Interleukin-10, Mean Arterial Blood Pressure and Insulin Resistance in Normal Pregnancy

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Abstract: Problem statement: The purpose of this study was to investigate whether serum of Interleukin-10 (IL-10) Concentration change during normal pregnancy and, if so, to relate these changes corresponding alterations in insulin resistance and blood pressure. **Approach:** This cross sectional study was carried out on 86 healthy pregnant women including 26, 23 and 37 individuals in the 1st, 2nd and 3rd trimesters, respectively and in 21 healthy non pregnant women. Serum IL-10 concentration was measured by Enzyme Linked Immunosorbent Assay (ELISA) method. Insulin resistance value was calculated using the homeostasis model assessment, HOMA-IR. **Results:** Serum IL-10 concentration was found to be significantly higher in patients in all gestational age as compared non pregnant women. Il-10 level was significantly increased with increase in gestational age. Pregnant women exhibited higher score of HOMA IR compared non pregnant women, but there were no difference in this score between pregnant subjects in different gestational age. There were not significant correlation between IL-10 level with IR and blood pressure. **Conclusion:** The results of the study show maternal IL-10 level increase with further increase in gestational age and there is no significant correlation between IL-10 level with Mean Arterial blood Pressure (MAP) and IR.

Key words: Enzyme Linked Immunosorbent Assay (ELISA), Mean Arterial blood Pressure (MAP), changes corresponding alterations, between pregnant subjects, Insulin Resistance (IR)

INTRODUCTION

Normal pregnancy is associated to change in proinflammatory and anti-inflammatory cytokines (Davila *et al.*, 2011; Russell *et al.*, 1997; Holmes *et al.*, 2003). Cytokine balance is important for normal processes of pregnancy and onset of labor (Arntzen *et al.*, 1997).

Interleukin-10 (IL-10) is an anti-inflammatory and immunomodulatory Th2 cytokines, that produce by a range of maternal and fetal cells (Vigano *et al.*, 2001; Hennessy *et al.*, 2003). IL-10 has a critical role in maintaining the balance of anti-inflammatory and proinflammatory milieu at the maternal-fetal interface (Kalkunte *et al.*, 2011). Metabolic Syndrome (MS) is a collection of risk factors including insulin resistance, central obesity, hypertension and dyslipidemia and itself is

a risk factor for coronary artery disease (Mahajan *et al.*, 2010). Pregnancy is associated to glucose metabolism disorders, Insulin Resistance (IR) (Hadden and McLaughlin, 2009; Johnson, 2008) and oxidative stress (Idonije *et al.*, 2011). The Insulin Resistance syndrome is comprised of a cluster of metabolic abnormalities that confer increased risk of diabetes, hypertension and cardiovascular disease (Grinspoon, 2006). Insulin Resistance (IR) may facilitate supply of appropriate nutrients particularly of glucose to fetus for fetal growth and metabolism. The mechanism responsible for insulin resistance has not been clearly stated. Recent researches have been shown IL-10 increase insulin sensitivity (Hong *et al.*, 2009; Ye *et al.*, 2006; Holmes *et al.*, 2003; Straczkowski *et al.*, 2005). Insulin sensitivity changes from an enhance state during early pregnancy to an insulin resistant state in late pregnancy (Kirwan *et al.*, 2002).

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Insulin resistance may facilitate supply of appropriate nutrients particularly of glucose to fetus for fetal growth and metabolism. The mechanism responsible for insulin resistance has not been clearly stated. Insulin resistance can be affected by Nitric Oxide (NO) (McGrowder and Brown, 2007). Recent researches have been shown adipokinins include leptin (Soheilykhah *et al*., 2011; Mohiti *et al*., 2009; Assal *et al*., 2007; EL-Ghaffar and EL-Said, 2006), resistin (Caja *et al*., 2005) and cytokines include TNf-α (Kirwan *et al*., 2002), IL-6 and IL-1β (Nov *et al*., 2010) play an important role in insulin resistance. Insulin sensitivity changes from an enhanced state during early pregnancy to an insulin resistant state in late pregnancy. Insulin sensitivity changes from an enhanced state during early pregnancy to an insulin resistant state in late pregnancy (Kirwan *et al*., 2002).

