ROLE OF MATERNAL SERUM FIBROBLAST GROWTH FACTOR 21 LEVEL IN PREDICTION OF PREECLAMPSIA

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Summary

Pregnancy complications resulting from hypertensive disorders are a serious problem that impact 2-10 % of all pregnancies globally. Preeclampsia is defined as new onset hypertension and proteinuria after 20 weeks of gestation which may be associated with other maternal organ dysfunction, such as liver or renal insufficiency, hematological or neurological complications, uteroplacental dysfunction and fetal growth restriction.

Aim of the Work: Primary outcome is to determine the correlation between fibroblast growth factor 21 level and preeclampsia & significance of serum fibroblast growth factor 21 levels as a predictive tool for preeclampsia. Secondary outcome is to establish the relationship between maternal serum fibroblast growth factor 21 level and Maternal complications:

- Eclampsia (tonic clonic fits after 20 weeks with elevated blood pressure and proteinuria)
- HELLP syndrome (hemolysis, elevated liver enzymes and low platelet occur as a complication of severe preeclampsia)
- placental abruption (bleeding after 20 weeks gestation due to premature separation of a normally situated placenta)

Patients and Methods: This was a Nested case-control study that was conducted on 90 primigravidas at Ain Shams University maternity hospital from April 2021 to April 2022. Ethical approval was obtained from the ethical committee at Ain-Shams university maternity hospital. Data was recruited from patient attending obstetric clinic at Ain Shams University Maternity Hospital. Blood samples were collected after 20 weeks to 28 weeks gestation. Patients was followed up until delivery and grouped according to development of preeclampsia.

Random samples from 45 patients (group who develop PE) and 45 random samples from control group were assessed for fibroblast growth factor 21. Serum fibroblast growth factor 21 levels were measured by a commercially available enzyme-linked immunosorbent assay kit.

Results: our study showed that there was a significant difference in FGF21 levels between the groups, patients with preeclampsia having higher levels than controls. 15.9 % of patients with preeclampsia experienced maternal complications compared to none in the control group. Meanwhile, 18.2 % of patients with preeclampsia experienced fetal complications compared to 0 % in the control group.

Conclusion: Serum FGF-21 levels are significantly higher in preeclamptic pregnant women compared to healthy normotensive pregnant women. So, it can be used as a predictor for preeclampsia and maternal complications

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Key words: Serum Fibroblast 21; Preeclampsia; Primigravida.

Introduction

Up to 10 % of pregnancies in developing countries result in complications due to preeclampsia (PE), where emergency care is frequently insufficient or nonexistent. [1]

The measurement of maternal blood pressure and proteinuria is essential for the diagnosis of PE; however, these parameters have poor predictive power for unfavorable outcomes for both the mother and the fetus. Thus, there is a pressing need for broadly applicable, reasonably priced tests that can reliably identify women who are at risk and identify which fetus may have complications. By giving these patients and their offspring the best prenatal care possible, we can prevent morbidity and improve the outcome of the newborn. [2]

Preeclampsia shares many risk factors with cardiovascular, chronic kidney disease and metabolic diseases. The relationship between preeclampsia and the level of fibroblast growth factor 21 has been rarely studied. [3]

Fibroblast growth factor family are responsible for cell growth and differentiation, wound repair, embryogenesis and angiogenesis. As a member of this family, fibroblast growth factor 21 is a metabolic regulator with beneficial effects on lipid and glucose metabolism, it also plays a role in many disease such as cardiovascular and chronic kidney disease. [4]

The study was to explore the possible role of fibroblast growth factor 21 in preeclampsia and provide new theoretical basis for the prevention and treatment of preeclampsia. [5]

Patient and methods

After ethical committee approval and informed consent from patients, this was a nested case-control study that was conducted on 500 healthy pregnant women, recruited from the Obstetrics outpatient clinic at Ain Shams University Maternity hospital from April 2021 to April 2022. Participants included in this study were 18-40 years old primigravida pregnant ladies in single viable fetus 20-28 weeks gestational age, with BMI (20-30) kg/m2. Pregnant ladies aged less than 18 or more than 40 years old, or with previously diagnosed maternal disease e.g DM, chronic hypertension, thrombophilias were excluded from the study. Any participant with fetal comorbidity e.g CFM were excluded too.

