Original Article

Studying the Immunological Dysregulation in Women with Gestational Diabetes Mellitus

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Abstract

Background: "Gestational diabetes mellitus (GDM)" is the most prevalent pregnancy-related metabolic condition, and its prevalence is rising globally. It is responsible for hyperglycemia in "pregnant women." Early detection of pregnant women at risk of GDM is the first step toward administering effective preventative measures to reduce maternal and neonatal problems. The accurate identification of GDM is still debatable, and interleukin-1-beta (IL-1β), IL-18, and nod-like receptor protein 3 (NLRP3) are the most recent markers utilized in the diagnosis of GDM. **Objective:** The goal of this study was to determine (NLRP3, IL-1β, and IL-18) levels in the serum of women with GDM to know their role in immunological dysregulation in women with GDM. **Materials and Methods:** In this study, 89 individuals were used from August 2022 to December 2022; a total of 29 healthy participants and 60 pregnant with GDM were recruited. Healthy pregnancy and research participants ranged in age from 23 to 47 years, and all of them were analyzed using serum blood samples (3 mL). **Results:** Sixty patient women affirmatives out of 89 using enzyme-linked immunosorbent assay (ELISA); we determined that our GDM participants met the inclusion criteria. The findings of the (NLRP3, IL-1β, and IL-18) ELISA kit indicated that the level of markers was greatest in GDM (P = 0.01). The detection of (NLRP3, IL-1β, and IL-18) is made possible by using ELISA kit. This suggests that the levels of these markers are higher in pregnant patients than in controls. **Conclusion:** Patients in Babylon Province with GDM had significantly higher than average levels of (NLRP3, IL-1β, and IL-18).

Keywords: Nod-like receptor protein 3 (NLRP3), interleukin-1-beta (IL-1β), interleukin 18 (IL-18), gestational diabetes mellitus (GDM)

Introduction

Gestational diabetes mellitus (GDM) is described as any degree of glucose intolerance that manifests itself or is first recognized during pregnancy.[1] GDM is an obstetric condition that affects 5%–10% of all pregnancies across the world.[2] Previous research has suggested that placental and adipose tissue both initiates and mediates this inflammatory response, which involves differential activation of maternal "innate" and "adaptive" immune cells.[3] Immunological dysregulation is blamed for the condition's worsening.[4] To avoid adverse pregnancy pathology, key maternal immune-mediated mediators such as macrophages, natural killer cells, and regulatory T cells must be meticulously balanced by the maternal immune system from the early stages of implantation and decadal formation to successful fetal delivery.[5] Several inflammatory cytokines, produced by the placenta and adipose tissue, help to activate inflammation and establish or worsen insulin resistance during pregnancy.[6] The pyrin domain-containing three of the nucleotide binding and oligomerization domain-like receptor family and has a role in the regulation of interleukin-1-beta (IL-1β) and IL-18 production. IL-1β and IL-18 are inflammatory cytokines that play a role in the development of maternal immune response (IR) during GDM.[7] The nod-like receptor protein 3 (NLRP3) can be activated by a wide range of illnesses.[8] The innate immune defense mediates the inflammatory response during *Helicobacter pylori* infection.[9] Along with

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toll-like receptors pathways, nod-like receptors have been proposed to mediate pro-inflammatory responses and maintain tissue homeostasis.[10] Excessive inflammation can lead to chronic or secondary illnesses that cause tissue damage and worsen the severity of the host's sickness. Innate immune receptors, which continually monitor the extracellular and intracellular milieus, serve as the first line of defense against pathogenic microbes.[11] Currently, the role of immune dysregulation as a causal mediator in GDM pathogenesis is unknown.[12] Secondary effects of GDM, including maternal and fetal cardiovascular and metabolic problems, are exacerbated by an aberrant maternal IR.[13]

Materials and Methods

Sample collection

The Babylon Health Department of the Ministry of Health gave its consent to the research using a tailored approval template. Before beginning sampling, patients gave their permission after being explained the study's goals. Sixty pregnant women had GDM.

Collection place

The study was done at an Al-Hilla Teaching Hospital Department of Gynecology and Al-Mahawill General Hospital.

Collection period

Between August 2022 and December 2022.

Sample type

Sixty blood samples were taken from persons study participants and were frozen at −20°C to prevent repeated thawing and freezing.

