

Exploring Interleukin 6 as a Promising Marker for The Diagnosis of Gestational Diabetes Mellitus

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Abstract

Gestational diabetes mellitus (GDM) is a condition in which the ability of the mother's pancreatic β -cells to function properly is compromised. This leads to inadequate insulin production and, therefore, poor glucose regulation throughout pregnancy. In recent years, there has been a significant surge in interest in determining the impact of inflammation on the progression of GDM.

Inflammatory factors may function as antagonists to insulin and induce insulin resistance. Interleukin -6 (IL-6), a kind of cytokine, significantly impacts the development of glucose intolerance and may be used as a possible indicator in the blood for early detection of glucose intolerance. This study aims to assess the potential role of IL6 as a prospective diagnostic marker for Gestational Diabetes mellitus.

Our study is a case-control study started from September 2022 to June 2023 and enrolled 200 pregnant women aged between 15 and 45 years; cases included 100 patients selectively collected with a confirmed diagnosis of gestational diabetes mellitus in Kerbala obstetrics and gynecology hospital, and the control group included 100 healthy pregnant women also gathered from the obstetrics and gynecology hospital in Kerbala governorate. Results show that the mean of Interleukin 6 was significantly higher in pregnant women with GDM, with p-values of 0.05 and 0.001. these results suggest that Interleukin6 (IL6) can be used as a prospective diagnostic marker for GDM.

استكشاف الإنترلوكين ٦ كعلامة واعدة لتشخيص داء سكري الحمل

ديمه ضياء عزيز, سامي الخطيب, نور ضياء عزيز

الملخص

يتم تعريف داء سكري الحمل (GDM) على أنه حالة تضعف فيها قدرة خلايا بيتا البنكرياسية لدى الأم على العمل بشكل صحيح، مما يؤدي إلى عدم كفاية إنتاج الأنسولين، وبالتالي ضعف تنظيم مستويات الجلوكوز طوال فترة الحمل. هناك زيادة كبيرة في الاهتمام بتحديد تأثير الالتهاب على حدوث سكري الحمل في السنوات الأخيرة. قد تعمل العوامل الالتهابية كمضادات للأنسولين وتحفز مقاومة الأنسولين مثل الإنترلوكين-6 (IL-6)، وهو نوع من السيتوكين، له تأثير كبير على تطور عدم تحمل الجلوكوز ويمكن استخدامه كمؤشر محتمل في الدم للكشف المبكر عن تحمل الجلوكوز. تهدف هذه الدراسة إلى تقييم الدور المحتمل للإنترلوكين ٦ كعلامة تشخيصية محتملة لمرض سكري الحمل.

دراستنا هي دراسة مراقبة الحالة بدأت من سبتمبر ٢٠٢٢ إلى يونيو ٢٠٢٣ حيث شملت ٢٠٠ امرأة حامل تتراوح أعمارهن بين ١٥-٤٥ سنة، وشملت الحالات ١٠٠ مريضة تم جمعها بشكل انتقائي مع تشخيص مؤكد لمرض سكري الحمل في مستشفى كربلاء للأمراض النسائية والتوليد وتضمنت المجموعة الضابطة ١٠٠ امرأة حامل سليمة أيضا تم جمع النساء الحوامل الأصحاء من مستشفى النسائية والتوليد في محافظة كربلاء. أظهرت النتائج أن متوسط إنترلوكين ٦ كان أعلى بشكل ملحوظ في النساء الحوامل المصابات بسكر الحمل مع قيم $p < 0.05$ و 0.001 . تشير هذه النتائج إلى أنه يمكن استخدام الإنترلوكين ٦ كعلامة تشخيصية محتملة لسكري الحمل.

