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Original Article

Comparison of Plasma Nitric Oxide Levels in Gestational Diabetic and Non-diabetic Pregnant Women Referred to Fatemieh Hospital in Hamadan, Iran

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Abstract

Background: Changes in plasma nitric oxide levels are an important predictor of cardiovascular disease progression in diabetic patients.

Methods: In this study, plasma nitric oxide levels were measured in pregnant women with gestational diabetes and non-diabetic controls. The present study was conducted on 50 pregnant women referred to Fatemeh Hospital in Hamadan who had gestational diabetes, and 50 non-diabetic pregnant women whose blood tests confirmed they were non-diabetic. The study population in both groups was matched in terms of age and gender. After collecting blood samples and separating plasma, plasma nitric oxide levels were measured using the Griess method, and the data obtained were analyzed using SPSS software.

Results: The results of the study indicated that plasma nitric oxide levels in pregnant women with gestational diabetes were 22.17 ± 1.46 µmol/L, while in non-diabetic pregnant women, the levels were 17.16 ± 1.15 µmol/L.

Conclusion: The results showed that plasma nitric oxide levels in pregnant women with gestational diabetes were higher than in non-diabetic pregnant women. Therefore, these patients are more sensitive to the risks associated with increased plasma nitric oxide.

Keywords: Gestational diabetes, Nitric oxide, Non-diabetic pregnant

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Introduction

Diabetes is one of the most common diseases in human societies, and managing its complications imposes significant costs on healthcare systems worldwide. The most prominent clinical symptom of this disease is increased blood sugar levels. Over time, it can lead to disorders in the cardiovascular, nervous, and metabolic systems of the body. One form of diabetes is gestational diabetes, which may develop during pregnancy. According to the World Health Organization (WHO), the prevalence of gestational diabetes ranges from 1.3 to 5%, with some regions reporting figures as high as 7%. As a result, this condition is considered one of the most common complications of pregnancy (1).

Most cases of gestational diabetes occur in individuals who did not have diabetes before pregnancy and develop this type of diabetes due to physiological changes during pregnancy. Typically, this form of diabetes resolves after delivery (2). During pregnancy, hormones make it difficult for the body's cells to use insulin, prompting the pancreas to produce and secrete more insulin. If the pancreas is unable to secrete sufficient insulin and blood sugar levels rise, gestational diabetes occurs. Many women with gestational diabetes recover completely after giving birth. However, having this condition increases the risk of developing gestational diabetes in subsequent pregnancies (3).

In pregnant women with gestational diabetes, there is a concern that elevated blood sugar may lead to high blood sugar in the fetus. As a result, the fetus's pancreas must produce and secrete more insulin to burn the excess glucose. This process leads to the production of increased body fat in the fetus and increased fetus weight, especially in the upper body, resulting in a condition known as macrosomia. During vaginal delivery, a macrosomic fetus may have difficulty passing through the birth canal, potentially resulting in a broken leg or damage to the fetal nervous system. However, most newborns recover without permanent complications (4).

The most important concern during pregnancy for mothers with gestational diabetes is excessive fetal growth,

which can lead to birth trauma. More than half of women with gestational diabetes eventually develop overt diabetes in later years, and emerging evidence suggests that a wide range of complications, including obesity and diabetes, can also occur in their children. If a specialist suspects that the fetus is too large, a cesarean section may be performed. Fortunately, if gestational diabetes is well-controlled, only a small percentage of babies are born too large. However, if gestational diabetes is poorly controlled, it can affect the baby's heart function. Studies show a significant relationship between severe gestational diabetes and the risk of stillbirth in the last two months of pregnancy, and gestational diabetes has been found to double the risk of preeclampsia. Therefore, it seems essential to identify and accurately determine the factors that affect gestational diabetes (4).

