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Predictive Power of Unconjugated Estriol in Diagnosis of Gestational Diabetes: A Cohort Study

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Abstract

Background: Gestational diabetes is the most common antenatal medical complication that is associated with adverse short- and long-term maternal, fetal, and neonatal outcomes. Reducing maternal and fetal complications requires the early diagnosis of gestational diabetes. Unconjugated Estriol (UE) has led to insulin resistance under *in vitro* conditions.

Objectives: This study aimed to determine the predictive power of unconjugated estriol in the diagnosis of gestational diabetes in Tehran, Iran.

Methods: The present historical cohort study was conducted on 523 pregnant women presenting to two university-affiliated hospitals in Tehran, Iran, 2017 - 2018. The level of unconjugated estriol was determined at the 14th - 17th week of pregnancy, and gestational diabetes was diagnosed at the 24th - 28th week of pregnancy using the oral glucose tolerance test with 75 grams of glucose. Data were collected through interviews and sampling was carried out using a convenience sampling method.

Results: Out of 523 pregnant women examined, 63 (12%) were placed in the gestational diabetes group and 460 (88%) in the non-gestational diabetes group. The best cutoff point for unconjugated estriol was determined using the ROC curve as 0.965 MOM. We obtained 66.66% sensitivity, 54.78% specificity, 16.8% positive predictive value, and 92.30 negative predictive value for the UE test.

Conclusions: Given the acceptable sensitivity (66.66%) and specificity (54.78%) obtained for the UE test and the area under the ROC curve of 0.60, it appears that the UE test can be considered a new, accessible, and reliable screening test for gestational diabetes.

Keywords: Cohort Studies, Estriol, Fetus, Gestational Diabetes, Glucose, Insulin Resistance, Pregnancy, Unconjugated

1. Background

Diabetes is a metabolic disease with a constantly increasing number of affected people such that it will be the seventh cause of death by 2030 (1). Gestational Diabetes Mellitus (GDM) is one of the main forms of diabetes that is defined as varying severities of carbohydrate intolerance that initially begins or is diagnosed during pregnancy (2).

One out of every ten pregnancies worldwide is accompanied by diabetes, and 90% of these cases are gestational diabetes (3). According to the International Diabetes Federation (IDF) report in 2017, one of every seven births was a product of gestational diabetes (4), and the rate was reported as 5.88% in Iran (5). With the current increase in the worldwide prevalence of obesity, the prevalence of gestational diabetes is also increasing (6).

Gestational diabetes is associated with adverse short- and long-term maternal, fetal, and neonatal outcomes (2). The most common short-term outcomes include macrosomia, polyhydramnios, preeclampsia, stillbirth, cesarean section, and postpartum hemorrhage (7, 8). In the long-term, gestational diabetes is associated with a risk of obesity, glucose intolerance, and neonatal metabolic syndrome (9). In addition, gestational diabetes is a risk factor for the development of type-II diabetes and hypertension in mothers (9).

One of the causes of gestational diabetes is insulin resistance (10). During a normal pregnancy, the increased secretion of diabetogenic placental hormones, including the growth hormone, corticotropin-releasing hormone (CRH),

placental lactogen, and progesterone, along with maternal hormonal changes lead to insulin resistance and these changes can cause the development of gestational diabetes (11).

The trend of changes leading to gestational diabetes begins during the weeks and months prior to diagnosis, and the factors related to these changes appear in the blood before clinical diagnosis (12). The effectiveness of early-pregnancy interventions has been formerly demonstrated in reducing the risk of developing gestational diabetes and its complications. In fact, pregnancy outcomes can be improved with the early, accurate diagnosis of gestational diabetes (13, 14).

After more than 50 years of research, there is still no general consensus on the best method for the screening and diagnosis of gestational diabetes (2). Gestational diabetes is usually diagnosed at the 24th - 28th week of pregnancy. The Oral Glucose Tolerance Test (OGTT) with 75 grams of glucose is currently the standard test for the diagnosis of gestational diabetes (15). Nevertheless, this test is associated with some problems, such as the patient's need for fasting prior to the test, heavy costs, time-consuming nature of the test, conflicting results in people from different races and ethnicities, and some patients' intolerance to high amounts of powdered sugar. Some studies have proposed using 50 grams of glucose in the OGTT for the diagnosis of gestational diabetes (16), but this test is limited in terms of sensitivity and specificity (17). Moreover, the OGTT is unable to diagnose mild glucose intolerance, and this deficiency could cause perinatal complications (18). Studies investigating the predictive power of fasting plasma glucose for gestational diabetes have concluded that fasting plasma glucose in early pregnancy is an ineffective screening measure for gestational diabetes (19). Moreover, despite the serious complications of gestational diabetes, its diagnostic test is carried out during late pregnancy, when it is too late to take preventive measures for the condition (20, 21). In addition, recent evidence suggests that gestational diabetes can be prevented by interventions performed in early pregnancy. Therefore, a simple and effective strategy in early pregnancy is required (22).

