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Original Article

Internal Medicine Section

Anti-Inflammatory Markers IL-10 and IL-35: Role in Developing Gestational Diabetes Mellitus

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ABSTRACT

Introduction: Inflammatory state is considered as the pathogenesis of Gestational Diabetes Mellitus (GDM). Cytokines can cause insulin resistance and maybe the molecular basis of inflammation in Diabetes Mellitus (DM).

Aim: To assess the level of Interleukin-10 (IL-10) in addition to a new anti-inflammatory cytokine marker Interleukin-35 (IL-35) in pregnant women with and without GDM.

Materials and Methods: Participants in the study included 29 pregnant women with GDM (case group) and 29 healthy pregnant women (control group). Blood levels of IL-10, IL-35, Erythrocyte Sedimentation Rate (ESR) and C-Reactive Protein (CRP) were measured in all participants. Independent t-test and Chi-square test were used for data analysis. Quantitative data between three gestational subgroups (<29, 29-32 and >32 weeks) in each GDM and control group were compared by ANOVA test. The p-value <0.05 was considered significant.

Results: The mean levels of IL-10 were 1.03±0.85 and 0.83±0.57 pg/mL (p=0.284) and the mean IL-35 concentrations were 10.2±8.1 and 8.8±4.3 pg/mL (p=0.437) in GDM and control groups, respectively. The mean CRP and ESR levels were higher in the GDM group than the controls but the differences were not statistically significant. In the GDM group, IL-10 was significantly lower at the early stage of pregnancy (<29 weeks) compared to the later stage (>32 weeks) (p=0.04), but this was not true in the control group. There was no significant difference between the mean level of IL-35 at different gestational ages in both GDM and control groups.

Conclusion: The present study showed the decreased level of anti-inflammatory marker IL-10 in the late stage of pregnancy in diabetic women especially during the last weeks of gestation. New inflammatory marker IL-35 was not statistically significant in GDM subjects.

Keywords: Cytokine, Immune system, Pregnancy

INTRODUCTION

GDM is defined as glucose intolerance that occurs during pregnancy. Timely diagnosis and appropriate knowledge about the risk factors associated with GDM are highly important due to materno-fetal complications and the risk of progression to type II DM [1]. The prevalence of gestational diabetes is high in the Asian population and is variable according to selected patients or different criteria [2]. In the north of Iran, GDM affects 4 to 38% of expectant mothers [3,4].

Similar to type II DM, GDM is caused by insufficient insulin secretion and increased insulin resistance [5]. Obesity is one of the major pathophysiological reasons for type II DM and GDM [6]. Evidence suggests a strong correlation between obesity (as a chronic low-grade inflammatory state) and increased levels of inflammatory markers [7]. On the other hand, some cytokines such as IL-6 and TNF-α can cause insulin resistance and stimulate the acute phase reactant response [8]. Some reports indicate a low level of anti-inflammatory marker IL-10 association with insulin resistance [9], metabolic syndrome [10] and type 2 DM [11].

The immune system plays an important role in normal pregnancy processes and the balance between pro-inflammatory and anti-inflammatory cytokines is necessary to promote natural pregnancy [12]. It is believed that anti-inflammatory status is dominant during the last two trimester of pregnancy [13].

IL-35, a newly discovered anti-inflammatory marker, is a member of the IL-12 and is produced by regulating T cells [14]. There is a preventive role of IL-35 in spontaneous abortion [15]. An article showed an association between IL-35 and high Body Mass Index, pre-eclampsia and blood sugar in pregnant women [16]. Given the important role of the immune system in the normal pregnancy process, the study was planned to evaluate the level of two anti-inflammatory markers, IL-10 and IL-35 in pregnant women with and without gestational diabetes.

MATERIALS AND METHODS

This case-control study was conducted in 58 pregnant women including 29 cases with GDM and 29 healthy, age-matched pregnant women as controls. The participants were selected from pregnant women attending the diabetes clinic and obstetric clinic in Sari, Imam Khomeini Hospital and between 2017-2018. The study was approved by the Ethical Committee of Mazandaran University of Medical Sciences (IR.MAZUMS.REC.1395.1910). The informed consent was obtained from all participants.

Inclusion criteria: Gestational age greater than 20 weeks and single-pregnancy.

Exclusion criteria: Women with a history of IBD or any inflammatory status, acute infection and diabetic ketoacidosis were excluded from the study.