Nitric oxide is a radical compound and an important reactive molecule with a short half-life, playing a significant role in many physiological processes. It is produced from arginine through the activity of the enzyme nitric oxide synthase, which is present in various tissues throughout the body. This enzyme exists in three forms in the body: 1) neural, present in tissues of the nervous system, 2) endothelial, located in vascular endothelial cells, and 3) inducible, expressed by cytokines and endotoxins in various body cells. The inducible form of this enzyme rapidly increases nitric oxide levels and increases its concentration in the body. Nitric oxide produced in the walls of blood vessels acts as an endothelium-dilating agent that increases the diameter of blood vessels and affects the cardiovascular system (5).

One of the most important functions of this compound in the body is its reaction with various radicals. This reaction is of great importance because it is an addition reaction that neutralizes these radicals, forming inactive compounds. Additionally, nitric oxide reacts rapidly with oxygen in the presence of air, producing a brown gas called nitrogen dioxide. Although nitric oxide at physiological levels can reduce the damage caused by these compounds by reacting with other radicals, an increase in the bloodstream and plasma can, in turn, cause damage to cells. Nitric oxide is synthesized in the vascular wall and acts as an endothelium-dilating agent, increasing the diameter of blood vessels and influencing the cardiovascular system (6) .

Given the importance of accurately measuring plasma nitric oxide levels as a critical laboratory and biochemical factor, and considering the studies conducted thus far, this study aimed to measure plasma nitric oxide levels in patients with gestational diabetes. No similar studies on the precise determination of this compound in gestational diabetes patients have been found. Therefore, this study strived to accurately measure plasma nitric oxide levels in people with gestational diabetes and compare it with normal and non-diabetic pregnant people, making it an important step towards identifying the mechanisms involved in gestational diabetes.

Materials and Methods

In this study, 50 pregnant women referred to Fatemieh Hospital in Hamadan in 2021 who had gestational diabetes, confirmed by their specialists, were selected as the test population. Blood sugar levels of the test population were measured at the beginning of pregnancy. The diagnosis was based on a blood sugar test performed between 24 and 38 weeks of pregnancy for women suspected of having gestational diabetes. The subjects studied were diabetic pregnant women who were either still sick or had not yet completely recovered and were undergoing treatment. Moreover, a control group consisting of 50 healthy, non-diabetic pregnant women, whose blood sugar tests confirmed their non-diabetic status and were verified by a specialist, was also included.

Plasma Separation

First, peripheral venous blood samples (5 mL) were collected from both test and control groups. The blood samples were immediately poured into tubes containing 18 mM sodium citrate buffer and centrifuged at 3500 rpm for 15 minutes at room temperature. The plasma was then separated for the measurement of nitric oxide. All plasma samples were stored at -20 ˚C until analysis.

Plasma Nitric Oxide Measurement

Plasma nitric oxide levels were measured using the Greiss method (7). For this, standard sodium nitrite solutions were prepared at concentrations of 3.125, 6.25, 12.5, 25, 50, and 100 µmol in phosphate buffer (pH = 7.4). Next, 2% sulfanilamide, 0.2% naphthyl ethylenediamine, and 5% phosphoric acid solutions were prepared separately. Each of the three solutions was mixed in a 1:1:1 ratio and added to each of the six sodium nitrite solutions. After mixing with a shaker, the absorbance of each sample was read at 540 nm wavelength using a spectrophotometer, and absorbance values were recorded in terms of the six sodium nitrite concentrations. Then, 25 µL of the triple test mixture (i.e., sulfanilamide, naphthyl ethylenediamine, and phosphoric acid) was added to 75 µL of each plasma sample. After mixing, the absorbance was measured at 540 nm using a spectrophotometer.

All steps were performed in triplicate, and the tests were repeated several times to ensure more accurate results.

Results

In this study, plasma nitric oxide levels were measured in gestational diabetes and non-diabetic pregnant women referred to Fatemeh Hospital in Hamadan.

Table 1 presents the statistical analysis of plasma nitric oxide levels in the test and control groups.

The information in Table 2 shows the comparison of plasma nitric oxide levels between two groups (gestational diabetes and non-diabetic pregnant women). According to the data in the table, the mean plasma nitrite oxide levels in the two groups were 22.17 ± 1.46 µmol/L for gestational diabetes and 17.16 ± 1.15 µmol/L for non-

diabetic pregnant women. The observed difference in plasma nitric oxide levels, exceeding 1%, was statistically significant based on the t-test. The observed difference in the amount of plasma nitric oxide levels at a level above 5% is significant.