Many clinical researchers are interested in studying changes in the biochemical markers that are routinely screened during pregnancy to detect adverse pregnancy outcomes early on and mitigate their complications (20). Unconjugated Estriol (UE3) is one of the biochemical markers assessed in the screening of Down's syndrome in the early second trimester of pregnancy (2) and has been considered by researchers as a new diagnostic technique for gestational diabetes (20). Estrogen is a steroid hormone that regulates glucose homeostasis by increasing insulin sensitivity, glucose-stimulated insulin secretion, and glucose transfer expression (22). In addition, postmenopausal estrogen replacement has reduced the risk of developing type II diabetes (23). Estrogen and its receptor are known as insulin sensitivity regulators (24). Meanwhile, estriol is a weak estrogen agonist that has a strong antagonistic activity along with estradiol (25). Unconjugated Estriol (UE) has led to insulin resistance under *in vitro* conditions (26). Moreover, the low levels of maternal serum UE in the second trimester have been associated with adverse pregnancy complications (27).

Few studies have investigated the predictive power of UE in gestational diabetes. A study conducted in 2017 showed a significant relationship between increased levels of UE and gestational diabetes (28). In contrast, another study reported reduced UE levels in women with gestational diabetes (29). The results of another study showed no relationships between UE levels and gestational diabetes (27).

The diagnosis of gestational diabetes using a test with high sensitivity and specificity and the prevention of its complications play major roles in improving maternal and neonatal health. Moreover, the evaluation of UE in the early second trimester can be used as a new and accessible marker of gestational diabetes.

2. Objectives

The present study was conducted to determine the predictive power of UE in diagnosing gestational diabetes in Tehran, Iran.

3. Methods

3.1. Study Design and Sampling Criteria

The present historical cohort study was conducted on 523 pregnant women who referred to the prenatal care clinics of Mahdieh and Taleghani hospitals in Tehran, capital of Iran, in 2017 - 2018.

Data were collected using a two-part form. The first part was to assess the inclusion criteria and the second part to gather demographic details, midwifery history, the checklist recording, the UE levels in weeks 14 - 17 of pregnancy, and the results of the gestational diabetes screening test with 75 grams of oral glucose in weeks 24 - 28 of pregnancy. Gestational diabetes was assessed based on the WHO instructions. The two-hour oral glucose tolerance test was performed in all the participants after taking 75 grams of glucose in weeks 24 - 28 of pregnancy (2 and 15). Fasting blood glucose of 92 or higher, blood glucose of 180 or higher after one hour of taking glucose, and 153

or higher after two hours of taking glucose were considered abnormal. The diagnosis of gestational diabetes was definitive, even with one abnormal blood glucose result (2, 15).

Sampling was of the multistage type. In the first stage, centers with the highest statistics of prenatal care referrals were selected from among all teaching hospitals affiliated to the Shahid Beheshti University of Medical Sciences. Then, a quota was given to each center based on the total number of pregnant women presenting to the center. Finally, purposive sampling was conducted in each center and continued until the required sample size was achieved.

3.2. Inclusion and Exclusion Criteria

The inclusion criteria included women with singleton pregnancy aged 20 - 40 years with a gestational age of 24 - 34 weeks (based on the first day of the last menstruation or the first-trimester ultrasound). The exclusion criteria were a history of known systemic diseases (i.e., diabetes, preeclampsia, hypertension, PCO, etc.), type II diabetes in first-degree relatives, BMI > 30 kg/m^2, a history of stillbirth, habitual abortion, fetal anomalies, fetal macrosomia, intake of medications affecting glucose metabolism, smoking, and drug use.

3.3. Sample Size

The sample size was determined as 523 women based on the prevalence of gestational diabetes of 6%, the type I error of $\alpha = 0.05$, required sensitivity of 80% (S^e), and the required accuracy of d = 0.1.

