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Clinical Implications of Interleukin-10 and Interleukin-6 Dysregulation in Gestational Diabetes

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Abstract

Gestational diabetes (GDM) can result in short-term and lasting health problems for the pregnant mother and the developing foetus. Instead of a sudden inflammatory response, GDM might trigger a chronic, low-level inflammation called "meta-inflammation." This persistent inflammation could play a role in the future development of diabetes, obesity, and cardiovascular issues for both the mother and child. Given this, the present study is an attempt to investigate the association between GDM and inflammation by evaluating the levels of interleukins. The study involved a total of 50 individuals, 25 pregnant women in gestational age 24 to 28 weeks as calculated by LMP and dating scan with gestational diabetes and 25 healthy pregnant women. Interleukin-10 and interleukin-6 levels were estimated by ELISA analysis. The plasma level of the anti-inflammatory marker IL-10 was significantly lower in the GDM group than in the healthy subjects group. Concomitantly the levels of pro-inflammatory cytokine IL-6 were found to be markedly increased in the GDM subjects in comparison to that of healthy subjects. Enhanced inflammatory response has been observed in GDM, suggesting that inflammatory markers could serve as predictive indicators for GDM. Delayed diagnosis could result in significant consequences for both the mother and the child, both in the short and long term. Hence, there is a pressing requirement for early markers of GDM to facilitate timely intervention and treatment. Exploring these inflammatory signals in greater detail presents an opportunity to enhance maternal health outcomes by creating focused and efficient therapeutic approaches.

Keywords: *Gestational Diabetes, Health, Interleukins, Meta-Inflammation, Pregnancy.*

Introduction

Pregnancy triggers a series of metabolic alterations in women's bodies involving carbohydrates, fats, and proteins. These changes are aimed at supplying the essential nutrients and oxygen necessary for the development of the foetus, as well as storing additional energy needed for childbirth and breastfeeding. Any significant disruptions in these metabolic processes can result in the condition known as gestational diabetes mellitus (GDM) [1]. The likelihood of experiencing GDM is elevated by 1.3 to 3.8 times in women who are obese in comparison to those with a normal BMI. Additionally, approximately 70% of women diagnosed with GDM might progress to developing type 2 diabetes after giving birth [2, 3].

Recent research has highlighted the impact of inflammation on the development and progression of gestational diabetes mellitus

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(GDM). In the context of GDM, it has been shown that inflammation plays a significant role in causing insulin resistance, disrupting normal glucose metabolism, and impairing the function of pancreatic beta cells [4]. Inflammatory molecules like tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β), along with other similar cytokines, have been identified in various tissues such as skeletal muscle, liver, and adipose tissue [5]. Moreover, it has been observed that women with GDM have ongoing mild inflammation in their adipose tissue, indicated by elevated levels of pro-inflammatory cytokines and markers of acute-phase response [6]. These findings suggest that inflammation contributes to insulin resistance in GDM and has the potential to disrupt the normal pathways of insulin signalling.

Furthermore, the functioning of pancreatic beta-cells, crucial for the release of insulin, can be influenced by inflammatory cytokines. Prolonged exposure to these cytokines can lead to a decline in beta-cell performance, resulting in reduced insulin production and an increased rate of beta-cell mortality [7]. Inflammation has also been proven to impact the operation of placental cells, modify placental metabolism, and change the transfer of nutrients. All of these effects can contribute to elevated blood sugar levels in foetuses and heighten the likelihood of unfavourable outcomes in gestational diabetes [8]. Given the complications of GDM and its association with inflammatory signalling, the goal of the present study was to investigate the clinical significance of imbalances in interleukin-10 and interleukin-6 in diabetes mellitus. This will be achieved by analysing samples from individuals who meet specific criteria for both cases and controls. The levels of these markers will be quantitatively assessed using the ELISA method.

Materials and Methods

Study Population

The study involved recruiting 25 pregnant women who were diagnosed with gestational diabetes (GDM) for the first time and 25 pregnant women with normal glucose tolerance (NGT). These individuals received regular outpatient check-ups at the Department of Obstetrics and Gynaecology in Saveetha Medical College and Hospitals located in Chennai.