Discussion

In gestational diabetes, instead of entering the cells and breaking down and producing energy, glucose remains in the bloodstream, which not only disrupts the metabolism of the mother and fetus but also leads to various acute and chronic metabolic complications. Cardiovascular disorders tend to appear gradually in people with gestational diabetes. As such, this study aimed to measure plasma nitric oxide levels in individuals with gestational diabetes and non-diabetic pregnant women.

Analysis of the data obtained in the results indicated that plasma nitric oxide levels were significantly higher in people with gestational diabetes compared to non-diabetic pregnant women. This difference was statistically significant, with a *P* value exceeding 1% as determined by the t-test.

In support of our findings, Ünüvar et al reported an increase in plasma nitric oxide in patients with gestational diabetes. They observed that impaired glucose metabolism in these patients led to vascular endothelial dysfunction and increased nitric oxide synthase enzyme function, thereby resulting in increased plasma nitric oxide levels. They also found that arginine, a precursor of nitric oxide, increased in cell culture media of subjects with gestational diabetes (8).

Similarly, Gao et al found that the plasma nitric oxide levels were significantly higher in women with gestational diabetes, with a statistically significant difference (*P* value < 0.05) when compared to non-diabetic pregnant women and the control group (9).

Furthermore, Valkonen et al demonstrated that the plasma nitric oxide levels were significantly elevated in women working in hospitals compared to the control group (*P* value < 0.05) (10). According to the findings of Siama et al, in radiation workers working in radiology and photography, there was a decrease in the activity of glutathione S-transferase and catalase, accompanied by an increase in lipid peroxidation reaction. They further highlighted that factors such as duration of employment, smoking, age, exposure dose, and patient volume were effective variables. Chronic low-dose exposure of hospital workers to ionizing radiation leads to reduced antioxidants in peripheral blood lymphocytes (11).

In a similar study, Ebrahimpour et al investigated radiology damage in 70 radiologists working in the radiology centers of government hospitals in Isfahan. They measured the levels of 8-hydroxy-2-deoxyguanosine (8OHdG), a marker of DNA oxidative damage, in urine samples of radiation-exposed workers. The results showed a significant difference ($P < 0.05$) in the average concentration of 8OHdG between radiation users and non-radiation users (12). Moreover, Kochanova and colleagues' study on radiologists indicated long-termgenomic instability, chromosomal aberrations, and genetic mutations in the DNA molecule among radiologists (13).

Given the role of radicals in the development of atherogenesis, carcinogenesis, and other degenerative diseases, the results of this study suggest that gestational diabetes is accompanied by increased plasma nitric oxide levels. Therefore, it is crucial to pay serious attention to this laboratory marker in people with gestational diabetes. The increase in nitric oxide levels may serve as an important prognosis for the development of additional disorders and acts as a warning that endangers the health of mothers with gestational diabetes and their newborns.

Table 1. Statistical Analysis of the Plasma Nitric Oxide Levels

Statistics (Plasma Nitric Oxide Levels)	Gestational Diabetes Women	Non-diabetic Pregnant Women
Number	50	50
Mean	22.17	17.16
SD	1.46	1.15
Standard error of the mean	0.11	0.13
Median	22.11	17.22
Mode	22.22	17.66
Minimum	19.10	15.88
Maximum	23.66	18.19

Note. SD, Standard deviation.

Table 2. Comparison of Plasma Nitric Oxide Levels Between Two Groups

Groups	Mean Difference	SE	Confidence Interval	t	*P* value
Gestational diabetes women	4.32	1.81	39.56-46.84	23.8	0.0001* (Significance)
Non-diabetic pregnant women	4.32	1.81	39.56-46.84	23.8	0.0001* (Significance)

Note. SE: Standard error. *Independent two-sample t-test.

Authors' Contribution

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Competing Interests

The authors declare no conflict of interests.

Ethical Approval

This study was approved by the Research Ethics Committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1386.199).

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