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Original Article

Biochemistry Section

Normal Pregnancy- A State of Insulin Resistance

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ABSTRACT

Background: Purpose of insulin resistance (IR) adapted by mother is to deliver enough quantity of nutrients to the growing fetus. Many maternal hormones and factors play role in causation of IR during pregnancy.

Aim: The study aims at evaluating IR at different trimesters of pregnancy.

Materials and Methods: Pregnant women at 1st, 2nd and 3rd trimester were grouped into groups I, II and III respectively (n=20 in each group). Healthy non-pregnant women were taken as controls (n=30). Fasting plasma glucose (FPG) and fasting serum insulin (FSI) were measured and IR indices such as fasting glucose to insulin ratio (FGIR), quantitative insulin sensitivity check index (QUICKI), log FSI and log HOMA1-IR were calculated. The student's t-test and one way Analysis of variance (ANOVA) were used for data analysis.

Results: The mean FSI, log FSI and log HOMA 1-IR were significantly higher in 2nd and 3rd trimesters while QUICKI was significantly lower in 2nd and 3rd trimesters of pregnancy when compared with controls. Also, mean FGIR was found to be significantly lower in 3rd trimester when compared with controls.

Conclusion: As pregnancy advances, IR increases. Increased IR is associated with poor maternal and fetal outcome. Screening of all pregnancy for IR and early intervention may help to reduce the associated complications.

Keywords: Gestation, Insulin insensitivity, Log HOMA 1-IR, Trimester, QUICKI

INTRODUCTION

Pregnancy can be associated with many metabolic, biochemical, physiological, hematological and immunological changes. With no complications at full term, these changes are reversible after delivery [1]. Healthy women pregnancy can be associated with resistance to the action of insulin on glucose uptake and utilization [2]. IR is defined as decreased ability of target tissues such as liver, adipose tissue and muscle to respond to normal circulating concentrations of insulin [3]. It is reported that pregnant women require an additional energy of 300 kcal/day over routine energy intake [2] while the average glucose utilized by a growing fetus at the 3rd trimester reaches approximately to 33 μmol/kg/min [4]. Maternal IR leads to more use of fats than carbohydrates for energy by mother and spares carbohydrates for fetus. Thus, the development of IR serves as a physiological adaptation of the mother to ensure adequate carbohydrate supply for the rapidly growing fetus [4].

As the pregnancy advances to third trimester, insulin sensitivity may gradually decline to 50% of the normal expected value [5]. This decline is reported to be mediated by a number of factors such as increase in the levels of estrogen, progesterone, human placental lactogen (hPL), among other factors [6].

Normally, insulin binding to insulin receptor causes phosphorylation of β-subunit of receptor and it further leads to phosphorylation of Insulin Receptor Substrate-I (IRS-I) at tyrosine residue which act as docking site for further signal transduction molecules [7].

Progesterone suppresses the phosphoinositol 3-kinase-mediated pathway by reducing the expression of IRS-1. Gradually increasing progesterone concentration with advancement of normal pregnancy is associated with increased inhibition insulin-induced GLUT4 translocation and glucose uptake [8]. Estrogen concentration is also high in pregnancy. 17β-estradiol diminishes insulin sensitivity at high concentrations [9].

hPL has both insulin-like and anti-insulin effects. In vitro, it has been shown to increase lipolysis and free fatty acids (FFAs) in adipocytes. Increased hPL level in pregnancy is found to increase glucose uptake, oxidation, and incorporation of glucose into glycogen, which may favor glycogen storage in the mother [10].

Human placental growth hormone (hPGH), a product of the human growth hormone variant gene, is not regulated by growth hormone-releasing hormone (GH-RH) and is secreted tonically rather than in a pulsatile fashion. hPGH has the same affinity for the growth hormone receptor as pituitary GH. The hPGH may also have the same diabetogenic effects as pituitary growth hormone such as hyperinsulinemia, decreased insulin-stimulated glucose uptake and glycogen synthesis, and impairment of the ability of insulin to suppress hepatic gluconeogenesis [10].

Other factors such as increased levels of serum cortisol, Tumor necrosis factor α (TNFα), ILs etc., can interrupt the insulin signaling pathway and can lead to IR during normal pregnancy [11].

Available literature [12-14] suggests that there is a rise in IR in 3rd trimester of pregnancy. However literature is less on the 1st and 2nd trimester. So the present study was undertaken to evaluate the status of IR in different phases of normal pregnancy.

MATERIALS AND METHODS

Case control study was carried out in antenatal clinic of tertiary care hospital attached teaching institute. Those participating as controls in the experiment were taken from households surrounding the hospitals. Approval from institutional ethical committee was taken in the year 2010 and each subject gave an informed consent for participation in study. A proforma was used to collect relevant patient details.

Cases: Sixty pregnant women of age between 18 and 45 years were taken as cases and divided into three subgroups as per trimester.

Group I: 20 healthy women in 1st trimester of pregnancy.