Examination

An NLRP3, IL-1β, and IL-18 enzyme-linked immunosorbent assay (ELISA) kit was used to determine the concentration of the NLRP3, IL-1β, and IL-18 in the serum samples. The test was carried out exactly as the manufacturer instructed, and the microplates were read. The microplates were read in 450 nm wavelength by using an ELISA reader. The absorbance of the occulted sample might be compared to the cutoff value to identify the value of NLRP3 inflammasome, IL-1β, and IL-18. Can use 3 mL of blood from patients and control groups for ELISA examination, following the manufacturer's instructions from Sun Long (Sunlong Biotech Co. ltd / Hangzhou City, Zhejiang Province, China). For this purpose, we employed SPSS Statistics. The analysis of variance test was used to analyze the demographics of the NLRP3, IL-1β, and IL-18, and significant differences in prevalence across body mass index (BMI) and throughout the age spectrum were defined as *P* values of 0.05 or below.

Ethical approval

The Declaration of Helsinki was strictly adhered to in order to ensure that the participants were not put in any unnecessary danger. Patient verbal and analytical permission was sought prior to sample collection. The study methodology, subject information, and consent form were reviewed and authorized by a local ethics committee, as shown by document BMS 510/16 (containing the number and the date on June 27, 2022).

Results

The comparison of age between patients with gestational diabetes mellitus and control group

It is case–control study that included 60 pregnant women who tested for GDM and ranged in age from 23 to 47 years. The 60 sample from 89 sample, who had GDM (patients group). This result indicates that the patients have mean age more than control group, the *P* value ≤0.01 [Figure 1].

Table 1 shows a highly significant difference in mean age between the case and control groups.

The comparison of body mass index between patients with gestational diabetes mellitus and control group

It is case–control study that included 60 pregnant women who tested for GDM and ranged in BMI from 22 to 41, but the control ranged from 20 to 40. This result indicates that the patients have a mean BMI more than control group (control = 31.38±1.13, GDM patients = 34.67±0.70) and $P \leq 0.05$ [Figure 2]. Table 2 shows a significant difference in mean BMI between the case and control groups, and Table 3 shows Correlation coefficient between immunity parameters with age and BMI Parameters.

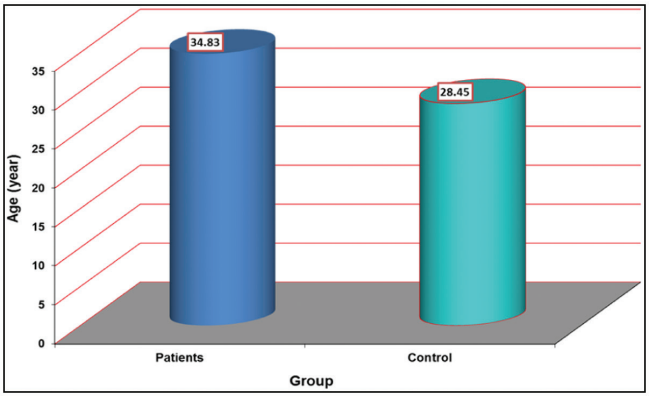

Figure 1: The frequency distribution of GDM patients according to age

Table 1: Distribution of GDM patients and control by age

Group means ± SD	Age (year)	Range	*P* value
Patient (*n* = 60)	34.83 ± 0.80	25–47	$P \leq 0.01$
Control (*n* = 29)	28.45 ± 1.16	23–45	

Fasting blood sugar and oral glucose tolerance test

The comparison of mean fasting blood sugar (FBS) between patients with GDM and control is shown in Table 4. The mean FBS of patients with GDM was 132.60 ± 3.52 mg/dL, and the mean FBS of control was 66.31 ± 1.86 mg/dL. In the present study, there is a highly significant difference in mean FBS between the "patient and control" group ($P = 0.01$) [Figure 3].

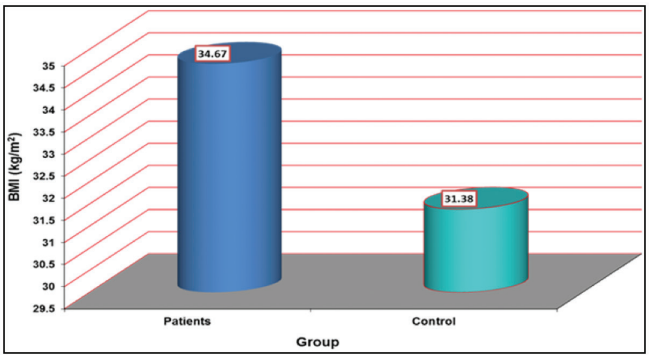

Figure 2: The frequency distribution of GDM patients according to BMI

Table 2: Distribution of GDM patients and control by BMI

	Control ($n = 29$)	GDM patients ($n = 60$)
BMI (kg/m^2)	31.38 ± 1.13	34.67 ± 0.70
P-value	$P \leq 0.05$	