Therefore, it is inspected, subsequent to change in insulin resistance during normal pregnancy, IL-10 level change too. In a recently published study, it has been reported that in normal pregnant women both maternal IL-6 (Jahromi *et al*., 2011a) and TNF-α cytokines are increased (Zareian *et al*., 2010; Elsamanoudy and Abbas, 2010) and IL-1β is decreased (Jahromi *et al*., 2011b), But it was not find any correlation between IR with IL-6 and TNF-α. (Jahromi *et al*., 2011a) and between IR with IL-1β (Jahromi *et al*., 2011b). Resistin is an insulin-sensitizing adipokine that can be implicated in endogenous glucose regulation (Mohiti *et al*., 2009).

However, at the time of this study data concerning the pattern of IL-10 secretion in normal pregnancies are still limited and partially contradictory (Russell *et al*., 1997) and its correlation with insulin resistance during normal pregnancy have not been studied. Therefore, the aim of this study was examine either serum IL-10 concentration change during normal pregnancy and if so, to relate those changes corresponding alterations in insulin resistance, mean arterial pressure and body mass index.

MATERIALS AND METHODS

This study was conducted at the department of Obstetrics and Gynecology of Honary Clinic, Jahrom, Iran. Subjects were 86 pregnant women with different gestational ages (first trimester: 26 sec trimester: 23, third trimester: 37 and 21 non pregnant women similar in age and Body Mass Index (BMI). All subjects met the following criteria: no history of pre- gestational diabetes; no history of liver, respiratory, thyroid or other illness and any current infectious condition. They were not on any drug therapy.

Body Mass Index (BMI, Kg m^{-2}) was calculated according to the maternal height and pre-pregnancy weight. Serum samples were analysed for concentrations of IL-10, insulin and glucose. Blood sugar was measured by Glucose Oxidase/Peroxidase (GOD-POD) method. Serum insulin was determined by ELISA (Diaplus; based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. During incubation insulin in the sample reacts with enzyme (HRP) conjugated anti-insulin antibody and anti-insulin antibody bound to micro-titration well. A sample washing step removed unbound enzyme labeled antibody. In the insulin ELISA, the bound HRP complex is detected by reaction with TMB substrate. The reaction is stopped by adding acid to give a colorimetric endpoint that is read using ELISA reader). Serum IL-10was measured by ELISA (Bendermed, Austria: Cat. No. BMS224INSTCE). An anti-human IL-10 coating antibody is adsorbed onto microwells. Human IL-10 present in the sample or standard binds to antibodies adsorbed to the microwells; a biotin-conjugated monoclonal anti-human IL-10 antibody binds to human IL-10 captured by the first antibody. Streptavidin-HRP binds to the biotin conjugated anti-human IL-10. Following incubation unbound biotin conjugated anti human IL-10 and Streptavidin-HRP is removed during a wash step and substrate solution reactive with HRP is added to the wells. A colored product is formed in proportion to the amount of soluble human IL-10 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from seven human IL-10 standard dilutions and human IL-10 sample concentration determined.

Mean Arterial Pressure (MAP) was calculated by formula as (Systolic pressure mmHg) + (Diastolic pressure^2 mmHg)/3.

Insulin resistance value were calculated using the homeostasis model assessment, HOMA- IR ,as (fasting insulin IU/L)×(fasting glucose mmol L^{-1}) /22.5 as previously reported by (Matthews *et al*., 1985).

Statistical analysis: All results are displayed as Mean ± SD (standard deviation of mean) and Min-Max. Resistin, Insulin Resistance (IR), BMI and body weight data were analyzed with One Way Analysis of Variance (ANOVA). Serum IL-1β and insulin concentration data were analyzed with non-parametric kruskal-wallis test (Vargha, 1998) followed by Mann Whitney U-test

(Rosner and Grove, 1999). Correlations were calculated using liner correlation (pearson). Statistical analysis was performed using SPSS 11 for windows. $p < 0.05$ was considered statistically significant for all analysis.

RESULTS

A total of 86 pregnant women and 21 non pregnant subjects participated in the study. Clinical and laboratory characteristics of pregnant women and controls are summarized in Table 1. BMI was found to be significantly increased in the 3rd trimester as compared with controls and women with 1st trimester of pregnancy Fig. 1a, Table 2. Pregnant women in the second and third trimester of pregnancy had significantly higher systolic pressure than non pregnant women Fig. 1b. IL-10 level was also significantly higher in patients in all gestational age as compared non pregnant women Fig. 1c. However, during pregnancy IL-10 level were significantly increased with increase in gestational age Table 2 Pregnant women exhibited higher score of HOMA IR compared non pregnant women, but there were no difference in this score between pregnant subjects in different gestational age Table 1 and 2. There were significant correlation between gestational age with BMI ($r = 0.28$, $p = 0.01$), diastolic pressure ($r = 0.28$, $p = 0.01$) and IL-10 level ($r = 0.56$, $p < 0.01$). There was not significant correlation between gestational age and IR. IL-10 level also correlates with BM ($r = 0.299$, $p = 0.004$).