All women included are subjected to detailed medical history including past medical history and the date of the first day of LMP to calculate the gestational age. They
also underwent physical examination including BMI, blood pressure measurement and abdominal examination to assess the fundal level and the viability of the fetus. Obstetric ultrasound was done to all of the women included in the study to assess fetal biometry and exclude any fetal abnormalities in addition to CBC and Serum fibroblast growth factor 21 analysis for samples of target patients.

Blood samples were withdrawn from healthy pregnant primigravidas 20-28 weeks gestational age. Samples were centrifuged and then the supernatant were freeze-dried at −80 °C. Patients were followed up until delivery and grouped according to development of preeclampsia. It targeted reaching a sample of (45) initially healthy primigravidas who subsequently develop preeclampsia and (45) healthy primigravidas who continued their pregnancies without any maternal or fetal complication. Criteria for the diagnosis of preeclampsia are illustrated in Fig1 and 2. Serum fibroblast growth factor 21 levels were measured by a commercially available enzyme-linked immunosorbent assay kit. The detection range was 31 ng/L–200 ng/L. The main outcome of the study is to determine the correlation between maternal serum fibroblast growth factor 21 level and preeclampsia & significance of serum fibroblast growth factor 21 levels as a predictive tool for preeclampsia. Secondary outcome is to establish the relationship between maternal serum fibroblast growth factor 21 level and maternal and fetal morbidities e.g. IUGR, oligohydraminos, abruptio placentae.

**Criteria for the diagnosis of preeclampsia**

**Systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg on two occasions at least four hours apart after 20 weeks of gestation in a previously normotensive patient:**

If systolic blood pressure is ≥160 mmHg or diastolic blood pressure is ≥110 mmHg, confirmation within 5 minutes is required.

Proteinuria ≥0.3 grams in a 24-hour urine specimen or protein (mg/dL)/creatinine (mg/dL) ratio ≥20.3

Dipstick ≥1+ if a quantitative measurement is unavailable.

**In patients with new-onset hypertension without proteinuria, the new onset of any of the following is diagnostic of preeclampsia:**

- Platelet count <100,000/microliter
- Serum creatinine >1.1 mg/dL or doubling of serum creatinine in the absence of other renal disease
- Liver transaminases at least twice the normal concentrations
- Pulmonary edema
- Cerebral or visual symptoms


**Figure 1: Diagnostic criteria of Preclampsia [6]**

The presence of one or more of the following indicates a diagnosis of "preeclampsia with severe features”

| Symptoms of central nervous system dysfunction: |
| New onset, headache, or visual disturbance, such as: |
| Severe headache (worse when lying flat) |
| Severe vertigo |
| Seizures |
| Altered mental status |

| Hepatic abnormality: |
| Severe persistent right upper quadrant or epigastric pain unresponsive to medication and not accounted for by an alternative diagnosis or serum transaminase concentration ≥2 times normal, or both |

| Severe blood pressure elevation: |
| Hypertensive urgency (systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥100 mmHg on two occasions at least four hours apart while the patient is on bedrest) |

| Thrombocytopenia: |
| Platelet count <100,000/microliter |

| Renal abnormality: |
| Progressive renal insufficiency (serum creatinine >1.1 mg/dL or doubling of serum creatinine concentration in the absence of other renal disease) |

| Pulmonary edema |

In contrast to older criteria, the 2013 criteria do not include proteinuria >5 grams/24 hours and fetal growth restriction as features of severe disease.


**Figure 2: Criteria of severity of Preclampsia [6]**
**Results**

Table (1) shows that there was no significant difference in age and BMI between the groups, but there was a significant difference in GA, with patients with preeclampsia having a lower GA than controls.