1. Introduction

Gestational Diabetes Mellitus (GDM) is a prevalent disorder during pregnancy, characterized by impaired glucose tolerance. It affects around 9-25% of pregnancies globally. However, the percentages may vary depending on the demographic and diagnostic criteria. Gestational diabetes mellitus (GDM) is defined as a condition where the ability of the mother's pancreatic β -cells to function correctly is compromised, leading to inadequate insulin production and, therefore, poor regulation of glucose levels throughout pregnancy [(Mechchate *et al.*, 2021), (Choudhury and Rajeswari, 2021)]

During pregnancy, the placenta secretes a hormone called human placental lactogen. It induces significant metabolic changes during pregnancy and has a structure comparable to the growth hormone figure (1). to ensure the fetus receives enough nutrients. This hormone can induce changes and modifications in the insulin receptors. The following molecular alterations seem to be associated with a reduction in glucose absorption by peripheral tissues:

1) The beta-subunit of the insulin receptor undergoes molecular changes. 2) The phosphorylation of tyrosine kinase is reduced. 3) The insulin receptor substrate-1 and phosphatidylinositol 3-kinase undergo remodeling. Elevated maternal glucose levels pass via the placenta and increase the fetus's blood sugar levels. The fetal pancreas is activated in response to high blood sugar levels. The anabolic characteristics of insulin stimulate accelerated development in embryonic tissues [(Spaight *et al.*, 2016), (Moyce and Dolinsky, 2018)].

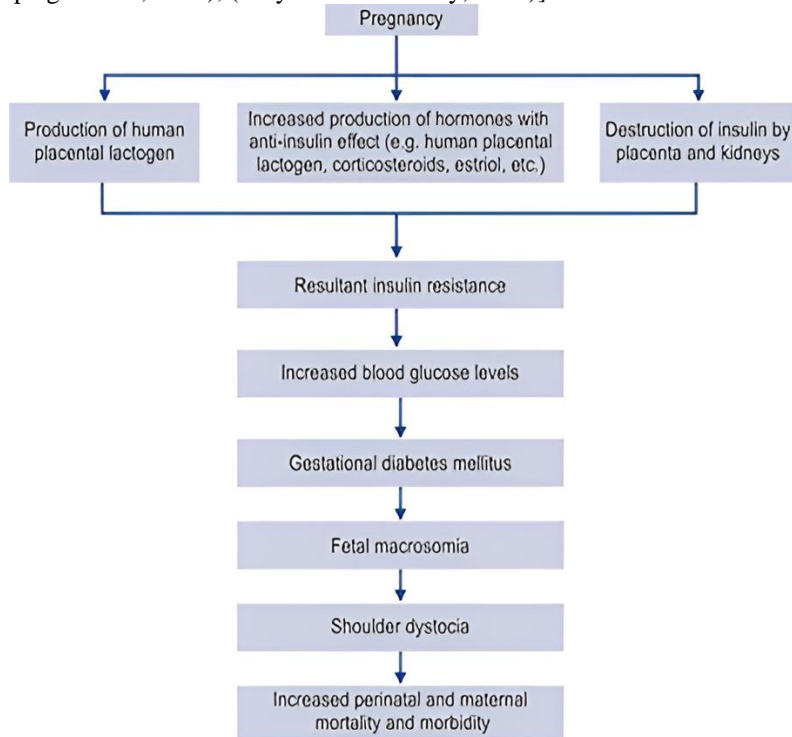


Figure 1: Pathophysiology of Gestational Diabetes Mellitus (Bedside Obstetric & Gynecology 2014).

The International Association of Diabetes and Pregnancy Study Groups (IADSPG) standards are the primary basis for diagnosing gestational diabetes mellitus (GDM). These protocols include doing an oral glucose tolerance test (OGTT) between the 24th and 28th week of pregnancy. The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) research, conducted in 2010, set out revised criteria for determining Oral Glucose Tolerance Test (OGTT) thresholds from the 24th to 28th weeks of pregnancy. The fasting plasma glucose level is 5.1 mmol/L, the one-hour plasma glucose level is ten mmol/L, and the two-hour plasma glucose level is 8.5 mmol/L (Kopylov *et al.*, 2020). Early and precise identification of women with a high susceptibility to developing GDM offers a chance to implement effective prenatal care strategies and future treatments aimed at mitigating the advancement of gestational diabetes. This, in turn, may help lower the related healthcare costs and adverse consequences. Nevertheless, there is ongoing

controversy over the diagnostic criteria for GDM. A prior investigation revealed a direct correlation between maternal blood glucose levels and unfavorable perinatal outcomes.

In recent years, there has been a significant surge in interest in determining the impact of inflammation on the progression of GDM.