There were not significant correlation between IL-10 level with IR, Systolic pressure, diastolic pressure, MAP and fasting insulin level.

Table 1: Clinical and laboratory characteristic of patients and control

	Pregnant women	Control
Number of case	86	21.0
Age (year)	26.4±4.1	27.2±5.6
Gestational age (week)	23.9±9.8	
HT (m)	1.6±0.06	1.58±0.07
WT (Kg)	64.99±11.5b	58.6±6.4
BMI (Kg m^{-2})	25.4±3.7a	23.4±3
SBP (mmHg)	117±7.8 b	110.7±5.9
DBP (mmHg)	72.8±6.9	70.9±11.4
MAP (mmHg)	86.5±6.70	85.1±8.4
BGL (mg 100^{-1})	81.5 ±8.7	80.2±8.7
Insulin (μLU mL^{-1})	10.9±6.00	8.7±1.9
IL-10 (pg mL^{-1})	0.851±0.702c	0.119±0.087
IR	2.1±0.7a	1.7±0.4

BMI: Body Max Index, HT: Height of Women, WT: Weight of body SBP: Systolic blood pressure DBP: Diastolic blood pressure MAP: mean arterial pressure BGL: Blood glucose level IL-10: Interleukin-10 IR: Insulin resistance [a] $p < 0.05$ (control) [b] $p < 0.01$ (control)[c] $p < 0.0001$ (control)
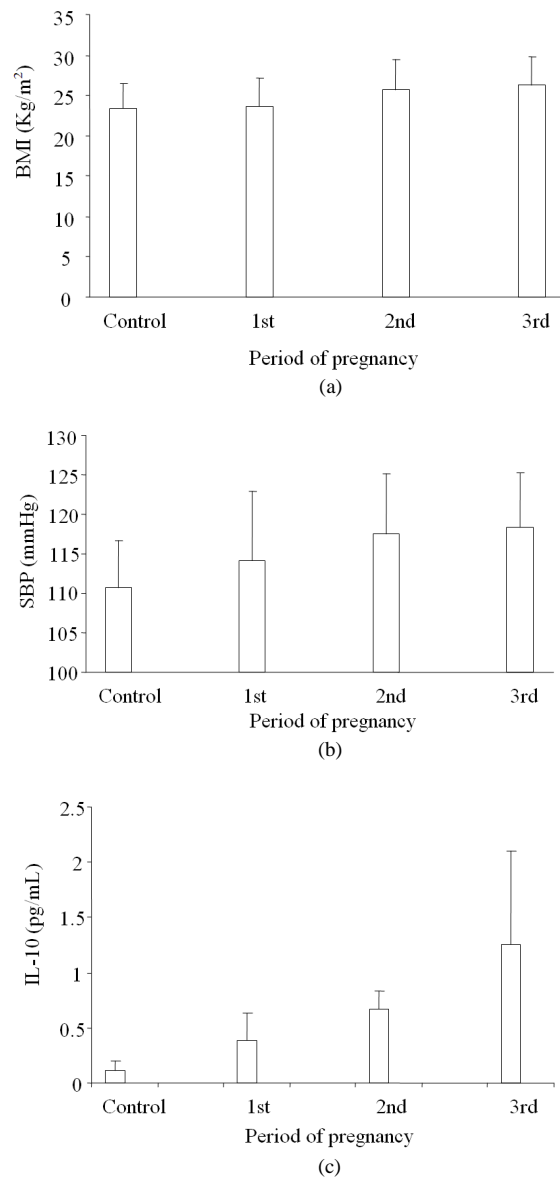

Fig. 1: (A) BMI in patients in different trimesters of pregnancy and controls. BMI were significantly higher in 3rd trimesters compared with the control (b: $p < 0.01$). (B) Systolic blood pressure in different trimesters of pregnancy and controls. Systolic blood pressure were significantly higher in the 2nd and 3rd trimesters as compared with control group (a: $p < 0.05$, b: $p < 0.01$). (C) Serum IL-10 level in different trimesters of pregnancy and control. Serum IL-10 level significantly higher in the 1st, 2nd and 3rd trimester as compared control (a: $p < 0.05$, b: $p < 0.01$, c: $p < 0.001$)