Inclusion and Exclusion Criteria

In the present study, we identified gestational diabetes mellitus (GDM) in participating women between the 24th and 28th weeks of their pregnancy, using the diagnostic criteria outlined by the American Diabetes Association (ADA) in 2014. Women with pre-existing diabetes before becoming pregnant or who had medical conditions, treatments, or therapies that could influence blood characteristics were not considered in our study. We evaluated pregnancies with normal glucose tolerance (NGT) during the 24th to 28th weeks, ensuring that they exhibited appropriate glucose levels at three specific times before and during the oral glucose tolerance test (OGT). These times included fasting plasma glucose (FPG) levels, as well as glucose levels at the 1-hour and 2-hour marks following the oral glucose intake.

Ethical Approval/Consent to Participate

Individuals participating in the study willingly enrolled and gave their explicit written consent. The research plan was authorized by the Institutional Ethics Committee of Saveetha Medical College and Hospitals, Chennai. Our investigation strictly adhered to the principles outlined in the 1964 Declaration of Helsinki, which underwent revisions in 2013.

Sample Size

The total sample size is 40. The sample size is calculated using G-Power assuming 95%

confidence interval with 80% power and 10%αerror.

Sample Analysis

Pregnant women with GDM and NGT were provided blood samples of 5mL each, which were collected in tubes containing ethylenediaminetetraacetic acid (EDTA). Following collection, the blood tubes were promptly placed in a refrigerator. To isolate the plasma, the samples were subjected to centrifugation at 1,500 rpm for 10 minutes at a temperature of 4°C, all within 15 minutes of the initial collection.

Elisa Analysis

ELISA analysis of interleukin-10 and interleukin-6 was carried out in the plasma collected from NGT and GDM subjects using commercially procured ELISA kits (Ray Biotech Life, USA).

Statistical Analysis

The data obtained after the sandwich ELISA procedure was presented as Mean±SEM and compared by unpaired student t-test. Non- Non-parametric Mann-Whitney sum rank "U" test and Kruskal-Wallis X^2. A p-value less than 0.05 was considered significant. Graph Pad Prism software version 7.0 was used.

Results

ELISA Analysis of Interleukin-10

Interleukin-10, an anti-inflammatory marker that plays a pivotal role in the regulation of innate immunity was evaluated by ELISA method in both GDM and NGT pregnant women. The results demonstrated significant ($p<0.001$) depletion in the levels of IL-10 in GDM women when compared to healthy subjects who did not have GDM (Fig 1).

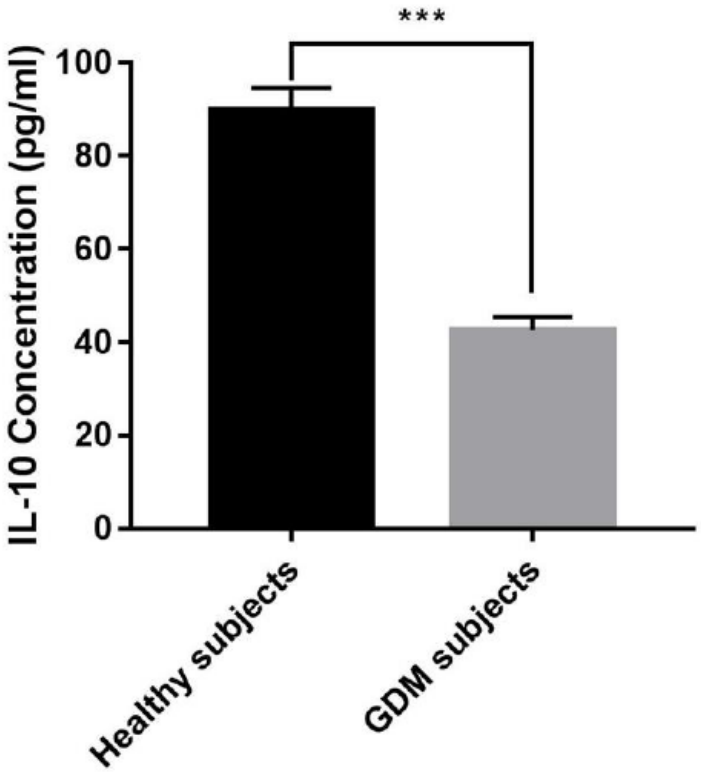

Figure 1. Bar Graph Showing the Levels of Interleukin-10 between Healthy Pregnant (NGT) and GDM Women. Normally Distributed Continuous Variables were Described by Mean± SEM and Student t-tests were performed. *p<0.05, **p<0.01, ***p<0.001 vs Control

ELISA Analysis of Interleukin-6

The interleukin-6, another important cytokine has been evaluated in healthy pregnant and GDM women. The plasma analysis of IL-6 showed that GDM women have significantly ($p<0.001$) higher concentrations of IL-6 when compared to healthy pregnant women (Fig 2).