The diagnosis of GDM was made if at least one value of the 75 g Oral Glucose Tolerance Test (OGTT) exceeded the recommended American Diabetes Association (ADA 2019) [17] thresholds: Fasting Blood Glucose (FBS) ≥92, one-hour Postprandial Plasma Glucose (2h PPG) ≥180 and two-hour Postprandial Plasma Glucose (2h PPG) ≥153 mg/dL.

Demographic characteristics were recorded at the beginning of the study. In all participants, the serum levels of cytokines, including IL-10 and IL-35 (e-bioscience, Germany, normal range <0.3 pg/mL and <4.5 ng/mL, respectively) were measured by Enzyme-Linked Immunosorbent Assay (ELISA) based on the manufacturer's protocol, in addition to ESR and quantitative CRP in fasting conditions (normal range <3.8 mg/L).

STATISTICAL ANALYSIS

Based on a 95% confidence level, the minimal sample size suggested to provide 90% power was 52 (n=26 per group) [18]. Quantitative and qualitative characteristics were compared between the cases and controls, applying independent t-test and Chi-square test, respectively. To investigate the differences in inflammatory and anti-inflammatory marker levels in different gestational age, all participants in the case group and control group were divided into three subgroups: less than 29, 29-32 and more than 32 weeks. The mean values of IL-10, IL-35, ESR and CRP were compared by one-way ANOVA and Tukey's post-hoc test. The differences in markers were analysed by Student t-tests and $p<0.05$ were considered significant.

RESULTS

The mean age of participants in case and control groups was 29.9±6.6 and 30.4±4.1 years, respectively (p=0.723). Demographic characteristics of participants are shown in [Table/Fig-1]. There were no significant differences between the two groups in any variables.

Variable	With GDM N (%)	Without GDM N (%)	p-value
Previous history of GDM	3 (10.3)	3 (10.3)	1.00
Family history of DM	11 (37.9)	12 (41.4)	0.79
Demographic characterstics	**Mean±SD**	**Mean±SD**	
Age (year)	29.96±6.6	30.4±4.1	0.72
Weight (kg)	73.1±13.9	70.7±12.1	0.47
BMI (kg/m^2)	27.2±4.8	26.5±4.2	0.57
Systolic BP (mmHg)	104.1±10.2	103.9±8.6	0.95
Diastolic BP (mmHg)	64.8±5.7	64.8±6.9	1.00
Gestational age (weeks)	33±4.1	34.7±2.4	0.06

[Table/Fig-1]: Baseline characteristics of pregnant women with and without gestational diabetes mellitus.

The inflammatory and anti-inflammatory markers had normal distribution according to the Kolmogorov-Smirnov statistical test. All markers (IL-10, IL-35, ESR and CRP) were higher in the case group than the controls but the difference between the two groups was not statistically significant. The mean levels of IL-10 were 1.03±0.85 and 0.83±0.57 pg/mL (p=0.284) and the mean IL-35 concentrations were 10.2±8.1 and 8.8±4.3 pg/mL (p=0.437) in GDM and control groups, respectively. The serum levels of CRP and ESR were not significantly different between the cases and controls [Table/Fig-2].

Variable	With GDM Mean±SD	Without GDM Mean±SD	p-value
ESR (mm/h)	41.9±14.9	40.9±19.5	0.821
CRP (mg/dL)	5.1±5.8	3.9±3.9	0.360
IL-10 (pg/mL)	1.03±0.85	0.83±0.57	0.284
IL-35 (pg/mL)	10.2±8.1	8.8±4.3	0.437

[Table/Fig-2]: Mean anti-inflammatory and inflammatory markers levels in pregnant women with and without gestational diabetes mellitus.

The anti-inflammatory markers (IL-10, IL-35) were also compared between three gestational age sub-groups in GDM and control groups separately. IL-10 was significantly different among the GDM gestational age subgroups, (p=0.04) and was higher in participants near the (>32 weeks compared to <29 weeks. This trend was not seen in healthy pregnant women (0.82). Although the level of anti-inflammatory marker IL-35, in both diabetic and healthy groups first decreased and then increased with the increasing gestational age, these changes were not statistically significant in both groups (0.58 and 0.93, respectively) [Table/Fig-3].

