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RESEARCH ARTICLE

SERUM ADROPIN AND ADIPOSE FATTY-ACID BINDING PROTEIN AT 6TH WEEK OF PREGNANCY ARE SIGNIFICANT PREDICTORS FOR DEVELOPMENT OF INSULIN RESISTANCE AT THE 24TH WEEK.

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Keywords:-
Insulin resistance, Gestational Diabetes Mellitus, Adropin, Adipose fatty-acid binding protein.

Abstract

Objectives: To estimate serum Adropin (ADR) and Adipose fatty-acid-binding proteins (AFABP) levels at 6th wk and its relations with insulin resistance (IR) judged by homeostasis model assessment of IR (HOMA-IR) score and their predictive value for later development of gestational diabetes mellitus (GDM).

Patients & Methods: 335 pregnant women gave blood samples for estimation of fasting blood glucose (FBG), and fasting serum insulin, ADR and AFABP. All women underwent the 75-Oral glucose tolerance test (OGTT) and HOMA-IR scoring at 6th and 24th week GA.

Results: At 6th wk GA, 77 women had IR score >2 and 24th wk 39 women developed GDM and 106 had IR >2. Mean 6th wk serum ADR levels were significantly lower, while serum AFABP levels were significantly higher in GDM than non-GDM women. Statistical analyses defined 6th wk GA high serum AFABP and insulin, BMI and 2-hr PPBG, and low serum ADR, as significant predictors for development of IR at the 24th wk GA.

Conclusion: Pregnancy induces maternal IR that was related to maternal BMI and may progress to GDM that was reported by a frequency of 11.9%. Development of GDM and IR were closely related to high serum AFABP and low serum ADR levels.

Introduction:-

During pregnancy glucose metabolism is governed by equilibrium between lactogenic hormones stimulating insulin production and counter-regulatory hormones inducing insulin resistance (IR) [(1)]. Multiple peptides play a major role in pathogenesis of metabolic disorders and carbohydrate metabolism [(2)] and substances of hormonal character secreted by adipose tissue (Adipokines) are of great importance [(3)]. Studies tried to understand the dynamic associations of adipokines with gestational diabetes mellitus (GDM) risk [(4)] documented disturbed adipokines levels early in pregnancy among women who later develop GDM than women completed their pregnancy free of GDM [(5)].

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Fatty-acid-binding proteins (FABP) are small intracellular proteins of 14–15 KDa expressed in several tissues [6]. Adipocyte FABP (AFABP) is mainly expressed in adipocytes and macrophages where it regulates adipocyte fatty-acid uptake, lipogenesis and delivery of lipids to nuclear receptors to mediate nuclear transcriptional programs [7]. In macrophages AFABP modulates inflammatory responses and cholesterol ester accumulation [8]. Despite being intracellular proteins, FABP are released into the circulation and its increased plasma levels had found in several clinical conditions and proposed as markers of tissue injury [6]. Plasma AFABP levels are increased in metabolic disorders as obesity, type-2 diabetes mellitus [9] and cardiovascular conditions [10], and in critically-ill patients and correlate with poor prognosis [11].

New peptides not secreted by adipose tissue have a significant role in metabolic regulations [3]. Adropin (ADR) is a secreted peptide translated from the Energy Homeostasis Associated (ENHO) gene [12] which comprised two-exons on human chromosome 9p13.3 [13] and linked to metabolic control and vascular function [14]. ADR is a 42-amino-acid peptide hormone which is highly conserved across mammalian species through open reading frame in exon 2 that encodes the full-length 75-amino acid peptide; 33-amino acid as secretory signal peptide and the biologically active ADR [13]. ADR is abundant in liver and secreted into the circulation and its plasma concentrations are highly regulated by energy intake [15] and functions to preserve the circulatory system through regulating endothelial function and activity of endothelial nitric oxide [16].

Hypothesis:-
The current study suggests a certain relation between development of gestational IR and DM on one-side and disturbed levels of plasma peptides with an action related to energy homeostasis on the other side.