Table 2: Clinical and laboratory characteristics of pregnant women with different gestational age

	1st trimester Mean ± SD	2nd trimester Mean ± SD	3rd trimester Mean ± SD
Number of cases	26	23	37.00000
Age (year)	25.5±4.1000	24.9±2.3000	27.6±4.7000
GA (week)	11.2±1.6000	22.2±2.7000	32.9±4.3000
WT (Kg)	59.2±10.200	65.7±12.600	68.2±10.6b0
HT (m)	1.58±0.0600	1.58±0.0600	1.6±0.0700
SBP (mmHg)	114.2±8.700	117.5±7.700	118.3±7a000
DBP (mmHg)	70.5±6.6000	71.7±7.1000	74.7±6.5000
MAP (mmHg)	85.1±6.9000	86.7±6.9000	89.4±6.2000
BMI (Kg m^{-2})	23.6±3.6000	25.7±3.7000	26.2±3.6b00
BGL (mg 100^{-1})	78.6±6.7000	79.4±6.7000	84.1±21.200
Insulin (µL mL^{-1})	10.5±3.4000	10.6±3.8000	10.05±3.900
IL-10 (pg mL^{-1})	0.383±0.247	0.672±0.156	1.257±0.847b
IR	2±0.600000	2.1±0.60000	2.1±0.80000

BMI: Body mass index GA: Gestational age WT: Wight of body HT: Height SBP: Systolic blood pressure DBP: Diastolic blood pressure MAP: Mean Arterial Pressure BGL: Blood Glucose Level IR: Insulin Resistance IL-10: Interleukin-10 [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$ (Significantly different from pregnant women in 1st trimester)

DISCUSSION

Glucose metabolism disorder is a common complication during pregnancy and its pathology is associated with IR and deficiency of insulin secretion2.In this study, insulin resistance significantly was higher in total group of healthy pregnant than in non pregnant women. In spite of previous report we did not found correlation between gestational age and insulin resistance. Previous researches have shown insulin resistance was significantly increased in late pregnancy compared with either control or early pregnancy (Kirwan *et al*., 2002; Melczer *et al*., 2002). This difference may be related to differences in dietary composition, life style between western and eastern societies (Clapp, 2006; Sivabalan *et al*., 2008), variability between insulin assays in different experimental researches, differences in the population studied and sampling time during pregnancy (Manley *et al*., 2008).

Cytokine proteins are soluble proteins that involved in the inflammation and many other processes. These proteins classifies based on their T-cell linage as Th1 or Th2. There are reports demonstrated that in normal pregnant women circulating Th2 cell-derived cytokines increased and Th1 cell derived cytokines decreased (Saito *et al*., 1999).

The results of present study are in consistent with a previous similar study that found an increase in IL-10 level in pregnant compared nonpregnant subjects (Holmes *et al*., 2003), but our study showed a significant correlation between serum IL-10 concentration and gestational age. Maternal serum IL-10 increased with further increase in pregnancy period. It has been indicated that Il-10 has a role in regulating placental development and programming (Roberts *et al*., 2003). Furthermore, the derived cytokine as IL-10 may be associated to normal human pregnancy and labor (Opsjłn *et al*., 1993). Test of correlation showed significant positive correlation between IL-10 level and BMI. Therefore, it seems, IL-10 produces not only by intrauterine tissue but also extra uterine tissues (Schaffler *et al*., 2006).

In a previous study, have been shown that administration of the IL-10 can normalize blood pressure in pregnancy-induced hypertensive rats (Tinsley *et al*., 2010). In other study antihuman IL-10 monoclonal antibody administration to baboons in early pregnancy, caused a significant increase in mean arterial pressure (Orangea *et al*., 2005).

Based on these observations it will be speculated that with further increase in IL-10 production during pregnancy period blood pressure decreases progressively, but the reports of the current study do not support of this speculation. In the study, in spite of significant increase in IL-10 level in pregnant compared nonpregnant subjects, MAP did not change significantly and there was a marginally significant correlation between IL-10 level and MAP in pregnant women($r = 0.22$, $p = 0.053$). Therefore, it seems endogenous production of IL-10 during normal pregnancy has a week effect on MAP

In our study, we could not find correlation between maternal IL-10 and insulin resistance.

CONCLUSION

In conclusion, the results of the study show maternal IL-10 level increases with further increase in gestational age. There is no significant correlation between IL-10 level with MAP and insulin resistance.

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