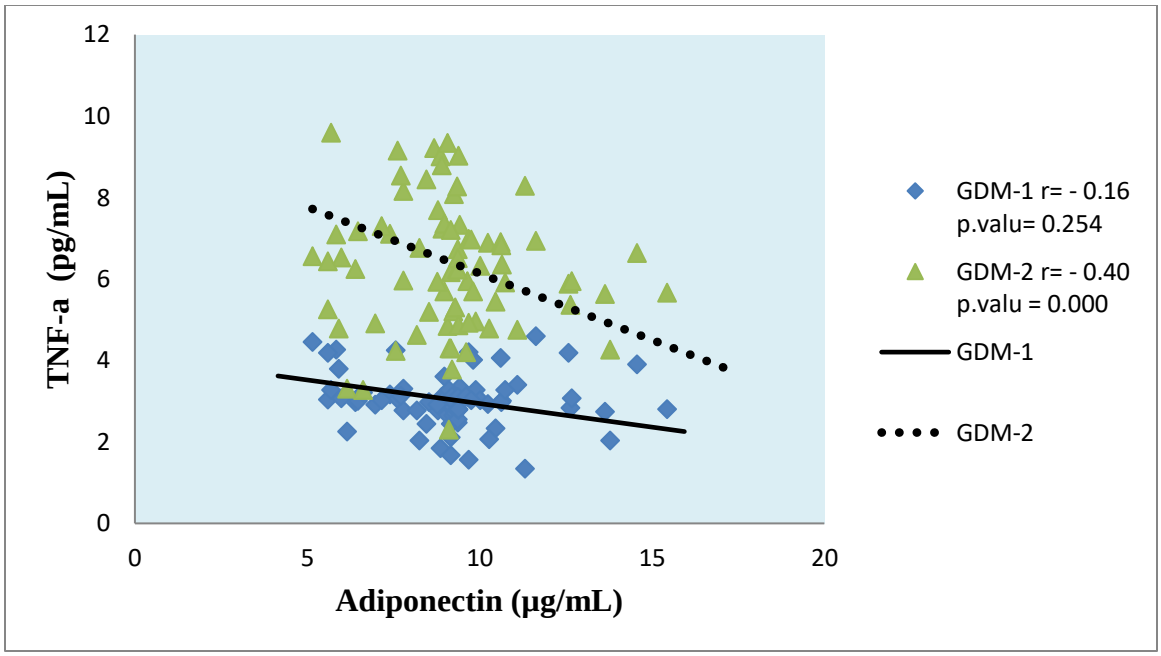

Figure (4): Correlation between serum adiponectin and serum TNF-a

CONCLUSION

Maternal late second trimester and early third trimester adiponectin concentrations were decreased in women with GDM compared with healthy pregnant. Therefore, adiponectin as an early biomarker may help to predict the development of GDM later in pregnancy. The present study found that the TNF-a levels were the most important pro inflammatory cytokines associated with increased risk of GDM. The presence of GDM in pregnant patients lead to disturbances in FBG, Insulin and HOMA-IR.

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