Table 2 shows that 15.9% of patients with preeclampsia experienced maternal complications compared to 7% in the control group. Meanwhile, 18.2% of patients with preeclampsia experienced fetal complications compared to 8% in the control group (table 2).

### Table 1

**Comparison between control and cases regarding age, GA and BMI**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cases</th>
<th>t test</th>
<th>p value</th>
<th>sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td></td>
<td>24.55</td>
<td>2.98</td>
<td>24.18</td>
<td>5.39</td>
<td>0.392</td>
</tr>
<tr>
<td>GA</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>t</td>
</tr>
<tr>
<td></td>
<td>38.16</td>
<td>2.21</td>
<td>34.97</td>
<td>2.82</td>
<td>5.902</td>
</tr>
<tr>
<td>BMI</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>t</td>
</tr>
<tr>
<td></td>
<td>26.53</td>
<td>2.18</td>
<td>26.28</td>
<td>2.31</td>
<td>0.528</td>
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</table>

### Table 2

**Comparison between groups regarding secondary outcome**

<table>
<thead>
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<th>Mean</th>
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<tbody>
<tr>
<td>Maternal comp</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Fetal comp</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
</tr>
</tbody>
</table>

### Table 3

**Median levels of fibroblast growth factor 21 (FGF21) between patients with preeclampsia and controls using Mann Whitney’s test.**

<table>
<thead>
<tr>
<th>FGF21</th>
<th>Controls</th>
<th>Median (IQR)</th>
<th>Range</th>
<th>Cases</th>
<th>Median (IQR)</th>
<th>Range</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(108.9-174.9)</td>
<td>59.78-216.5</td>
<td></td>
<td>(446.95-791.15)</td>
<td>229.1-1447</td>
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</tbody>
</table>

The Mann Whitney’s test was used to assess the statistical significance of the differences between the groups. The results indicate that there was a significant difference in FGF21 levels between the groups, with patients with preeclampsia having higher levels than controls.

### Table 4

**Median levels of fibroblast growth factor 21 (FGF21) between patients with and patient without maternal complication using Mann Whitney’s test.**

<table>
<thead>
<tr>
<th>FGF21</th>
<th>No maternal complication</th>
<th>Maternal complication</th>
<th>Mann Whitney’s test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Range</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td></td>
<td>(350.9-674.6)</td>
<td>229.1-1353</td>
<td>(1019-1264)</td>
</tr>
</tbody>
</table>

The Mann Whitney’s test was used to assess the statistical significance of the differences between the groups. The results indicate that there was a significant difference in FGF21 levels between the groups, with patients with maternal complication having higher levels than those without.

### Table 5

**Median levels of fibroblast growth factor 21 (FGF21) between patients with and patient without fetal complication using Mann Whitney’s test.**

<table>
<thead>
<tr>
<th>FGF21</th>
<th>No fetal complication</th>
<th>Fetal complication</th>
<th>Mann Whitney’s test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Range</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td></td>
<td>(398.55-855.3)</td>
<td>229.1-1353</td>
<td>(542.85-682)</td>
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</table>
Table (5) shows the results of a Mann Whitney’s test comparing the median levels of fibroblast growth factor 21 (FGF21) between patients with and without fetal complication among cases. The median and interquartile range (IQR) are reported for both groups. The Mann Whitney’s test was used to assess the statistical significance of the differences between the groups. The results indicate that there was no significant difference in FGF21 levels between the groups.