Design:-
Prospective comparative clinical trial

Setting:-
Benha University Hospitals

Aim of work:-
This study aimed to estimate serum ADR and AFABP levels and its relations with IR as judged by the score of homeostasis model assessment of IR (HOMA-IR) in pregnant women. The study also targets to evaluate the predictive value of estimated serum ADR and AFABP levels early in pregnancy for later development of GDM.

Patients & Methods:-
After approval of the study protocol by the Local Ethical Committee, all pregnant women who attended the Antenatal Outpatient Clinics (OPC), at Benha University Hospitals since June 2015 for assurance of diagnosis of being pregnant and signed written fully informed consent to participate in the study were evaluated for eligibility for study inclusion. All women underwent evaluation for demographic data including age, weight and height, and for baseline clinical and obstetric data. Body mass index (BMI) was calculated in kg/m^2 as weight (kg)/ height (m^2) [17].

Exclusion criteria include manifest DM, previous GDM in multipara women, morbid obesity with BMI>35 kg/m^2 [18] and liver, or renal diseases. All pregnant women gave blood samples for estimation of random blood glucose to assure absence of manifest DM and women were asked to attend the OPC overnight fasting on the next day to give blood samples for estimation of fasting blood glucose (FBG), and fasting serum insulin (FSI), ADR and AFABP. Then, all women underwent the 75-Oral glucose tolerance test (OGTT) which consists of estimation of FBG and postprandial blood glucose (PPBG) levels at one and two hours after taking a 75-gm oral glucose diet. All women were asked to attend the OPC overnight fasting during the period between 24^{th} and 28^{th} week GA to give blood samples for re-estimation of FSI and to repeat the 75-OGTT.

Insulin resistance (IR) was evaluated using the homeostasis model assessment of IR (HOMA-IR) score calculated according to the formula: fasting serum insulin (µU/ml) x [fasting plasma glucose (mg/ml)/18])/22.5; HOMA-IR score of >2 is considered abnormal [19]. HOMA-IR score was determined twice at 6^{th} and 24^{th} week GA. The results of the 75-OGTT were interpreted for diagnosis of GDM according to the recommendations of the International association of diabetes and pregnancy study groups [20] as follows: FBG ≥92 mg/dl, 1-h BG ≥180 mg/dl and 2-h BG ≥153 mg/dl.

Investigations:-

Sampling:-

Venous blood samples (5 ml) were collected from the antecubital vein under complete aseptic conditions and were divided into two parts:

1. The first part was put in a tube containing sodium fluoride (2 mg sodium fluoride/ ml blood) to prevent glycolysis for estimation of blood glucose levels.
2. The second part was collected in plain tube, allowed to clot, centrifuged at 1500×g for 15 min and the serum samples were collected in clean dry Eppindorff tube to be stored at –70°C until assayed.

Estimated parameters:-

1. Blood glucose levels were estimated using glucose oxidase method [21].
2. ELISA estimation of serum insulin levels using ELYSA kit (Enzymuntest Insulin, ES 600, Boehringer Mannheim) [22], ADR using ELYSA kit (MyBioSource Inc., San Diego, California, USA) [23] and FABP4 using ELYSA kit (MyBioSource Inc., San Diego, California, USA) [24].

Statistical analysis:-

Obtained data were presented as mean±SD, numbers and percentages. Results were analyzed using One-way ANOVA with post-hoc Tukey HSD Test and Chi-square test (X^2 test). Possible relationships were investigated using Pearson's linear regression. Sensitivity & specificity of estimated parameters as predictors were evaluated using the receiver operating characteristic (ROC) curve analysis judged by the area under the curve (AUC) compared versus the null hypothesis that AUC=0.05. Statistical analysis was conducted using the IBM SPSS (Version 23, 2015) for Windows statistical package. P value <0.05 was considered statistically significant.