Table 3: Correlation coefficient between immunity parameters with age and BMI

Parameters	Age	BMI
IL-1β	0.03 NS	0.13 NS
IL-18	−0.07 NS	−0.04 NS
NLRP3	−0.06 NS	−0.11 NS

NS: non-significant

Table 4: Distribution of GDM patients and control by OGTT and FBS

Group	OGTT (mg/dL)	FBS (mg/dL)
Patients	208.78 ± 5.96	132.60 ± 3.52
Control	85.68 ± 1.26	66.31 ± 1.86
P-value	($P \leq 0.01$)	

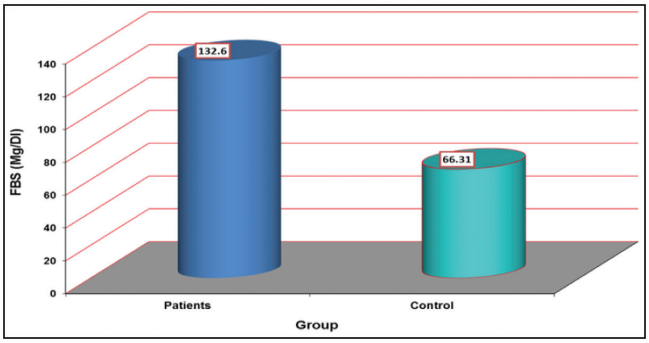

Figure 3: The frequency distribution of GDM patients according to FBS

The comparison of mean oral glucose tolerance test (OGTT) between patients with GDM and control is shown in Table 4. The mean OGTT of patients with GDM was 208.78 ± 5.96 mg/dL, and the mean OGTT of control was 85.68 ± 1.26 mg/dL. In the present study, there is a highly significant difference in mean OGTT between the "patient and control "group ($P = 0.01$) [Figure 4].

Association of interleukin-18, interleukin-1-beta, and NLRP3 level with gestational diabetes mellitus patients

Mean differences between GDM patients and controls are seen in Table 5, with GDM patients having higher IL-18, IL-1β, and NLRP3 than control. The present investigation found a significant (*P*-value 0.01) inverse association between NLRP3 and IL-1β while a non-significant (*P*-value >0.05) inverse correlation between IL-18 and IL-1β in patients with GDM [Figure 5-7.

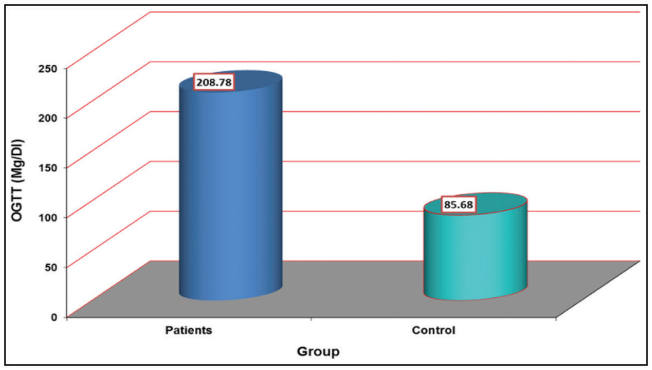

Figure 4: The frequency distribution of GDM patients according to OGTT

Table 5: Mean difference of IL-18, NLRP3, and IL-1β in GDM patients and control

Parameters	Control ($n = 29$) Means ± SD	GDM patients ($n = 60$) Means ± SD	*P* value
IL-18	20.13 ± 1.06	58.87 ± 4.79	<0.01
NLRP3	41.15 ± 2.66	76.39 ± 6.71	<0.01
IL-1β	6.12 ± 0.37	38.68 ± 5.02	<0.01

