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CORRELATION OF NFκB AND ADIPONECTIN LEVELS WITH HOMA-IR IN PATIENTS WITH GESTATIONAL DIABETES MELLITUS.

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ABSTRACT

Objective: To investigate the correlation of serum Tumor Necrosis Factor α (TNF-α), and Adiponectin (ADP) with insulin resistance index (HOMA-IR) in patients with Gestational Diabetes Mellitus (GDM) and to analyze the relationship between the levels of TNF-, TNFR1 as well as ADP during pregnancy period and adverse pregnancy outcomes as well as postpartum progression to type 2 diabetes mellitus (T2DM).

Method: A total of 100 pregnant women who received regular prenatal examination in our hospital from January 2017 to September 2017 were selected as the research objects, among whom 50 patients diagnosed with gestational diabetes mellitus were set as patient group with other 50 normal pregnant women as control group. The selected patients were examined on Fasting Plasma Glucose (FPG), Fasting Serum Insulin (FINS). HOMA-IR calculated by formula, which was followed by the analysis on the correlations of HOMA-IR with various indexes, of pregnancy outcome with serum index and of postpartum progression to T2DM with serum index. Serum adiponectin and NFB was estimated by manual ELISA method.

Results: Compared with the control group, the levels of FPG, FINS, LDL, HOMA-IR were significantly increased while adiponectin in patient group. Multivariate Logistic regression analysis showed: in patient group, the levels of FPG, FINS, HbAlc, TG, HDL, LDL, TNF-a and TNFR1 showed a positive correlation with HOMA-IR. ADP showed a significant negative correlation with HOMA-IR. The adverse pregnancy outcome was positively correlated with HOMA-IR, TNF-a and TNFR1 while negatively correlated with ADP. The postpartum progression to T2DM was positively correlated with HOMA-IR while negatively correlated with ADP.

Conclusion: In patients with gestational diabetes the levels of TNF- are relatively high andADP level moderately low, which is closely related with insulin resistance.

KEYWORDS : Gestational diabetes mellitus, Adiponectin, Insulin resistance

INTRODUCTION:

With change in lifestyle and food habits, diabetes mellitus (DM) is a common prevalence in developing as well as underdeveloped nations1. Gestational diabetes mellitus (GDM) is defined as glucose intolerance of various degrees that is first detected during pregnancy2. GDM is detected through the screening of all pregnant women for clinical signs and symptoms and testing for abnormal glucose tolerance. GDM results from the same physiological and genetic abnormalities that characterize diabetes without pregnancy. Women with GDM are at high risk for having or developing diabetes when they are not pregnant. GDM provides an opportunity to study the early pathogenesis of diabetes and to develop methods to prevent the disease. In gestational diabetes, the placenta faces a variety of structural and functional changes3. The nature and extent of changes depend on variety of factors including the quality of glycemic control achieved during the different periods in placental development, the modality of treatment, and the time period of severe divergence from metabolic control of a non-diabetic environment3.

NF-κB and its target genes such as TNF- and IL-6 have been documented to cause insulin resistance. In addition NF-κB has been found to cause insulin resistance and diabetic complications .

Many other acute phase proteins and pro-inflammatory markers has been studied in correlation to GDM. However, the exact cause as well as the mechanism of insulin resistance is yet not clear. Many of the questions remain unanswered like the exact trigger for the development of GDM and why does it not happen in all individuals? Our study explores the interplay of multiple molecules in causation of GDM.