Results:-

The study included 348 women eligible for evaluation, 13 women were excluded and 335 women were included in the study (Fig. 1). At the 24th wk GA, OGTT defined 39 GDM women (GDM group) for a frequency of GDM of 11.6%, while 296 women (88.4%) achieved the 24th wk free of GDM (Non-GDM group). Baseline body weight (BW) and BMI were significantly higher in GDM than non-GDM women; otherwise there was non-significant (p>0.05) difference between studied women as regards baseline demographic and clinical data (Table 1)

Table 1:- Demographic and clinical data determined at the 6th week GA

Data			Non-GDM group (n=296)	GDM group (n=39)	P value
Age (years)			27.9±2.8	28.7±5.5	0.087
BMI data		Weight (kg)	83±6.6	87.7±7.6	0.003*
		Height (cm)	169.8±3.6	170.2±3.2	0.089
		BMI (kg/m^2)	28.8±2.6	30.2±2.6	0.007*
Obstetric history	Gravidity	Primigravida	131 (44.3%)	19 (48.7%)	0.781
		Multigravida	165 (55.7%)	20 (51.3%)	
	Parity	Primipara	136 (45.9%)	18 (46.2%)	0.682
		Multipara	160 (54.1%)	21 (53.8%)	
Blood pressure	Systolic		115±4.3	114.7±6.2	0.376
	Diastolic		78.1±6.9	76.9±4.1	0.298
Random blood glucose (mg/dl)			86.1±8.9	87.9±15.1	0.372

Data are presented as mean±SD, numbers & percentages; BMI: Body mass index; *: indicates significant difference

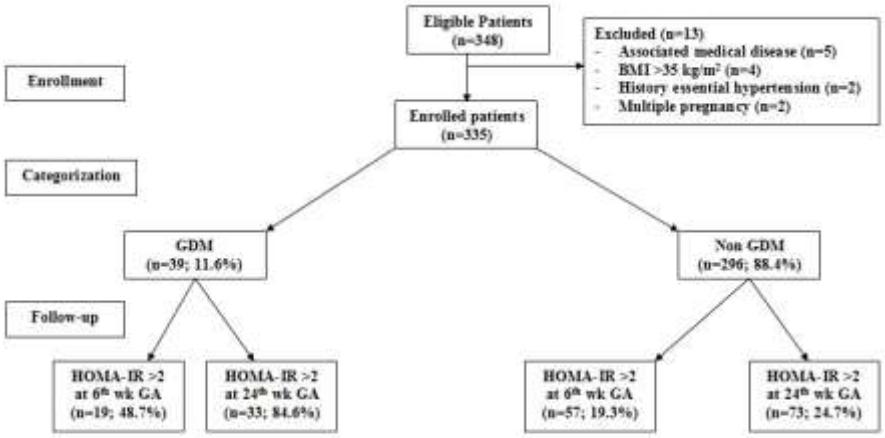

Fig. (1): Flow chart of the study

Mean FBG and PPBG levels estimated at the 6th wk GA were non-significantly (p>0.05) higher in GDM women compared to non-GDM women. At the 24th wk GA, FBG and PPBG levels were significantly higher in GDM women compared to their corresponding measures at the 6th wk GA and to corresponding measures of non-GDM women at 24th wk GA. On the other hand, FBG levels at the 24th wk GA were non-significantly (p>0.05) higher, while PPBG measures were significantly higher in non-GDM women compared to their 6th wk GA levels. At the 24th wk GA, mean FSI levels were significantly (p<0.05) higher in all women compared to their 6th wk GA levels with significantly higher (p<0.05) levels in GDM women than non-GDM women. Calculated HOMA-IR score defined 77 and 106 IR women, at the 6th and 24th wk GA, respectively with significantly higher frequency among GDM than non-GDM women, (p=0.001, respectively). Mean HOMA-IR score was significantly higher (p<0.05) in all women at the 24th wk GA compared to their respective 6th wk GA score with significantly higher score in GDM than non-GDM women (Table 2). Moreover, 6th wk GA serum ADR levels were significantly (p<0.05) lower (Fig. 2), while serum AFABP levels were significantly (p<0.05) higher in GDM than non-GDM women.