Group II: 20 healthy women in 2nd trimester of pregnancy.

Group III: 20 healthy women in 3rd trimester of pregnancy.

Controls: Thirty age matched healthy non-pregnant women without any significant illness were taken as controls.

The women with history of hypertension, diabetes mellitus, insulin therapy, hypoglycemic or hypolipidemic drugs intake, smoking, alcoholism, liver, cardiac or renal diseases or any other major illness were excluded from the study. Women with molar pregnancy, twins or multiple fetuses were also excluded from the study.

		1st Trimester (Group I) (n=20)	2nd Trimester (Group II) (n=20)	3rd Trimester (Group III) (n=20)	Controls (n=30)
POG (weeks)	Mean±SD	10.4±0.82	16.35±0.93	34.35±2.76	-
Age (years)	Mean±SD	22.6±2.60	22.45±1.82	22.05±3.52	22.83±2.98
	t test (p)*	0.771	0.576	0.283	-
Fasting Plasma Glucose (mg/dl)	Mean±SD	81.59±7.31	82.26±9.65	79.52±13.41	81.34±5.31
	t test (p)*	0.898	0.704	0.569	-
Fasting S. Insulin (µIU/ml)	Mean±SD	6.50±1.56	7.81±2.41	9.68±2.76	6.01±1.95
	t test (p)*	0.336	< 0.05†	< 0.001‡	-
FGIR	Mean±SD	13.25±3.27	11.98±5.65	8.95±3.52	14.97±5.14
	t test (p)*	0.155	0.065	< 0.001‡	-
QUICKI	Mean±SD	0.369±0.016	0.361±0.025	0.350±0.021	0.376±0.02
	t test (p)*	0.229	< 0.05†	< 0.001‡	-
log (Fasting S. Insulin)	Mean±SD	0.8±0.11	0.87±0.16	0.97±0.14	0.76±0.14
	t test (p)*	0.225	< 0.05†	< 0.001‡	-
log (HOMA 1-IR)	Mean±SD	0.1±0.12	0.17±0.18	0.26±0.16	0.06±0.14
		0.249	< 0.05†	< 0.001‡	-

[Table/Fig-1]: Comparison of parameters among study groups., ** p-value of unpaired student's t-test between respective case groups and controls., POG – period of gestation; HOMA1-IR – Homeostatic Model Assessment for IR., † Significant, ‡ Highly Significant*

Sample collection

Five ml of venous blood was collected 12 hours post overnight fasting. Of this, 2 ml was placed in fluoride EDTA vial for plasma collection while the remaining 3 ml was placed in a plain vial for the collection of serum. Plasma and serum were separated by centrifugation and used for the estimation of plasma glucose and serum insulin concentration.

Sample analysis

Concentration of FPG was determined by using analytical kit from ERBA Diagnostics Mannheim GmbH in semi-autoanaylzer (CHEM-5 plus V2, Erba Mannheim) according to glucose oxidase and peroxidise (GOD-POD) method [15]. FSI concentration was estimated using Human insulin chemiluminescence immunoassay (CLIA) [16] kit from Acculite-Monobind in Lumax CLIA microplate reader.

IR indices which include FGIR, QUICKI, log FSI and log HOMA 1-IR were calculated from the values of FPG and FSI concentration based on the methods [17] using the formulas:

QUICKI = 1/ (log FPG in mg/dl + log FSI in µIU/ml)

Log HOMA 1-IR = log { (FPG in mg/dl x FSI in µIU/ml) / 405}

STATISTICAL ANALYSIS

Values are presented as mean ± SD and the statistical analysis was done using SPSS 17.0 software. Student's unpaired t-test was used for comparison between two groups while one way ANOVA test was used to compare all the groups simultaneously.

RESULTS

[Table/Fig-1] shows that the mean levels of FSI, log FSI and log HOMA1-IR were significantly higher in 2nd and 3rd trimesters of pregnancy when compared with healthy non-pregnant controls. The mean level of QUICKI was significantly lower in 2nd and 3rd trimesters of pregnancy when compared with controls. The mean level of FGIR was significantly lower in 3rd trimester when compared with controls but in the 2nd trimester, the decrease observed was not statistically significant. There was no significant difference in all the parameters in 1st trimester when compared with controls. No significant difference was found in FBG in all the study groups.

	F value	p value
FBG (mg/dl)	0.335	0.800
Fasting S.Insulin (µIU/ml)	12.541	< 0.001*
FGIR	7.127	< 0.001*
QUICKI	6.218	< 0.001*
log (Fasting S.Insulin)	9.909	< 0.001*
log (HOMA 1-IR)	7.189	< 0.001*

[Table/Fig-2]: One way ANOVA test for parameters among study groups
** Highly Significant*

One way ANOVA test in [Table/Fig-2] shows significant difference in levels of all the parameters among study groups except FBG which was non-significant.