Research group	Parameter	<29 weeks Mean±SD	29-32 weeks Mean±SD	>32 weeks Mean±SD	p-value
Without GDM (n=29)	IL-10 (pg/mL	0.64±0.21	1.01±0.03	0.83±0.61	0.82
	IL-35 (pg/mL)	9.2±0.0	7.60±0.85	8.92±4.69	0.93
With GDM (n=29)	IL-10 (pg/mL)	1.69±1.3	1.01±0.53	0.75±0.55	**0.04**
	IL-35 (pg/mL)	8.87±3.6	6.86±3.09	11.24±9.73	0.58

[Table/Fig-3]: Anti-inflammatory markers in diabetic and non-diabetic pregnant women with different gestational age.

DISCUSSION

The current study performed in 58 pregnant women with and without GDM at the gestational stage between 20 to 38 weeks to determine the potential link between GDM with inflammatory markers (ESR and CRP) and anti-inflammatory cytokines (IL-10 and IL-35).

The role of pro-inflammatory cytokines such as TNFα, IL-6, and IL-I was previously evaluated in GDM; however, few studies investigated the role of anti-inflammatory cytokines such as IL-10 and IL-35 in the pathogenesis of GDM [19].

The present study, found a trend of increasing inflammatory markers (ESR and CRP) in the GDM group compared to control, although this difference was not significant. Previous findings by Wolf M et al., and Qui C et al., [20,21] demonstrated a positive relationship between CRP and gestational diabetes. Indeed, high levels of serum CRP in the early stages of pregnancy could increase the risk of GDM, independent of other risk factors such as age, parity, smoking and race. Though, this correlation was restricted to lean women (Body Mass Index <25 kg/m^2) [21]. In the Rosario-Capellan study, serum CRP and ESR levels were measured for predicting GDM and just ESR levels at 12 weeks were reported as a predictor of GDM [22]. The reason for the discrepancy between the present study results with the above studies could be due to differences in the gestational stage and BMI value.

In order to have a healthy pregnancy process, there needs to be a balance between pro-inflammatory and anti-inflammatory cytokines [5,19]. A decrease in serum level of IL-10 is reported to be associated with insulin resistance, metabolic syndrome, and type II DM [10,23,24]. Since pregnancy modifies the immune system [22], a decrease in anti-inflammatory factors could lead to some disorders including GDM. The present study showed a trend of decreasing IL-10 levels with increasing the gestational stage in women with GDM.

An increase in IL-10 level during the first and second trimesters of normal pregnancy was reported by Chatterjee P et al., [25]. Also, some authors have shown that IL-10 production decreased before and during labour [25,26]. The gestational age of most of the participants in the present study was more than 27 weeks and the women in the first trimester of pregnancy were not included in the study according to the ADA definition of gestational diabetes. In the healthy group, IL-10 levels increased at first but decreased [17] during the last weeks of the third trimester, but these changes were not statistically significant. There was a declining trend of IL-10 level during all weeks of the third trimester in the GDM group. This may be one of the reasons for the increase in pre-term labour in diabetic women.

IL-35, a newly found anti-inflammatory cytokine, has been shown to inhibit T-cell proliferation and to suppress the immune response. The potential therapeutic effect of IL-35, have been demonstrated in rats for disorders such as inflammatory bowel disease [27] and multiple sclerosis [28]. Moreover, it was shown that in type I diabetes models, the concentration of IL-35 is reduced, which indicates the role of this anti-inflammatory cytokine in this condition [29]. As beta cells dysfunction is associated with the pathogenesis of GDM [5], the potential link between IL-35 and GDM was investigated in the current study.

With increasing gestational age, the level of anti-inflammatory marker IL-35 reduced first and then increased, which can be partly

explained by a low number of cases in each group. In a study by Cao W et al., in 2018, lower levels of IL-35 and higher serum levels of IL-17, as well as, high BMI, were observed in women with GDM and preeclampsia [16]. The difference of result between current research and Cao W study, maybe because of matching the BMI and blood pressure between the GDM and healthy group in the present study.

Limitation(s)

The anti-inflammatory markers were not evaluated in each trimester for every participant.

CONCLUSION(S)

The present study showed a decrease in the IL-10 level in late pregnancy age, especially in the weeks leading up to delivery, in women with gestational diabetes. New anti-inflammatory marker IL-35 was not significantly different in subjects with GDM compared to controls and also in different gestational ages. More comprehensive studies are required with a high sample size to investigate inflammatory markers in each trimester.

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