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Determination of Preterm Labour with pIGFBP-1 and Cervical Biometrics

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Abstract

Introduction: A staggering 30% of deaths in neonates are caused by preterm births. The most common cause of perinatal morbidity and mortality around the world is due to preterm births, also referred to as premature. Hence, the ability to predict preterm births would result in significantly reduced fatalities.

The likelihood of predicting a premature birth can be measured by the cervical length; however, there are other diagnostic procedures which utilize the contents of the cervico-vaginal fluid (CVF) for a more precise diagnosis. The latter also aids in detecting other pregnancy related anomalies. The phosphorylated insulin-like growth factor binding protein-1 (pIGFBP-1) is an example of a clinical biomarker which is frequently tested as it proves to be an indicator to predict preterm labour.

Aim: Thus, in this study, our aim was to evaluate the accuracy of pIGFBP-1 as a marker of preterm labour when coupled with cervical biometrics.

Materials and methods: A cross-sectional study of 32 pregnant women was conducted. The patients were split into 2 groups in which Group A consisted of those at risk of preterm labour and Group B – of women with normal and uncomplicated pregnancies. All participants were tested for pIGFBP-1 along with gathering data of transvaginal measurements of their cervical length.

Results: We found in all participants in the study a moderate association between the expression of pIGFBP-1 in the CVF and the onset of preterm birth. Eight patients showed positive results amongst the symptomatic patients and as predicted, within 14 days, 6 of those patients did go into preterm labour.

Conclusion: Appropriate scanning for pregnant women can be of excellent value as it has the potential to reduce the number of premature babies being born; this would also mean that the health complications associated with premature births can be prevented.

INTRODUCTION

In 2014, 14 835 606 babies (10.6% of all live births) were born before completing 37 weeks of gestation. What is alarming, though, is that WHO Global Preterm Birth estimates that there is a gradual increase in the percentage of preterm labour. There are screening programs available which are globally promoted to combat this phenomenon which is

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the leading cause of worldwide mortality and morbidity.[1] However, despite all that is put to find the best method for predicting and decreasing the rate of preterm labour, the results have not been satisfactory and the rate is rising.

The exact pathogenesis of preterm labour is not well understood, but the etiology is widely studied, and it is associated with many known risk factors. High risk factors such as obstetrical history of a previous preterm birth, infections as well as cervical, vascular or placental insufficiencies, are all well documented and studied.

Regardless of cause, the terminal process, which is activated in preterm labour, is the same. It is initiated by activation of the membranes, increase in the uterine contractility, dilation of the cervix followed by effacement. This is a process which the obstetrician must be familiar with and understand the mechanism of to take preterm labour. If an obstetrician can accurately predict this, postponing preterm labour is made far easier.

Measuring the cervical length has been the main method used in the routine practice for predicting preterm births. For a more precise prognosis, however, the cervico-vaginal fluid (CVF) provides a versatile diagnostic method used in many conditions.[2-5] The fetal fibronectin (fFN) found in CVF is the most commonly used clinical biomarker test for the prediction of preterm labour. Phosphorylated insulin-like growth factor binding protein-1 (pIGFBP-1) is another biomarker with a potential of everyday use in practice.

pIGFBP-1 is a protein produced by the decidua – tiny amounts are released into the cervix and found in the CVF once the membranes have been activated and the process of labour starts. If this is found in a pregnant woman before she has reached 37 weeks of gestation, it would be classified as abnormal findings thus indicating a risk of preterm labour.[6]

Tests were carried out during pregnancies where unphosphorylated and phosphorylated IGFBP-1 were detected. The amniotic fluid consists of high concentrations of unphosphorylated IGFBP-1 (npIGFBP1), so its presence in the cervical vaginal secretions would signal premature rupture of the amniotic sac resulting in a preterm birth. The role of pIGFBP-1 has been studied and it was found to be a predictor of preterm birth as it predicts cervical maturation.[7]

Studies have shown pIGFBP-1 to have adequacy to predict premature labour to 80%–90% sensitivity with a comparable specificity of 75%–90% for a 10% screen-positive rate. The outcome of these studies provides results which prove a better prognosis for symptomatic patients, predicting preterm birth within a range of 7-14 days.[8,9]

AIM

The study was conducted to evaluate the cervical biometrics and the pIGFBP-1 at the population studied as screening methods for preterm labour. The ultimate aim is to decrease the rate of preterm births, fetal morbidity and mortality in the area.