METHODOLOGY:

We used a prospective, case-control study design to compare maternal plasma adiponectin, IL-6, TNF-α and NF-κB concentrations in 50 cases with 50 controls at 20-24 weeks of gestation. Antenatal women with FBG 140 mg/dl were taken as cases. All known cases of diabetes mellitus or any other endocrinal disorders were excluded from the study. Pregnant females without deranged blood glucose levels and normal GCT were taken as controls. The place of study was department of Obstetrics and Gynaecology, Lady Harding Medical College and Smt. Sucheta Kriplani Hospital. Fasting samples were collected in plain and EDTA vacutainers. Samples from EDTA vacutainers were to measure fasting blood glucose immediately and from plain vacutainers, serum was separated and stored at -20C for further analysis. Serum adiponectin and NF-κB levels were measured using manual ELISA method. For adiponectin Human ELISA Adiponectin kit by BioVendor was used. For NFκB Human Nuclear Factor- kappa B ELISA kit by SiNCERE was used. HOMA-IR was calculated using the formula:

HOMA-IR = fasting insulin(microU/L) × fasting glucose (nmol/L) / 22.5.

RESULTS:

Following the inclusion criteria 50 cases and 50 controls were enrolled in the study. The mean serum levels of adiponectin is 1.37g/ml in cases and 1.60g/ml in controls. Mean serum levels of NFκB were 0.13 ng/ml in cases and 0.12 ng/ml and controls. Difference is found to be statistically significant in both adiponectin and NFκB. Receiver operating characteristic (ROC) curve analysis was done for adiponectin and NFκB. Adiponectin was found to have area under curve (AUC) as 0.698 with sensitivity and specificity of 64% and 66 %respectively. However, NFB had AUC of 0.681 with sensitivity and specificity of 84% and 46 %respectively.

Table 1: Distribution of adiponectin and NFκB among cases and controls

PARAMETER	GROUP				P VALUE	ODD'S RATIO
	CASES (50)		CONTROL (50)			
	MEAN	S.D.	MEAN	S.D.		
NFκB (ng/ml)	0.13	0.03	0.12	0.03	0.001**	1.02
TNF-α	58.31	14.53	44.53	9.42	0.0001**	1.103

**p<0.001= highly significant *p<0.05=significant

The table illustrates the distribution of adiponectin and NFκB levels among cases and controls. The comparison of both the parameters between the groups was found to be highly significant.

Table 2. Correlation between NFκB and TNF-a with HOMA-IR

STUDY GROUPS	PARAMETERS	HOMA-IR	
		Pearson's correlation	p value
	NFκB (ng/ml)	-0.059	0.686
	TNF-α	0.118	0.414

**p<0.001= highly significant *p<0.05=significant

The table illustrates the correlation of calculated HOMA-IR with NFκB and adiponectin. It was not found to be significant.

Table 4. ROC analysis of NFκB and adiponectin

Parameters	Area under curve	95% Confidence interval	Sensitivity	Specificity	Negative predictive value
NF-κB	0.681	0.580-0.770	84%	46%	96.3%
Adiponectin	0.698	0.599-0.786	64%	66%	94.3%

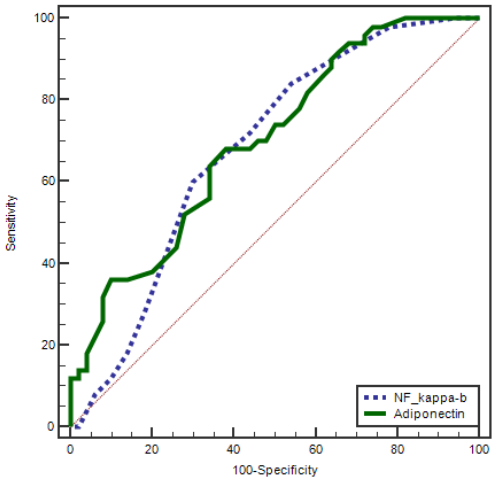

Figure 1: Area under curve of ROC analysis of NFκB and adiponectin

STATISTICAL ANALYSIS

The data was analyzed by appropriate statistical methods using 20 version of Statistical Package for Social Sciences. Quantitative data was expressed as mean±SD of mean and qualitative data as percentages. The p value of 0.05 was considered as significant. All the data were compared by using student t-test.