Table 2:- Results of OGTT and HOMA-IR scoring of studied women at the 6th and 24th week GA

Variable	Time / Group	6th wk GA		24th wk GA	
		Non-GDM (n=296)	GDM (n=39)	Non-GDM (n=296)	GDM (n=39)
OGTT	FBG	91.2±13.7	95.6±7.6	91.8±5.7	102.8±10.4*†
	1-hr PP	157±19.8	168.7±30.6	167.7±15.6*	214.8±15.9*†
	2-hr PP	117.7±11.1	119.2±7.6	121.5±14.3*	192.9±19.8*†
FSI		7.7±2.3	8.3±1.5	8.6±3.1*	13±4.2*†
HOMA-IR score	≤2	239 (80.7%)	19 (48.7%)	223 (75.3%)	6 (15.4%)
	>2	57 (19.3%)	20 (51.3%)	73 (24.7%)	33 (84.6%)*†
	Mean score	1.7±0.6	2±0.5	2±0.7*	3.3±1.1*†

Data are presented as mean±SD, numbers & percentages; GA: Gestational age; GDM: Gestational diabetes mellitus; OGTT: Oral glucose tolerance test; FSI: Fasting serum insulin; HOMA-IR: homeostasis model assessment of insulin resistance *: indicates significant difference

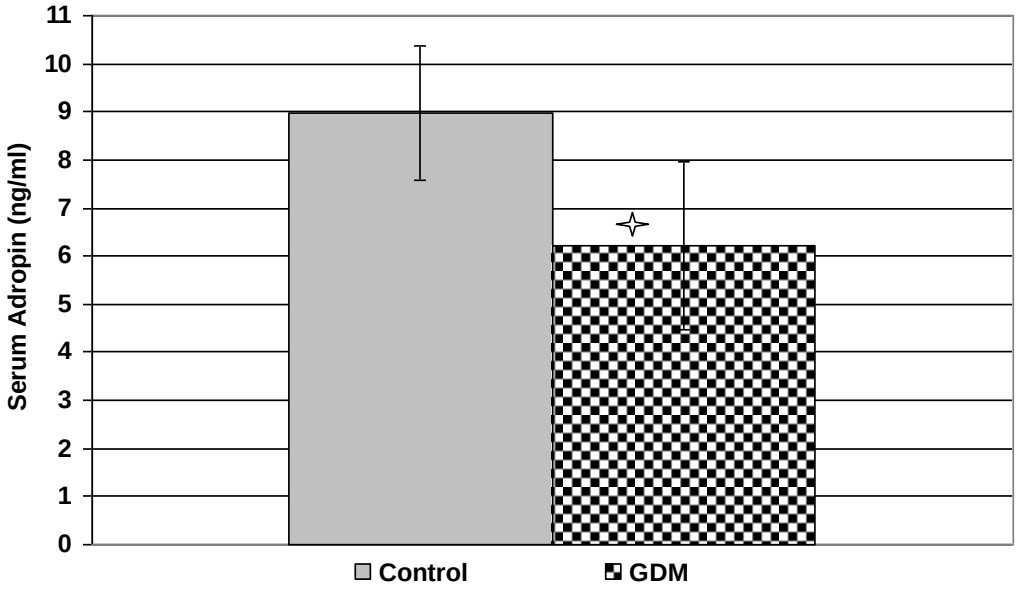

Fig. (2): Mean (±SD) Adropin estimated at 6th week GA in women of both group (✧: significant difference)