SD = standard deviation; $P < 0.01$: significant

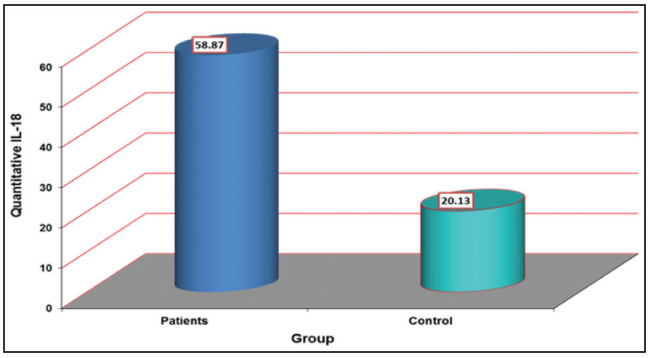

Figure 5: IL-18 level in GDM patient and the control

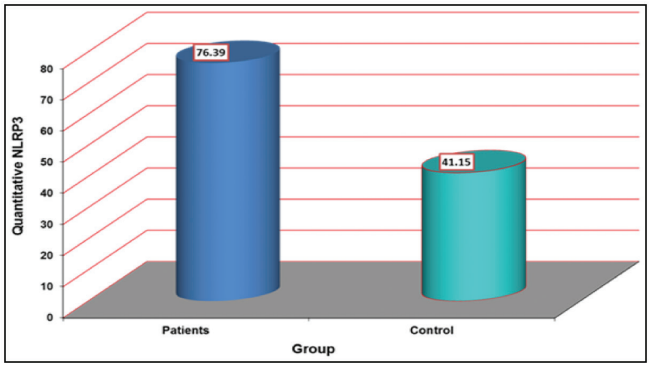

Figure 6: NLRP3 level in GDM patient and the control

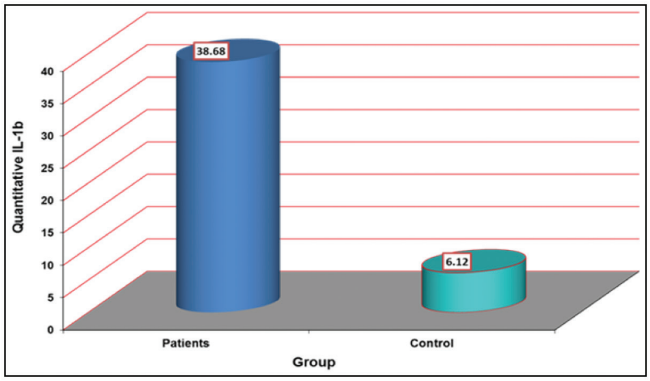

Figure 7: IL-1β level in GDM patient and the control

Discussion

In this research, we looked into the relationship between (IL-1β, IL-18, and NLRP3). Patients with GDM express this protein.[14] The findings suggest a significant correlation between IL-1β, IL-18, and NLRP3 inflammasome and GDM patients. GDM is characterized with an ongoing inflammatory disorder triggered by a variety of pro-inflammatory and anti-inflammatory chemicals.[15] By means of adiposity and obesity, the two disorders of metabolism manifested oxidative stress-induced beta-cell dysfunction and insulin resistance.[16] The IL-1β is a cytokine that plays roles in both the onset and persistence of the inflammatory process in pancreatic tissue. The function of the IL-1β protein in the etiology of GDM.[16] Since IL-18, IL-1β, and NLRP3 can be used as a prognostic biomarker for GDM, the research has looked at their predictive utility in GDM.[17] In our investigation, we also discovered a significant rise in serum IL-1β protein production in GDM patients. Because of its pro-inflammatory properties, IL-1β plays a role in the tissue devastation associated with GDM.[18] In pregnancy placental cells, NLRP3 was found to be involved in the production of IL-18 and IL-1β.[19] The placenta is a higher specific organ that acts as a bridge between "maternal and fetal" distribution during pregnant women.[20] The placenta's critical role in the incidence and progression of GDM.[21] IR is now the most important pathophysiological feature of GDM, and it is also prevalent during normal pregnancy.[22] Interfering with insulin receptor transduction by placental hormones, cytokines, and gaseous signaling transmitters can cause IR.[23] In addition, an abnormal glucose metabolism may be caused by a hormonal, cytokine, and gaseous signaling transmitter imbalance in the placental.[24] Through the modulation of inflammatory processes, cytokines from the "IL-1 family" are crucial in controlling the inflammatory response.[25] Numerous tissues and cells contain the inflammasome. The proteins NLRP3 and pro-caspase-1 make up the NLRP3 complex.[26] It is overproduced in GDM in the patients' pancreas tissue and peripheral blood, where it appears to have an important role in regulating the levels of inflammatory substances such as ILs, chemokines, and adhesion-associated molecules. A previous study has found that the level of expression of the IL-1β protein in GDM patients is elevated.[27] Raised serum IL-1β levels in GDM patients were associated with a number of disease-related parameters.[28] When diabetes complicates pregnancy, the risk of infection rises. The results of studies on the link between diabetes and poor pregnancy outcomes have been fairly consistent.[29,30] High blood glucose levels can cause maternal and fetal problems like spontaneous abortion, fetal deformity, preeclampsia, stillbirth, macrosomia, neonatal hypoglycemia, and neonatal hyperbilirubinemia, all of which are harmful to the mother's and infant's health. An infection could play a role in the etiology and pathophysiology of GDM pregnancy complications and poor fetal development.[31,32]

Conclusion

This research found that GDM patients had higher levels of IL-18, IL-1β, and NLRP3 than controls and also found a strong relationship between age, BMI, and GDM.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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