Inflammatory factors may function as antagonists to insulin and induce insulin resistance. Interleukin-6 (IL-6), a kind of cytokine, has a significant impact on the development of glucose intolerance IL-6 causes insulin resistance by impairing the phosphorylation of insulin receptor and insulin receptor substrate-1 and may be used as a possible indicator in the blood for early detection of glucose intolerance (Farrar *et al.*, 2017).

In individuals with type 2 diabetes, inflammatory cytokines may lead to insulin resistance by inhibiting several pathways in the tissues essential for effective insulin signaling. Insulin resistance is linked to the atypical release of proinflammatory cytokines, such as IL-6 (Murthy *et al.*, 2018). During pregnancy, the placenta secretes IL-6, which is essential for normal placenta development and successful pregnancy; it may cause a long-lasting inflammatory response in fat tissue and aid in the incidence of insulin resistance during pregnancy.

2. Materials and Methods

2.1 Ethical Statement

The study was conducted with the endorsement of the Scientific and Ethical Committees at the Faculty of Medicine, University of Kufa, Iraq.

2.2 Patient Selection and Studying Design

The present study is a case-control study started from September 2022 to June 2023 and enrolled 200 pregnant women aged between 15 and 45 years (childbearing age); cases included 100 patients selectively collected with a confirmed diagnosis of gestational diabetes mellitus in Kerbala Obstetrics and Gynecology Hospital.

The (control group) included 100 healthy pregnant women also collected from the obstetrics and gynecology hospital in Kerbala governorate.

Included patients: All pregnant women in the second and the third trimester (because GDM occurs in these trimesters) who have a confirmed diagnosis of gestational diabetes mellitus.

Excluded patients: pregnant women who have diabetes (both types I and II), pregnant women in the first trimester with diabetes, pregnant women with a baby with congenital anomalies, obese pregnant women, and pregnant women who smoke.

2.3 Research Methods

After skin sterilization, five milliliters of blood were aspirated from the anti-cubital vein and split into two halves. The first two milliliters of blood were used to detect the blood group type and measure HbA1c.

Allow the remaining three milliliters of blood to clot for 10-20 min. At room temperature, gel tubes to obtain serums by centrifuging at (2000-3000) r.p.m. for 20 minutes. After that, the obtained serum is stored at -15 C to perform the biochemical tests for Interleukin 6 (IL6) measurement.

2.3.1 Measurement of IL6 by Enzymes Linked Immunosorbents Assay (ELISA-Kits, Elab-science Cat.No. : E-EL-H6156

Principle of the Assay

We used the Sandwiches-ELISA principles with this ELISA kit. These kit's (micro) ELISA plates already have an antibody specific to human IL-6. Before combining the specified antibody with the samples or standards, they added it to the wells of the (micro) ELISA plates. The next step is to incubate every microplate well after adding the detection biotinylated antibodies specified to humans IL-6 and avidins-horseradish peroxidases (HRP). Wash to remove the waste product. Each one is filled with the substrate solutions. Only wells containing Human IL-6 will appear in blue. The components include a detection antibody that has been biotinylated, as well as an Avidin-HRP conjugate. Using a stop solution halts the enzymatic-substrate interaction, resulting in a yellow color change.

The spectrophotometric measurement of (OD) optical density is obtained at 450 nm wavelength with a tolerance of ± 2 nm. The concentration of Human IL-6 is directly proportional to the OD value. By comparing the samples' optical density (OD) with the standard curve, it is possible to ascertain the concentration of human IL-6 in the samples.