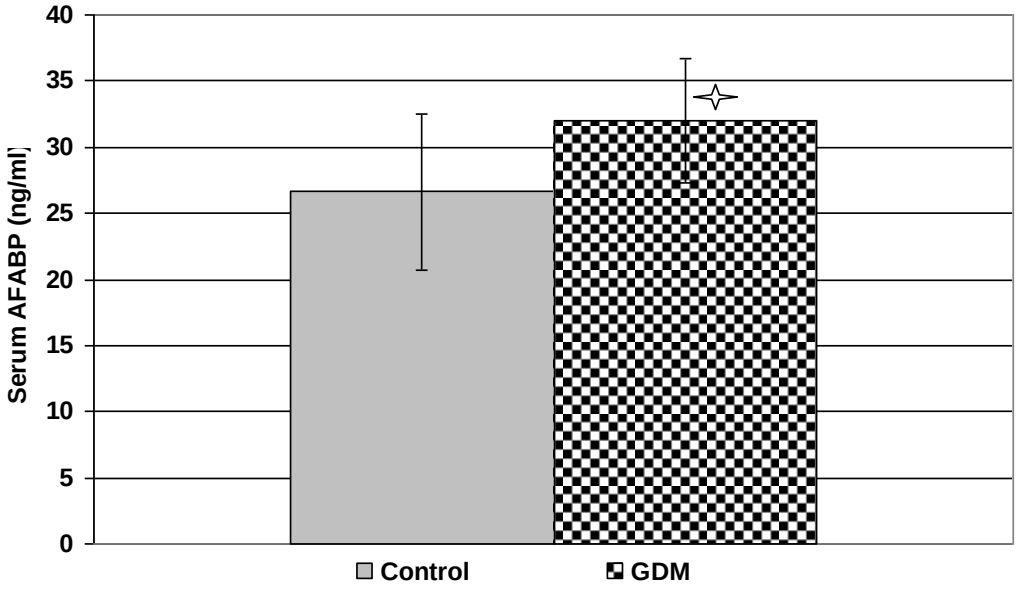

Fig. (3): Mean (±SD) AFABP estimated at 6th week GA in women of both group (✧: significant difference)

Development of DGM was significantly and negatively correlated with 6th wk serum ADR level, but positively correlated with baseline BW, 6th wk serum levels of AFABP and HOMA-IR score, in decreasing order of significance. The 6th wk serum ADR levels showed negative significant correlation with BW, BMI, 6th wk serum AFABP level and 2-hr PPBG, and with 24th wk FBG, PPBG, FSI levels and HOMA-IR score. Also, 6th wk serum AFABP levels showed positive significant correlation with age, 24th and 6th wk 2-hr PP blood glucose level, 24th wk FBG and HOMA-IR score and 6th wk and 24th wk fasting serum insulin levels (Table 3). Regression analysis of the

6th wk GA demographic and laboratory data defined serum ADR and AFABP levels as the persistently positive predictors for development of GDM at the 24th wk GA (Table 4).

Table 3:- Pearson's correlation between demographic data and laboratory findings in studied women

		GDM		6th wk ADR level		6th wk AFABP level	
		r	p	r	p	r	p
Age (years)		0.118	0.031	-0.071	>0.05	0.274	<0.001
Body weight (kg)		0.215	<0.001	-0.146	0.007	0.075	>0.05
BMI		0.150	0.006	-0.174	0.001	0.127	0.020
6th wk ADR level		-0.516	<0.001			0.276	<0.001
6th wk AFABP level		0.274	<0.001	-0.151	0.006		
6th wk GA	FBG	0.099	>0.05	0.066	>0.05	0.093	>0.05
	2-hr PP	0.045	>0.05	-0.114	0.036	0.171	0.002
	FSI	0.087	>0.05	-0.028	>0.05	0.120	0.027
	HOMA-IR	0.133	0.015	0.017	>0.05	0.079	>0.05
24th wk GA	FBG	0.486	<0.001	-0.294	0.001	0.143	0.009
	2-hr PP	0.837	<0.001	-0.444	<0.001	0.253	<0.001
	FSI	0.391	<0.001	-0.226	<0.001	0.114	0.038
	HOMA-IR	0.477	<0.001	-0.272	<0.001	0.134	0.014