3.4. Data Collection Tools

The data collection tools included a demographic and obstetrics questionnaire, a blood glucose kit/device, and a UE kit/device. The validity of the demographic and midwifery questionnaire was assessed using the content validity method. In both centers, the blood sugar level was determined using Pars Azmun kits, Hitachi Auto-Analyzer 911 (made in Japan), and glucose oxidase method. The validity of the UE kit was measured based on the kit's manufacturer (i.e., LDN Co., Germany) in both centers. This test was conducted using the ELISA method by utilizing an ELISA reader (Hyperion Co., France). To ensure the reliability of the UE and blood glucose measuring devices, they were calibrated every morning by medical engineers. All UE and blood sugar tests were carried out by the same devices and the same laboratory technicians in both centers. Concurrent observation (inter-rater consistency) was used to determine the reliability of the technicians. For this purpose, ten blood sugar samples and 10 UE samples were concurrently tested by the two similarly-experienced technicians. The results were assessed using Pearson's correlation test (Pearson correlation coefficient = 0.95).

3.5. Data Collection

The participants' data were collected by an expert midwife. After explaining the study objectives and obtaining the informed consent of pregnant women who had visited these centers at their 24th - 34th week of pregnancy and met the inclusion criteria (ensured through the completion of the first part of the data form), the second part of the data form (demographic and midwifery details) was completed. The results of the UE level test at the 14th - 17th week of pregnancy conducted at the two centers' laboratories were recorded in the relevant checklist. We also used the relevant checklist to record the results of the screening test for gestational diabetes, which was carried out using the OGTT with 75 grams of glucose at the 24th - 28th week of pregnancy as per the center's routine. After reaching the required sample size, i.e., 523 pregnant women, the relationship between different values of UE and gestational diabetes was assessed by using statistical methods and the best UE cutoff point was determined. Then, the participants were divided into two groups based on the cutoff point. The statistical relationship between UE and the incidence of gestational diabetes was then assessed in the two groups.

3.6. Data Analysis

Data were analyzed using IBM SPSS Statistics Software, version 20 (IBM Corp., Armonk, N.Y., USA) and R software. Frequency tables were drawn using descriptive statistics at a confidence interval of 95%. The differences between the groups with and without gestational diabetes were assessed using the independent *t*-test for quantitative variables, the chi-square test for qualitative variables, the Mann-Whitney test for ordinal variables, and logistic regression. The ROC curve was used to determine the UE threshold and to determine the sensitivity and specificity of the UE test. Meanwhile, the UE optimal cutoff point for the prediction of gestational diabetes was obtained using the Youden index (J).

4. Results

Out of 523 participants, 63 were assigned to the gestational diabetes group and 460 to the non-gestational diabetes group. There were no significant differences between the participants in terms of education, occupation, housing status, gestational age, gravidity, parity, and the number of abortions.

Table 1 presents the confounding variables and the demographic and midwifery details. The mean ± standard deviation of serum UE was 1.15 ± 0.60 multiples of the median (MOM) in the gestational diabetes group and 1.05 ± 0.58 MOM in the control group. The lowest and the highest serum UE levels were 0.49 MOM and 4.05 MOM in the gestational diabetes group and 0.01 MOM and 5.06 MOM in the control group, respectively; therefore, a significant difference was observed between the two groups in terms of serum UE level (P = 0.01).

The diagnosis of gestational diabetes was made by assessing the UE level using the ROC curve (Figure 1).

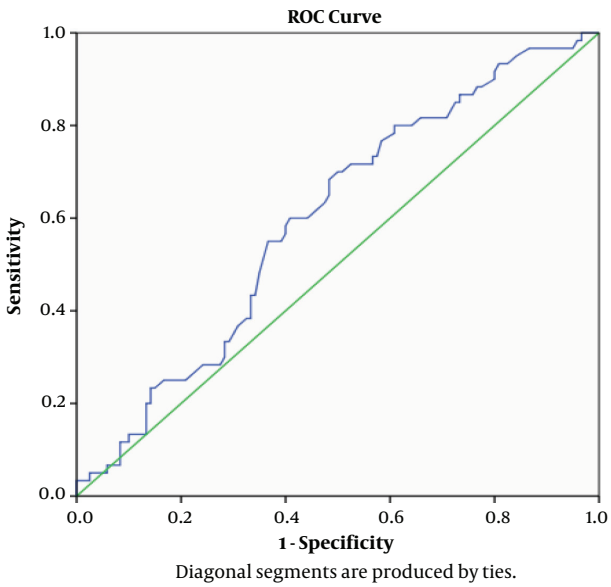

Figure 1. The UE curve for the prediction of gestational diabetes in the study participants

Using the ROC curve and after determining the minimum and maximum threshold levels of UE (Table 2), the appropriate UE threshold level for the diagnosis of gestational diabetes was obtained as 0.965 MOM. The area under the ROC curve was 0.60, which indicated the acceptability of the UE test for predicting gestational diabetes.