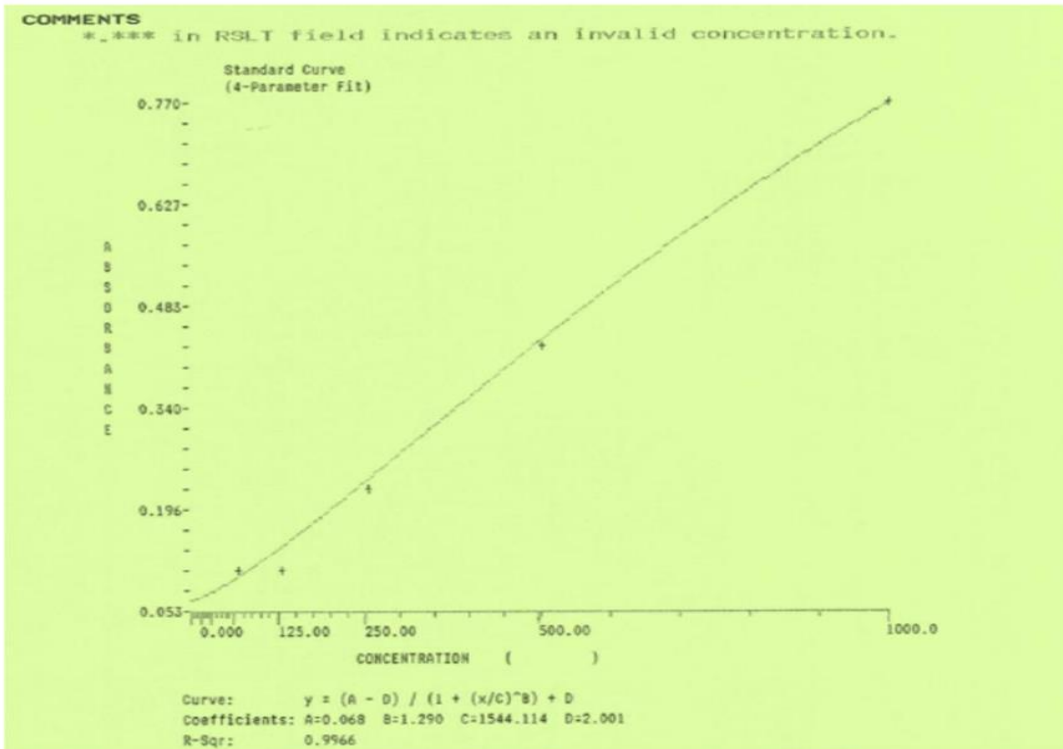


Figure 2: IL6 Standard Curve

2.3.2 Measurement of Glycosylated Hemoglobin (HbA1c) by HbA1c HPLC Kit , Eagle Biosciences Catalog Number: A1C31-H100

Principle of the assay :

First, the blood cells are lysed to determine HbA1c. An incubation step is performed on the samples at 37 °C to remove the unstable aldimine format. We inject the centrifuged liquid, the supernatant, into the HPLC equipment. It takes 5 minutes for the gradient separation to complete at 30°C using HPLC. A UV detector records the chromatograms.

We can measure concentrations using the supplied blood calibrator by integrating the corresponding peak heights and regions.

2.4 Statistical Analysis

Data was analyzed using SPSS version 26, a statistical tool for the social sciences. Presenting descriptive statistics in the form of frequency tables, The representation of continuous variables includes portraying them as the mean value offset by the standard deviation and utilizing the Student T test to identify associations between categories of data and continuous variables. Statistical analysis by Receiver operative characteristic curve (ROC) was used to evaluate the performance of IL6 in detecting GDM—a P-value of 0.05 or less determined statistical significance.

3. Results

3.1 level of Interleukin 6 in the two groups :

The mean interleukin 6 was significantly higher in pregnant women with GDM (p-values 0.05 and 0.001, table (1)) than in healthy pregnant women.

Table 1: The Level of Interleukin 6 in Patients and Controls

| Variable | Studied group | | | | P value |
|----------|-------------------|---------|------------------|----------|---------|
| | Pregnant with GDM | | Control pregnant | | |
| | Mean± SD | Range | Mean± SD | Range | |
| IL6 | 41.7±23.9 pg/ml | 10-98.1 | 4± 3.2 pg/ml | 0.1-20.6 | <0.001* |
| | | | | | |

*Student T test, Significant ≤ 0.05 .