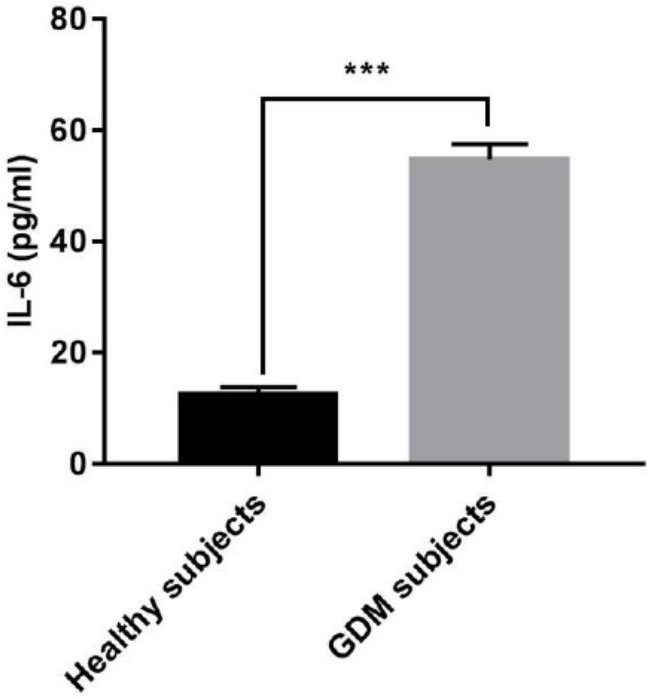

Figure 2. Bar Graph Showing the Levels of Interleukin-6 between Healthy Pregnant (NGT) and GDM Women. For Normally Distributed Continuous Variables were Described by Mean± Sem and Student's t-test was performed. *p<0.05, **p<0.01, ***p<0.001 vs control

Discussion

Both gestational diabetes mellitus (GDM) and type 2 diabetes mellitus share a connection with the inflammatory process [9, 10]. The disruption of glucose metabolism observed in GDM might be due to inflammation. Research has revealed that inflammatory molecules can enhance the liver's production of glucose through gluconeogenesis and decrease the absorption of glucose in fat tissues, leading to elevated blood sugar levels [6]. Several studies have even indicated that markers of inflammation can predict the development of GDM as early as the first trimester of pregnancy. Key inflammatory cytokines like tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) play a significant role in increasing insulin resistance, contributing to the occurrence of GDM [11, 12]. In agreement with the earlier published results, the present study demonstrated a considerable decrease in the anti-inflammatory cytokine IL-10 with a concomitant increase in pro-inflammatory cytokine IL-6.

Previous studies have associated IL-10, a cytokine linked to both type 1 and type 2 diabetes mellitus, with diabetes [13, 14]. A study conducted in Taiwan focused on this cytokine and its relationship with gestational diabetes in pregnant women. This research found that pregnant women with gestational diabetes (GDM) had higher levels of IL-10 in their blood plasma compared to healthy pregnant women [15]. The study also revealed that IL-10 levels varied during pregnancy in GDM women. These levels increased in early pregnancy, decreased by term, and slightly rose again after delivery. These fluctuations might

be influenced by increased cortisol levels during pregnancy [16]. It's worth noting that a recent meta-analysis did not find a significant link between IL-10 levels and susceptibility to GDM [17]. However, the low serum IL-10 levels alongside high HbA1C and blood glucose levels [18]. Conversely, other studies discovered higher serum IL-10 levels in patients with type 2 diabetes and pregnant women with GDM [19, 20]. Nonetheless, our study's results align with subsequent research that indicates decreased IL-10 concentrations in the blood plasma of GDM patients.

Because it tends to cause inflammation and potentially induce insulin resistance, it was expected that individuals dealing with gestational diabetes mellitus (GDM) would display higher amounts of the cytokine IL-6 in comparison to pregnant women who do not have this condition [21]. This aligns with previous research that demonstrated elevated levels of IL-16 in cases of GDM [22-24]. Correspondingly, the current study also exhibited elevated IL-6 levels in women with GDM. These findings imply that mild inflammation plays a role in promoting insulin resistance in gestational diabetes and could serve as an early predictive marker for GDM during the initial stages of pregnancy [25].

Conclusion

The inflammatory response is enhanced in GDM; hence, inflammatory markers could be used as predictive markers for GDM.

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Conflict of Interest

The authors declare no conflict of interest.

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