DISCUSSION

The diabetic environment is basically a network of substances (hormones, nutrients, cytokines) with altered concentrations[1]. The abnormal maternal metabolic environment may generate stimuli within the adipose tissue and the placental cells resulting in the increased production of inflammatory cytokines whose expression is minimal in normal pregnancy[4]. An important hypothesis is that changes in circulating TNF-α and adiponectin link inflammation to metabolic changes by increasing insulin resistance in the mother[4].

Similarly, the fetal environment is also changed in diabetes. The elevated levels of insulin and other cytokines have been documented[5]. The major mechanism of gestational diabetes mellitus is interplay between pro-inflammatory and anti-inflammatory molecules. NF- kappa B, TNF-α and IL-6 are considered as pro- inflammatory molecules and adiponectin is considered as anti-inflammatory molecule.

NFκB were found to be highly significant in cases and controls (p value=0.001). NFκB proteins comprise a family of structurally related eukaryotic transcription factors that are involved in control of normal cellular processes such as immune and inflammatory responses, developmental processes, cellular growth and apoptosis. NFκB regulates host inflammatory and immune responses and cellular growth properties by increasing the expression of specific cellular genes. NF-κB is a key regulator of inflammation and plays a key role in various inflammatory diseases[6]. In support of this Gerondakis et al proposed that NFκB regulates host inflammatory and immune responses and cellular growth properties by increasing expression of specific cellular genes encoding for cytokines, major histocompatibility complex (MHC), proteins for antigen presentation and receptors for neutrophil adhesion and migration[7]. NF-κB is activated by inflammatory mediators such as TNF-α which can phosphorylate IRS-1 on serine residues and inhibit its function which can explain its effect on insulin resiatance. Other inhibitory kinases such as Mtor, p70S6K1 and protein kinases C are also increased in insulin resistance states by conditions of nutrient excess.

Globular adiponectin is a cleavage product of adiponectin. In a study conducted by Tomizawa A et al, it was found that globular adiponectin strongly activates, thereby inducing the expression of various pro-inflammatory and adhesion molecule genes and requires a longer incubation period to show inhibition against cytokine induced NF-κB activation [8]. Xuemei Wang et al. found in a study that exogenous adiponectin decreases inflammation of adiponectin by inhibiting the expression of NF-κB nuclear protein and pro-inflammatory factors regulated by NF-κB [9]. In a study by Noriyuki Ouch et al, it was seen that adiponectin specifically suppressed TNF-α induced IB--B activation through a cAMP-dependent pathway in human aortic endothelial cells (HAECs) which further shows that adiponectin acts as an endogenous modulator for endothelial inflammatory response [10].

This study aimed at estimating the concentrations of adiponectin and NF-κB. Significant increase in adiponectin and decrease in NF-κB was observed in cases compared to controls.

Adiponectin levels were found to be highly significant in cases and controls (p value = 0.0001). Adiponectin, a protein secreted exclusively by adipocytes, has emerged as an important potential mediator of the insulin resistance that is so characteristic of obesity, type 2 diabetes, and atherosclerotic cardiovascular disease[10]. Consistent with the established inverse relationship between plasma adiponectin concentration and insulin resistance, hypoadiponectinemia has been documented[11]. Moreover, in the Indian population, low baseline adiponectin concentration predicts the subsequent development of insulin resistance, whereas conversely, elevated baseline adiponectin levels have been shown to be protective against the future development of type 2 diabetes[11]. Taken together, these observations suggest that hypoadiponectinemia may be an important factor in the development of insulin resistance and the pathophysiology of type 2 diabetes[11]. Gestational diabetes mellitus (GDM) identifies a population of young women at high risk of developing type 2 diabetes, representing an early stage in the natural history of the disease[12].

The present study investigated the relationship between adiponectin and glucose tolerance in pregnancy. Our objective was to determine the extent to which low maternal plasma adiponectin is predictive of gestational diabetes mellitus (GDM). Our findings are consistent with other reports suggesting an association between hypoadiponectinemia and risk of type 2 diabetes[13]. Our findings extend the literature to include GDM.

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