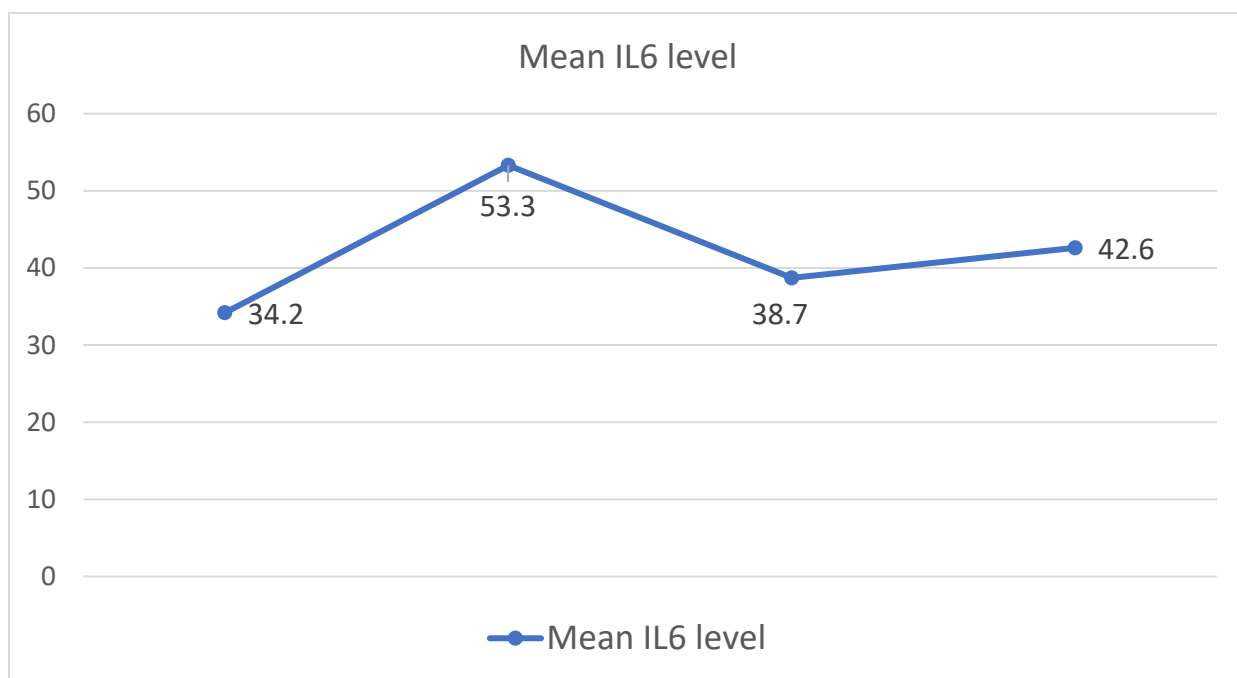


Figure 3: Mean Level of IL6

3.2 Evaluation of Interleukin 6 for diagnostic purposes using a Receiver Operating Characteristic (ROC) profile.

The ROC curve for IL6 was shown in Figure (4), obtained from the values of OGTT and IL6 tests with an area under the curve 0.992 and p-value <0.001 and with the cut of the value of 10.1 had a sensitivity of 99% and specificity of 10%.

4. Discussion

Gestational diabetes is an inflammatory disorder triggered by generating inflammatory cytokines such as interleukin-6 (HAMZA and MAJEED, 2021). Siddiqui et al. mentioned a valuable (case-control study) to detect the probable correlation between IL-6 and GDM in Indian women (Siddiqui *et al.*, 2019). The study's findings indicated that GDM women exhibited substantially elevated serum levels of IL-6 compared to control women. Additionally, our research revealed that pregnant GDM patients had a considerably higher mean IL6 level than pregnant healthy women. In addition, levels of IL-6 were connected with weight before pregnancy, blood sugar levels during fasting, and blood sugar levels after eating.

Furthermore, intestinal flora imbalances with higher inflammatory markers like IL6 and TNF α were likely to occur in GDM patients. These imbalances damaged the patients' immune systems and may have played a significant part in the development of diabetes. These results are based on a study by Yu et al. that examined how patients with GDM's gut flora and other inflammatory markers changed over time. Serum levels of proinflammatory factors, such as IL-6, were considerably higher in the patients than in the controls (Yu, Liu and Dong, 2018).

Zhao et al. study, which looked into the possible relationship between inflammatory markers and Chinese women's glucose intolerance and GDM, found that ladies who are expecting a child and have gestational diabetes or glucose intolerance had IL-6 levels significantly higher than those of the group of healthy individuals and that inflammatory cytokines were positively correlated with body mass index (BMI) and hemoglobin A1c (HbA1c) (Zhao *et al.*, 2018).