Table 6

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FGF21</td>
<td>p value</td>
<td>FGF21</td>
</tr>
<tr>
<td>CFFDNA</td>
<td>0.011</td>
<td>0.956</td>
<td>0.994</td>
</tr>
<tr>
<td>Age</td>
<td>0.12</td>
<td>0.438</td>
<td>-0.074</td>
</tr>
<tr>
<td>GA</td>
<td>-0.093</td>
<td>0.548</td>
<td>-0.42</td>
</tr>
<tr>
<td>BMI</td>
<td>0.306</td>
<td>0.043</td>
<td>-0.06</td>
</tr>
</tbody>
</table>

Table (6) shows the Spearman correlation coefficients and p-values for the association between fibroblast growth factor 21 (FGF21) levels and age, gestational age (GA), body mass index (BMI), and cell-free fetal DNA (CFFDNA) in patients with preeclampsia and controls. The results show that there was a significant positive correlation between FGF21 levels and CFFDNA in patients with preeclampsia (rs = 0.994, p < 0.001) but not in controls. There was also a significant negative correlation between GA and FGF21 levels in patients with preeclampsia (rs = –0.42, p = 0.005) but not in controls (rs = –0.164, p = 0.385).

Figure (3) shows the results of a receiver operating characteristic (ROC) curve analysis for cell-free fetal DNA (FGF21) to differentiate between patients with preeclampsia and controls. The area under the curve (AUC) is a measure of the accuracy of the test, the results indicate that FGF21 has an AUC of 1.0, indicating perfect discrimination between patients with preeclampsia and controls.

The table shows the results of a receiver operating characteristic (ROC) curve analysis for fibroblast growth factor 21 (FGF21) to detect maternal complications among patients with preeclampsia. The area under the curve (AUC) is a measure of the accuracy of the test, the results indicate that FGF21 has an AUC of 0.977, indicating excellent discrimination between patients with and without maternal complications. It also shows the optimal cutoff point for FGF21, which is >918.5, with a corresponding sensitivity of 100 %, meaning that it correctly identifies all patients with maternal complications. It also has a specificity of 97.3 %, meaning that it correctly identifies most of the patients without maternal complications. The +PV and -PV are 87.5 % and 100 %, respectively, meaning that there are few false positives and no false negatives in the test.
Discussion

Preeclampsia (PE) complicates up to 10% of pregnancies in developing nations, despite the fact that emergency care is often inadequate or nonexistent in these areas. [7]

While measuring maternal blood pressure and proteinuria is necessary for the diagnosis of PE, these indicators have a relatively low sensitivity and specificity in terms of predicting adverse outcomes for the mother and the fetus. [8]

Thus, there is a pressing need for tests that can reliably identify women who are at risk and predict which fetus may have complications. These tests also need to be widely applicable and reasonably priced. By guaranteeing that these patients and their offspring receive the best prenatal care available, this will enable the prevention of morbidity and the improvement of neonatal outcome. [8]

Many of the risk factors for preeclampsia are also associated with cardiovascular disease, chronic renal disease, and metabolic disorders. Preeclampsia and the level of fibroblast growth factor 21 have only seldom been examined in conjunction with one another as a link. [7]

Our study was done among Egyptian primigravidas pregnant in single fetus 20-28 weeks gestational age attending to maternity hospital – Ain shams university, Measuring the maternal serum fibroblast growth factor 21 and establishing the relationship between maternal serum fibroblast growth factor 21 and preeclampsia.

We concluded that there was no significant difference in age and BMI between the groups, but there was a significant difference in GA, with patients with preeclampsia having a lower GA than controls (Table 1).

Our study’s findings concurred with those of Jiang et al. [7], who investigated the relationship between PE and the serum level of fibroblast growth factor (FGF) 21. His study included 80 cases in total, including 49 cases in the PE group and 31 cases in the control group (normal pregnancy). PE was split into two groups: late-onset PE (LOPE) and early-onset PE (EOPE). Prior to delivery, biochemical parameters and the serum level of FGF21 were measured. Regarding mother age, the multipara ratio, and pre-pregnancy BMI, there were no statistically significant differences between the groups (P > 0.05).

Our study showed that there was a significant difference in FGF21 levels between the groups, patients with preeclampsia having higher levels than controls. The median level of FGF21 in patients with preeclampsia was 585.5 pg/ml, while the median level of FGF21 in the control group was 131.7 pg/ml (Table 3).