Data are presented as mean±SD, numbers & percentages; GA: Gestational age; GDM: Gestational diabetes mellitus; ADR: Adropin; AFABP; Adipocye fatty acid binding protein; FBG: Fasting blood glucose; 2-hr PP: 2-hour postprandial blood glucose; FSI: Fasting serum insulin; HOMA-IR: homeostasis model assessment of insulin resistance; p value <0.05 indicates significance

Table 4:- Regression analysis of baseline demographic data and laboratory findings in studied women as predictors for development of GDM at the 24th wk GA

	Model 1		Model 2		Model 3	
Variables	β	p	β	p	β	p
6th wk ADR level	-0.477	<0.001	-0.469	<0.001	-0.485	<0.001
6th wk AFABP level	0.218	<0.001	0.218	<0.001	0.201	<0.001
BMI	0.181	<0.001	0.199	<0.001		
6th wk FBG level	0.107	0.018				

Verification of baseline demographic and laboratory data using ROC curve analysis defined 6th wk GA high serum AFABP and insulin, BMI and 2-hr PPBG, and low serum ADR, as significant predictors for development of IR at the 24th wk GA. Regression analysis defined high serum insulin (β=0.411, p=0.0004) as positive and low serum ADR (β=-0.322, p=0.0007) as negative significant specific predictors for development of IR at the 24th wk GA.

Discussion:-

The body responses to metabolic demands of pregnancy still a challenging concern for physicians [25] and became a stressful target with the global increasing prevalence of obesity [26]. The current study assured pregnancy imposed glucogenic stresses on maternal body as evidenced by the reported high 24th wk blood glucose levels in all the study population in relation to their baseline 6th wk levels. Moreover, at 24th wk, 39 women (11.6%) had abnormal OGTT and considered as having GDM. Diagnosis of GDM relied on the results of OGTT that was interpreted according to the recommendations of the International association of diabetes and pregnancy study groups [20]; similarly multiple studies used this diagnostic policy for GDM [27, 28, 29]. The reported figure for frequency of GDM goes in hand with **Ma et al.** [30], **Karcaaltincaba et al.** [31] and **Arbib et al.** [32] who reported frequencies of 12.2%, 11.1% and 9.9%, respectively for GDM. Moreover, **Huhn et al.** [33] and **Brown & Wyckoff** [34] documented that the introduction of the IADPSG criteria resulted in an absolute increase of GDM prevalence of 8.5% [33] and a 1.03-3.78-fold rise versus baseline criteria [34].

As another evidence for the metabolic burden of pregnancy, IR defined by HOMA-IR score of >2 was detected in 77 and 106 women at the 6th and 24th wk, respectively, with an increase by 37.7%, irrespective of the development of GDM. Moreover, frequency of IR among GDM women was significantly higher than among non-GDM women.

Development of IR and DGM showed positive significant correlation with baseline maternal BMI and development of GDM showed positive significant correlation with maternal age.

These findings go in hand with **Shepherd et al.** [35] who detected reduced risk of GDM with their proposed diet and exercise interventions for pregnant women versus women received no intervention. Also, **Pan et al.** [36] reported that women with GDM were older and had higher BMI than women free of GDM and **Li et al.** [37] found the levels of FBG, PPBGG, FSI, lipid profile and HOMA-IR score were significantly higher in GDM than in normal glucose tolerance women with a positive correlation between HOMA-IR and BMI and concluded that with the increases of FBG, the progression of IR is increased and pancreatic β-cell function progressively declines. Recently, **Lindsay et al.** [38] reported a high rate of late IR among pregnant women of moderately older age and high rate of obesity.

The 6^{th} wk serum levels of ADR and AFABP levels were significantly lower and higher, respectively in GDM than non-GDM women and showed significant correlation with GDM, maternal age and BMI. ROC curve analysis defined high 6^{th} wk serum AFABP and insulin and low serum ADR levels as significant predictors for IR development at 24^{th} wk GA, however, Regression analysis defined high 6^{th} wk serum insulin as positive and low ADR levels as negative significant predictors for IR development at the 24^{th} wk GA.