Based on the results of the ROC curve and the threshold value of 0.965 MOM, the UE test turned out positive for 42 participants (66.7%) in the gestational diabetes group and 208 (45.22%) in the non-gestational diabetes group (Table 3). According to the data presented in Table 3, this test had 66.66% sensitivity, 54.78% specificity, 16.8% positive predictive value, 92.30% negative predictive value, 1.21 positive likelihood ratio, and 0.73 negative likelihood ratio. The cutoff point was 0.97 estimated by the Youden index. The sensitivity, specificity, positive and negative predictive values, DLR-positive, DLR-negative, and optimal criterion were 67%, 55%, 17%, 92%, 1.47, 0.61, and 0.21 in detecting gestational diabetes by evaluating UE of the early second trimester of pregnancy, respectively.

The confounding variables (BMI and mother's age) were controlled using the logistic regression analysis, which showed that the odds of developing gestational diabetes increased by 18% per each unit of increase in BMI and by 5% per each year of increase in age. After controlling for confounding variables, the odds of developing gestational diabetes increased 2.67 times with each unit increase in the UE level (Table 4).

5. Discussion

The present findings showed that the UE test had an acceptable predictive power for diagnosing gestational diabetes. Maternal UE levels can be used to predict fetal adaptation and health (25). Unconjugated Estriol (UE) is currently used as a component of Down syndrome diagnostic tests in pregnancy (30). Moreover, the serum UE concentration is associated with factors such as the mother's weight, gestational age, and race (31).

Limited studies have been conducted on the predictive power of estriol as an indicator of gestational diabetes. In line with the present findings, Hur et al. (2017) investigated the relationship between serum UE levels and gestational diabetes in South Korea and showed that the UE levels of > 2 MOM in the early second trimester had a significant relationship with the development of gestational diabetes (20).

In a study conducted by Settiyanan et al. (2016) in Thailand to determine the relationship between abnormal UE values at the 15th - 21st week of pregnancy and adverse pregnancy outcomes, no significant relationship was found between UE levels and gestational diabetes, which is inconsistent with the present findings. This difference can be explained in a number of ways. First, in Settiyanan et al. study, participants were grouped using UE percentiles (normal, percentile ≤ 5, and percentile ≥ 95), but in the present study, grouping was based on the cutoff point of the study population and using the ROC curve. Besides, Settiyanan et al. study did not discuss their method of diagnosing gestational diabetes while differences in diagnostic methods can directly affect the study results. Other possible reasons for the disparity between the study findings could be the racial differences, as race dictates different rates of gestational diabetes in different countries (28).

Unlike the present study, in a study conducted in Turkey, Sayin et al. (2008) found a relationship between lower UE levels and gestational diabetes. Their study was conducted to investigate the results of a triple marker

Table 1. Distribution of Women with Gestational Diabetes and the Control Group in Terms of Demographic and Midwifery Details[a]

Group/Characteristic	Women with Gestational Diabetes (N = 63)	Control Group (N = 460)	Statistical Test	P Value
Age, y	30.2 ± 4.78	5.38 ± 28.56	Independent *t*	0.022
Education (high school)	37 (58.7)	268 (58.3)	Mann-Whitney	0.128
Mother's job (including housewifery)	57 (90.5)	426 (92.6)	Chi-square	0.550
Spouse's job (self-employed)	44 (69.8)	369 (80.2)	Chi-square	0.146
Gestational age	31.25 ± 4.36	3.47 ± 32.41	Independent *t*	0.142
Gravidity	2.00 (1.00 - 3.00)	2.00 (1.00 - 3.00)	Mann-Whitney	0.486
Parity	1.00 (1.00 - 2.00)	1.00 (1.00 - 2.00)	Mann-Whitney	0.591
Number of abortions	1.00 (1.00)	1.00 (1.00)	Mann-Whitney	0.273
First-trimester BMI	25.98 ± 2.57	3.26 ± 24.38	Independent *t*	0.000
Housing status (renting)	43 (68.3)	325 (70.7)	Chi-square	0.703

[a]Values are expressed as mean ± SD, No. (%), or median (IQR).