MATERIALS AND METHODS

At Dr. Georgi Stranski University Hospital for Active Treatment in Pleven, Bulgaria, a prospective cohort study was conducted with 32 pregnant women. They were divided into two groups: Group A consisted of women who were at risk of premature labour, and Group B consisted of participants with non-complicated pregnancies. The criterion for women to be put into group A was to have at least one of the following symptoms: uterine contractions, vaginal discharge or abdominal pressure. The criterion for Group B was that patients were not at risk of preterm birth from their history. They had no changes in status during prenatal checks and were patients that were hospitalized for routine examinations. Women that had an amniotic sac rupture or leakage of amniotic fluid prior to the test were not included in the study, along with patients that had cervical dilation greater than 3 cm, to keep the independent variables to a minimum.

Group A was made up of 12 women and Group B of 20 women. Both groups had the pIGFBP-1 test performed, followed by a transvaginal ultrasound measurement of their cervical length. The women involved in the cohort study were between 24 and 32 weeks of gestation when the test for pIGFBP-1 was conducted.

The pIGFBP-1 testing was made for all participants using the patented product Actim Partus, produced by MEDIX BIOCHEMICA.[10] It is a rapid immunochromatographic strip test which detects the presence of phosphorylated IGFBP-1 in the CVF by monoclonal antibodies 1. A concentration greater than or equal to 10 µg/L of pIGFBP-1 in the CVF is considered to be a positive test result, which indicated that the woman is at risk of going into preterm labour.

To establish indicators leading to the occurrence of preterm birth, a binary logistic regression analysis was performed. Different indicators were assessed, including cervical length, pregnancy complaints as well as previous history of preterm labour. The cervical length was measured by transvaginal ultrasound of the cervix following the protocol of the Fetal Medicine Foundation.[11] During every examination there were 5 criteria to be looked for:

1. Cervical length, to be measured on the line passing through the cervical canal that is hypoechogenic compared to the cervical walls;
2. The diameter of the internal opening of the cervical canal;
3. The width of the cervix around the internal opening of the cervix;
4. The width of the front uterine wall where the lower uterine segment is;
5. The angle between the cervix and the posterior uterine wall.

The following ultrasound findings are specific to patients with insufficiency of the cervix, which makes them more vulnerable to go into premature labour.[12-14]
- A cervical length of less than 25 mm;
- An obtuse posterior angle that is greater than 90°;

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- An internal opening of the cervix of a diameter larger than 6 mm.

The results from the tests were double blinded; therefore, all the women in group A received tocolytics and corticosteroids because of the risk of preterm labour. Administering this therapy was due to following the standard protocol; as all of them received it, the effects of the therapy are negligible due to it being a controlled variable.

The research study was evaluated at the beginning and strictly monitored by the Ethics Committee for Research Studies of the Medical University of Pleven (No. 565-КЕНИД/ 07.05.2019). The data were analysed using the statistical package of IBM SPSS Statistics 25.0 and MedCalc Version 14.8.1. For a significance level at which the null hypothesis is rejected, $p<0.05$ was assumed.

Eventually, the newborns from both groups were weighed and those categorized to have a low birth weight less than 2500 gram or more. Indicators that may influence the weight status of the newborn were gathered, such as ethnicity, educational background and BMI of the mother in addition to the method of conception for their pregnancy.[14-16] Normal conception and past procedures on the cervix were also considered as influencing factors on the baby's birth weight.

RESULTS

The mean age of the studied contingent was 26.18±6.50 years (range 14-52 years). In this group the completed gestational week correlates poorly with the BMI and the age of the patient and sequence of the pregnancy. The correlation with the BMI is directly proportional, and with the age and the sequence of pregnancy – inversely proportional ($p<0.001$). Three patients in group A and 5 in group B had a history of prior preterm birth.