The findings of Stepan et al. [9] were consistent with our study, which demonstrated that the serum FGF21 level in PE patients was significantly higher than that of a normal pregnancy (maternal FGF-21 serum concentrations in PE patients were nearly three times higher (309.6 ng/l) than in healthy age-matched pregnant women (105.2 ng/l)).

Similarly, Buell et al. [8] sought to examine the serum FGF-21 profile during each trimester of pregnancy in healthy and mildly preeclamptic pregnant women. In a nested case-control study within a longitudinal cohort study comprising healthy (n = 54) and mildly preeclamptic (20) pregnant women, women three months postpartum (n = 20), and eumenorrheic women during the menstrual cycle (n = 20), serum FGF-21 levels were ascertained by ELISA. They demonstrated that during the three trimesters of pregnancy, maternal levels of FGF-21 were significantly higher in preeclamptic pregnant women than in healthy normotensive pregnant women. Moreover, FGF-21 serum levels peak in the third trimester of pregnancy after being considerably lower in the first and second trimesters of pregnancy in both healthy (p < 0.0000) and preeclamptic pregnant women (p < 0.0036).

Jiang et al. [7] likewise demonstrated that serum FGF21 level of PE group (462.53 ± 188.00 ng/L), EOPE group (510.76 ± 172.26 ng/L) and PE group with complication (517.26 ± 195.88 ng/L) was respectively higher than that of control group (366.85 ± 191.49 ng/L). FGF21 level of EOPE group was significantly higher than that of LOPE group (403.33 ± 193.36 ng/L). When compared with PE

<table>
<thead>
<tr>
<th></th>
<th>AUC</th>
<th>95% CI</th>
<th>P value</th>
<th>sig.</th>
<th>cutoff point</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>+PV</th>
<th>-PV</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGF21</td>
<td>0.977</td>
<td>0.879 to 0.999</td>
<td>&lt;0.001</td>
<td>S</td>
<td>&gt;918.5</td>
<td>100</td>
<td>97.3</td>
<td>87.5</td>
<td>100</td>
</tr>
</tbody>
</table>

Figure 4: ROC curve of FGF21 to detect maternal complications among cases.
group who had no complication (376.11 ± 139.84 ng/L), PE group with complication had higher FGF21 level.

Also, Abd Elmagid et al. [10] examined the serum levels of fibroblast growth factor-21 in patients with mild and severe preeclampsia; the severe group showed a significant increase, nearly two times higher than the control group and 1.5 times higher than the mild group.

In contrary to our research, Nitet et al. [11] found no significant difference in serum FGF21 level between PE patients and normal pregnant women. Circulating FGF21 was examined in maternal and cord blood samples of 10 mother-baby dyads in each group. FGF21 levels in maternal blood were comparable in women with and without PE (PE 0.44(0.3-1.00) ng/ml vs. control 0.38(0.21-0.66), P=0.38). In most cord blood samples, FGF21 was below the detection threshold (0.03ng/ml), but in two cord blood samples from normotensive pregnancies and four cord blood samples from PE pregnancies, it was slightly above the detection threshold. This contradictory results could be explained by variations in characteristics of the study participants such as BMI, insulin resistance, gestation of blood sampling and parity.

Our study showed that 15.9 % of patients with preeclampsia experienced maternal complications compared to none in the control group. Meanwhile, 18.2 % of patients with preeclampsia experienced fetal complications compared to 0 % in the control group (Table 2).

We used the results of a receiver operating characteristic (ROC) curve analysis for FGF21 to differentiate between patients with preeclampsia and controls. The area under the curve (AUC) is a measure of the accuracy of the test, the results indicate that FGF21 has an AUC of 0.977, indicating excellent discrimination between patients with and without maternal complications. The table also shows the optimal cutoff point for FGF21, which is >918.5, with a corresponding sensitivity of 100 %, meaning that it correctly identifies all patients with maternal complications. It also has a specificity of 97.3 %, meaning that it correctly identifies most of the patients without maternal complications. The +PV and -PV are 87.5 % and 100 %, respectively, meaning that there are few false positives and no false negatives in the test (Fig 4).