DISCUSSION

IR is the condition in which there is a decreased in the action of insulin on body tissue at normal concentration of plasma insulin. This can be as a result of a number of factors such as defective molecular structure of insulin, defective receptor functioning or defective signal transduction pathway. In the acquired condition, the defect is either in receptor affinity for insulin or as a result of some interruption in signalling pathway of insulin down the receptor [18]. To compensate this IR , there is increased production of insulin from beta cells of islet of langerhans leading to hyperinsulinemia. Insulin producing capacity of pancreatic beta cells is not infinite. Gradually beta cell functioning also declines leading to a reduction in insulin production and the condition progresses to glucose intolerance and subsequently, to diabetes mellitus [18].

Many researchers [19,20] have noted that age is one factor affecting insulin sensitivity and that with an increase in age, there is a progressive increase in IR . In the present study, there was no significant difference in age of mother in all the case groups (p>0.05). Age matched cases and controls were taken in order to remove one of the main confounding factor.

The present study looks at the status of IR in different trimesters of pregnancy. The average period of gestation in each groups were 10.4±0.82 weeks, 16.35±0.93 weeks and 34.35±2.76 weeks for groups I, II and III respectively. Diseases such as hypertension, diabetes, etc are associated with presence of IR [21]. Such subjects were excluded from study to remove the bias. No significant difference in FPG concentration was seen amongst all the study groups. Also, FSI concentration were significantly lower in controls when compared to cases in groups II and III. Non-significant but higher levels of FSI were noted in group I when compared with controls.

Serum insulin levels were significantly higher in 3rd trimester when compared with 2nd trimester and those of the 2nd trimester were significantly higher when compared with 1st trimester (p<0.05). These findings therefore suggest that there is progressive rise in insulin secretion as the pregnancy advances, indirectly signifying an increase in IR as the pregnancy advances. Similar finding had been reported by Stanley K et al., [12]. Catalano P et al., found significant 65% increase in both basal insulin and C-peptide concentrations in all subjects with advancing gestation [22]. In the present study, it was observed that the mean fasting insulin levels were 61% higher in 3rd trimester and 29% higher in 2nd trimester of pregnancy when compared with non-pregnant controls.

The mean plasma glucose concentrations in all study groups are not significantly different but there is a significant decrease in FGIR in 3rd trimester when compared with controls. It shows increase in insulin requirement to maintain the similar plasma glucose concentration in women of group III [23]. In the study done by Buchanan T et al., researchers found that there is gradual decline in insulin sensitivity as the pregnancy advances. So the amount of insulin produced in response to glucose concentration also gradually increases [24]. In normal pregnancy, there is an approximate 50% decrease in insulin-

mediated glucose disposal and a 200% to 250% increase in insulin secretion to maintain euglycemia in the mother [25].

QUICKI has proved to be a versatile tool in measuring IR. It has shown linear relation with other gold standard technique such as euglycemic hyperinsulinemic clamp testing, frequently sampled intravenous glucose tolerance test (FSIVGTT) [17,23,26]. In this study, significantly lower levels of QUICKI were obtained in 2nd and 3rd trimester as compared with non-pregnant controls thus suggesting the presence of IR .

Log FSI and log HOMA1-IR can also be used to evaluate the IR [17]. Evaluation with these markers has also shown presence of IR in women at 2nd and 3rd trimester of pregnancy when compared with controls.

Women with increased IR are more prone to develop preeclampsia & gestational diabetes. Preeclampsia is associated with increased expression of TNFα and other inflammatory marker which causes IR [27-28]. Increased IR leads to dyslipidemia [29] that can worsen the placental ischemia leading to vicious cycle of ischemia-inflammation-IR-dyslipidemia-ischemia. In prospective studies it is proven that most women, who develop gestational diabetes, have increased IR caused by alteration in insulin signaling pathway, abnormal subcellular localization of GLUT4 transporters, increased expression of the membrane glycoprotein PC-1 or reduced insulin-mediated glucose transport [30].

Increased IR is also associated with occurrence of premature labour, antepartum or postpartum hemorrhage and fetal complications like intrauterine growth retardation or fetal overgrowth and prematurity. Presence of IR also increases risk of development of metabolic syndrome, diabetes mellitus, hypertension, hyperlipidemia, and cardiovascular disorders later in life [21,28].

Screening for IR can be advised to all pregnant women. Insulin sensitivity can be improved in these women by modifying diet, lifestyle, amount and type of physical activity. Balanced diet providing required quantity of macro and micro nutrients with good amount of dietary fibers can be prescribed [31]. Avoidance of sedentary lifestyle and increasing amount of activity should be advised before, during and after pregnancy. Mild exercises such as walking, climbing stairs etc can be advised for women with increased IR during pregnancy [32]. Such intervention should be done at an early stage well before the IR related complication develops.

CONCLUSION

As pregnancy advances, IR increases. Increased IR is associated with poor maternal and fetal outcome. Screening of all pregnancy for IR and early intervention may help to reduce the associated complications.

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