Table 2. Sensitivity and Specificity of Serum UE Levels Based on Different Cutoff Values in Study Participants

Unconjugated Estriol	Sensitivity	Specificity
0.925	0.683	0.496
0.935	0.683	0.483
0.945	0.683	0.476
0.955	0.667	0.463
0.965	0.667	0.452
0.975	0.635	0.443
0.985	0.619	0.426
0.995	0.603	0.411
1.005	0.587	0.393

Table 3. Relative Frequency of Serum UE Based on the Threshold Value (the Cutoff Point) of 0.965 MOM in the Gestational Diabetes and Control Groups

Unconjugated Estriol	Women with Gestational Diabetes	Women without Gestational Diabetes (Control Group)
0.965 ≥	21 (33.3)	252 (54.78)
0.965 <	42 (66.7)	208 (45.22)
Total	63 (100)	460 (100)

Table 4. Determining the Odds of Developing Gestational Diabetes in Terms of Some Risk Factors Using the Logistic Regression

Variable	P Value	Odds Ratio	Confidence Interval
UE	0.001	2.67	1.51 - 4.73
BMI	0.001	1.18	1.07 - 1.30
Age	0.049	1.05	1 - 1.11

screening test at the 16th - 18th week of pregnancy, and using the ROC curve, the threshold for the diagnosis of gestational diabetes was measured as 0.88 MOM and the area under the ROC curve was measured as 57%. They reported UE test sensitivity of 36.2% and specificity of 78.5%. The possible reasons for the differences in results can be the diagnosis of gestational diabetes using the OGTT with 100 grams of glucose and determining the UE cutoff point using MedCalc V. 4.30. In the present study, the cutoff point was found using SPSS V. 20 and R software. Furthermore, Sayin et al. study was retrospective and aimed to investigate several adverse pregnancy outcomes, which may have reduced the accuracy of the study, as not all the confounding factors of gestational diabetes were controlled. Moreover, in Sayin et al. study, the UE level was measured by radioimmunoassay, but the present study used the ELISA method (29).

During pregnancy, the maternal hormones are regulated by the pituitary gland and the placenta and these hormones have major roles in the mother's development of insulin resistance (32). Estrogen receptors are expressed in the pancreatic Langerhans islet beta cells and beta-17 estradiol is also associated with the increased biosynthesis and secretion of insulin (33). Moreover, estriol has an antiestrogenic activity together with estradiol (34). Estriol inhibits the bond between estradiol and estrogen receptors (35). In an *in vitro* environment, estriol is known as an insulin resistance factor (26). Consequently, increased estriol in pregnancy could likely be associated with insulin resistance.

The UE test is often performed in the early second trimester to screen for Down syndrome. It removes the need for incurring costs and running tests in research centers for the diagnosis of gestational diabetes. Meanwhile, the OGTT is carried out at the 24th - 28th week of pregnancy at extra costs and has a poor predictive power in early pregnancy (19). Given the increasing prevalence of gestational

diabetes in Iran and across the world and the need for its early diagnosis to reduce adverse maternal and fetal complications, a convenient marker such as UE examined in early pregnancy can be extremely helpful.

The strengths of this study include the use of the latest gestational diabetes diagnostic criteria, controlling for confounding variables, the use of R software in conjunction with SPSS, and the use of the Youden index for determining the best cutoff point. In addition, all the participants underwent the two-hour glucose tolerance test for the diagnosis of gestational diabetes by taking 75 grams of oral glucose. The study limitations include recording the patients' disease history in a self-report way, which could be associated with misunderstandings about the medical status along with the UE level.

5.1. Conclusions

Given the need for the prevention and early diagnosis of gestational diabetes to reduce the resultant maternal and fetal complications, UE can be used as a gestational diabetes predictive factor. Measures should be taken to train all health service providers in this regard so that the risk of gestational diabetes is taken into account when noticing an increase in UE levels in the test results of pregnant women; therefore, immediate preventive and medical measures can be taken to reduce the complications. Since the prevalence of gestational diabetes is rising, further prospective studies with larger sample sizes are recommended for achieving more powerful results.

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Footnotes

Authors' Contribution: Study concept and design and analysis and interpretation of data: Azam Amirian, Nourossadat Kariman, Nasrin Borumandnia, Mehdi Hedayati, and Zohre Sheikhan; drafting of the manuscript: Azam Amirian and Nourossadat Kariman; critical revision of the manuscript for important intellectual content: Azam Amirian, Nourossadat Kariman, and Nasrin Borumandnia; statistical analysis: Azam Amirian and Nasrin Borumandnia.

Conflict of Interests: There are no conflicts of interest to disclose.

Ethical Approval: This study was approved by the Research Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (code number: IR.SBMU.PHNM.1396.777).

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Patient Consent: After explaining the study objectives, informed consent was obtained from all participants.

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