The results of our study were in accordance with Buell et al. [8] showed that the area under the receiver operating characteristic curve (AUC ROC) for predicting the development of the adverse maternal outcome of mild preeclampsia (dependent variable) from serum levels of FGF-21 levels (independent variable) was determined in the 1st (0.681 (95 % confidence interval 0.537-0.826)), 2nd (0.644 (95 % confidence interval 0.501-0.788)) and 3rd (0.680 (95 % confidence interval 0.523-0.836)) trimesters of pregnancy.

In correlation with our study, there were other studies done among primigravidas pregnant in single fetus 20-28 weeks gestational age measuring cell free fetal DNA and establishing its relation to preeclampsia [10-20]. The Spearman correlation coefficients and p-values for the association between fibroblast growth factor 21 (FGF21) levels with age, gestational age (GA), body mass index (BMI), and cell-free fetal DNA (CFDDNA) in patients with preeclampsia and controls was shown in Table 6. The results showed that there was a significant positive correlation between FGF21 levels and CFDDNA in patients with preeclampsia (rs = 0.994, p < 0.001) but not in controls. There was also a significant negative correlation between GA and FGF21 levels in patients with preeclampsia (rs = −0.42, p = 0.005) but not in controls (rs = −0.164, p = 0.385).

The cut-off points in the levels of FGF-21 obtained through the different ROC curves in each trimester of pregnancy could contribute to the risk prediction of preeclampsia and further studies are needed to confirm a relationship between FGF-1 and Log (sFlt-1)/Log (PlGF) ratio and the outcome of preeclampsia [21-26].

**Conclusion**

Finally, our results showed that serum FGF-21 levels are significantly higher in preeclamptic pregnant women compared to healthy normotensive pregnant women. So, it can be used as a predictor for preeclampsia and maternal complications.
Decrease during Early Pregnancy in Normotensive Pregnant Women but Are Higher in Preeclamptic Women—A Longitudinal Study.


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РОЛЬ МАТЕРІНСЬКОГО РІВНЯ СИРОВАТКОВОГО ФАКТОРА РОСТУ ФІБРОБЛАСТІВ 21 У ПРОГНОЗУВАННІ ПРЕЕКЛАМПСІЇ

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Університет Айн Шаме
(Кайр, Єгипет)

Резюме.

Вступ. Ускладнення вагітності внаслідок гіпертонічних розвалід є серйозною проблемою, яка стосується 2-10 % усієї вагітності у світі. Прецлампсія визначається як гіпертонія та протеїнурія, які виникають після 20 тижнів вагітності, що можуть бути пов’язані з дисфункцією інших органів матері, наприклад, печінковою або нирковою недостатністю, гематологічними або неврологічними ускладненнями, матково-плацентарною дисфункцією та обмеженням росту плода.
Мета роботи: встановити зв’язок між рівнями сироваткового фактора росту фібробластів 21 матері та інсультами у якості предиктору прееклампсії.

Пациєнти та методи: дослідження типу «випадок-контроль», яке було проведено на 90 першовагітних у пологовому будинку університету Айн Шамс з квітня 2021 року по квітень 2022 року.

Результати: наше дослідження показало, що існувала значна різниця в рівнях FGF21 між групами, а саме, пацієнтки з прееклампсією мали вищі рівні, ніж у контрольній групі, 15,9 % пацієнток з прееклампсією мали материнські ускладнення порівняно з відсутністю жодного випадку у контрольній групі. Водночас у 18,2 % пацієнток із прееклампсією спостерігалися ускладнення у плода порівняно з 0 % випадків у контрольній групі.

Висновок: рівень FGF-21 у сироватці крові значно вищий у вагітних з прееклампсією порівняно зі здоровими вагітними жінками, які мають нормальний тиск. Отже, його можна використовувати як предиктор розвитку прееклампсії та ускладнень у матері.

Ключові слова: сироватковий фібробласт 21; прееклампсія; примігравіда.

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