Zhang et al. found a significant difference in the serum and placental levels of inflammatory and placental indicators, such as IL-6, in women with gestational DM compared with women whose pregnancies were normal and healthy (Zhang *et al.*, 2017). This finding helped to clarify the relationship between inflammatory and metabolic biomarkers in women with GDM in Mongolia.

In recent years, much attention has been focused on how inflammation contributes to the onset of GDM. Inflammatory substances acting as antagonists to insulin can cause insulin resistance. As a cytokine, IL-6 is involved in the pathophysiology of glucose intolerance and may be used as a serum marker for early detection of glucose intolerance (Kleiblova *et al.*, 2010).

The inflammation of pancreatic macrophages and adipocytes, which increases IL-6 production, explains the link between IL-6 and gestational diabetes; thus, reduced insulin production brought on by the death of pancreatic β -cells results in elevated blood glucose levels (Tutar *et al.*, 2022).

The gold standard test, OGTT testing with 75 grams of oral glucose (O'Malley *et al.*, 2020), is conducted nearly late in pregnancy and necessitates an overnight fast. Furthermore, taking glucose during pregnancy is unpleasant for the expectant mother and requires the drawing of blood three times. On the other hand, measuring IL-6 levels is simple, inexpensive, and comfortable for a pregnant lady, and it does not present the difficulties mentioned above. As a result, IL-6 can be utilized as a marker to determine a pregnant woman's risk of GDM.

An appropriate measure like IL-6 is desperately needed, given the rising incidence of gestational diabetes globally and the importance of prompt identification and treatment of GDM to minimize harmful consequences for both the mother and the fetus.

The Receiver Operating Characteristic ROC curve is a graph that may be used to analyze how well a binary diagnostic classification system is performing. Using the ROC curve, another way to assess a test's diagnostic efficacy is to compare its findings to those of other tests.

The ROC curve offers several benefits. Initially, the whole range of test results can be distinguished between normal and abnormal using the detailed depiction provided by the ROC curve. Sorting the data in a histogram format is not essential to constraining the ROC curve; it displays the sensitivity and specificity for all possible cutoff values using the test data.

Finally, because the ROC curve depends only on sensitivity and specificity, samples can be obtained irrespective of the presence of a disease in the community because it is not impacted by prevalence (Joo, Cho and Kim, 2020).

Understanding the concept of sensitivity and specificity, which are used to assess a diagnostic test's performance, is a prerequisite to comprehending the ROC curve. The percentage of people who test +ve for a target disease who genuinely have it is known as sensitivity. In contrast, the percentage of people who try -ve for the disease is known as specificity (Jung, Lee and Park, 2019).

Area under the curve (AUC) is a commonly used tool to assess diagnostic tests' accuracy. Near the top left corner of the graph, where the sensitivity =1 and false positive rate =0 (specificity =1), the test's accuracy rises as the ROC curve moves closer to that area.

AUC = 1.0 is the perfect ROC curve. Nonetheless, when there is a one-to-one correspondence between the x- and y-axes and the actual positive rate is equal to the false positive rate (AUC=0.5), it would be like tossing a coin or using any other haphazard method. It would be inappropriate to ascertain the existence or absence of illness.

Thus, the AUC needs to be more than 0.5 for any diagnostic method to be relevant, and it should generally be more than 0.8 to be deemed appropriate. Furthermore, the ROC curve with the most excellent AUC was thought to perform the best diagnostics when comparing the results of two or more diagnostic tests (Hajian-Tilaki, 2013).

Our investigation revealed that the ROC curve for IL6 was displayed in Figure (4). The area under the curve analysis was 0.992, with a sensitivity of 99%, a specificity of 10%, and a cutoff value of 10.1. Since more information

is needed regarding the IL6 ROC curve for GDM, our work may be considered one of the first in this area. The considerable AUC value shows a high accuracy of the IL6 test, meaning it can be used to predict GDM in women.

5. Conclusion

The current study's results indicate that Interleukin6 (IL6) can be used as a prospective diagnostic marker for GDM.

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Conflict of Interest

The authors declare no conflict of interest.

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