These findings coincided with **Fasshauer et al.** [39] who documented that adipokines, adiponectin, leptin and AFABP seem to be the most probable candidates involved in GDM pathogenesis. Also, **Zhang et al.** [40] detected higher level of AFABP during mid- and late stages of pregnancy in GDM women and concluded that AFABP might be closely related to obesity and IR in pregnancy, and is a major risk factor for GDM. Thereafter, **Li et al.** [41] detected significantly higher expression of AFABP in serum, placenta and decidua of pregnant GDM women than in normal pregnant women.

The relation between AFABP and development of IR and GDM could be attributed to the findings provided by **Garin-Shkolnik et al.** [42] who proposed that suppression of peroxisome proliferator-activated receptor γ by FABP4 in visceral fat may explain the role of FABP4 in development of obesity-related morbidities, including IR. Thereafter, **Li et al.** [41] found serum of GDM women induced significantly increased expression of FABP4 mRNA in human pre-adipocytes and **Svensson et al.** [43] reported change of adipose tissue morphology and function during pregnancy, irrespective of glycemic status and supposed that increased fat mass and the proportion of very large adipocytes may contribute significantly to gestational IR and were strongly associated with late high HOMA-IR score.

In line with findings concerning serum ADR, **Aydin et al.** [44], **Celik et al.** [45] and **Beigi et al.** [46] detected lower ADR levels in GDM women with significant difference versus control women. Moreover, **Celik et al.** [45] detected lower ADR in cord blood independent on the maternal levels and **Beigi et al.** [46] defined a significant association between ADR levels and GDM.

On contrary to the obtained results, **Dąbrowski et al.** [47] reported significantly higher ADR concentrations in GDM patients than in control group. However, against these data **Gao et al.** [48] tried ADR treatment of diet-induced obesity with IR mice and found ADR treatment enhanced glucose tolerance, ameliorates IR and promotes preferential use of carbohydrate over fat in fuel selection and indicated a negative relationship between ADR level and high blood glucose level and IR; thus supporting the findings of the current study. Thereafter, **Tuna et al.** [15] experimentally detected high serum ADR levels among animals received calorie-restricted diet, while was significantly lower among animals maintained on normal calorie intake, thus supporting the inverse correlation between serum ADR levels and calorie intake.

Moreover, in line with the results of the current study and against that of **Dąbrowski et al.** [47] multiple clinical trials approved the negative relation between ADR serum level and blood glucose and IR whereas **Stevens et al.** [49] reported that ADR levels in humans are sensitive to dietary macronutrients, perhaps due to habitual consumption of carbohydrate-rich diets suppressing circulating ADR levels. **Zhang et al.** [50] found IR index is a negative independent risk factor of ADR levels in obese adolescents. **Yosaee et al.** [51] reported that subjects with metabolic syndrome had significantly lower ADR levels and adropin/leptin ratio and ADR levels were correlated with metabolic syndrome and hence is a potentially protective agent against its development.

Dąbrowski et al. [47] considered the high ADR levels as one of the multiple adaptive responses on adverse glucose metabolism during pregnancy; however, **Hill** [52] documented that as gestation progresses several parallel mechanisms contribute to increasing maternal β-cell mass through reactivation of β-cell proliferation and an expansion and differentiation of resident β-cell progenitors and these pathways could potentially be modulated during pregnancy to increase β-cell mass and prevent the onset of GDM.

Conclusion:-

Pregnancy induces maternal IR that was related to maternal BMI and may progress to manifest as GDM that was reported by a frequency of 11.9%. Development of GDM and IR were closely related to high serum AFABP and low serum ADR levels. High 6th wk GA serum insulin, despite of normal range FBG and low serum ADR are significant predictors for development of GDM and IR at or later than the 24th wk GA.

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