In all 32 women participating in the study, we found a moderate association between the expression of pIGFBP-1 in the CVF and the onset of preterm birth. Of these 32 women, 8 from both groups tested positive for pIGFBP-1, and 6 of these women went into preterm labour within 14 days of the test. Of the 12 women included in Group A, 4 proceeded to have a preterm birth. Out of the 20 women in Group B, only 2 had preterm birth (**Table 1**).

The Cramér's V scale is an effect size measurement for the chi-square test of independence. It measures how strongly two categorical fields are associated.[11] The scale ranges from 0, which is no association, to 1, which is complete association. The effect size for pIGFBP-1 and preterm birth in this study is 0.441. This indicates that the association is moderate, which suggests that if a woman tests positive for pIGFBP-1, there is a relative chance of her going into preterm labour. The presence of pIGFBP-1 in the CVF is related to an increase in incidence of preterm labour; meaning a negative expression (i.e.: the absence of pIGFBP-1) increases the incidence of full term labour ($p<0.023$).

The presence of pIGFBP-1 in the CVF increased the risk of preterm birth 8.3-fold, compared to women that had a negative result for pIGFBP-1 (**Table 2**). The assessment of the results of pIGFBP-1 as an indicator of preterm birth showed a low sensitivity of 56% but a higher specificity of 78%. However, the cohort group was very small; the data were accumulated from 32 patients, which is not sufficient to initiate a regression model. A continuation of the study with more patients would provide a stronger correlation to construct a regression model.

Other parameters noted to have a correlation to preterm birth are a shorter cervical length, complications during pregnancy and history of previous preterm births (**Table 3**). Cervical length was found to be a crucial component in predicting the incidence of preterm labour. A cervical length of ≤25.6 mm in women compared to women that

Table 1. Participants and groups included in the study

Groups	Participants	pIGFBP-1 positive	Preterm birth
Group A	12	6	4
Group B	20	2	2
Total	32	8	6

Table 2. Analysis of the correlation between preterm birth and pIGFBP-1 expression (Cramer's V = 0.441)

Indicator	Preterm labour				p
	No		Yes		
	n	%	n	%	
Expression of pIGFBP-1					0.023
Negative	20	87.0	4	44.4	
Positive	3	13.0	5	55.6	

Table 3. Risk ratio and 95% CI of the studied indicators as factors for the occurrence of premature birth

Indicator	Criteria	OR	95% CI		p
			Lower limit	Upper limit	
Cervical length (mm)	≤ 25.6 / > 25.6	29.70	4.92	179.29	<0.001
Complications during the pregnancy	Yes / No	5.46	3.65	8.17	<0.001
Previous preterm labour	Yes / No	2.36	1.41	3.93	0.001

have a longer cervix, had a 30 times greater risk of experiencing preterm labour (*p*<0.001). There were 2 patients whose cervical length was less than 10 mm and they both tested positive for the presence of pIGFBP-1 and consequently went into spontaneous preterm labour within a few hours. There was a high predictive value in the cases of negative results, meaning women that have a negative test for cervical measurement, i.e. greater than 10 mm, have a higher probability of not having a preterm birth, with respect to other risk factors.

The group A women, who experienced one or more of the risk factors: a short cervical length, complications in pregnancy or previous preterm births, were also associated with a 5.5 times higher prevalence rate of preterm labour. Previous history of preterm labour played a key role in predicting the occurrence of preterm labour in their current pregnancy. Women are 2.5 times more likely to have preterm birth in their current pregnancy if they have a history of preterm birth.[17]

From the history that was taken of the women participating in the study, the indicators that influenced the value of complete gestational week were as follows:

1. Education: the average value of weeks in patients with secondary and higher education were significantly higher than that of women that were illiterate.
2. Prior preterm: the average complete gestational weeks in patients that did not have previous history of preterm birth was statistically higher than in women with one previous preterm birth.
3. BMI of women while they are pregnant can impact the occurrence of preterm birth. Lower BMI during pregnancy was shown to increase the incidence of preterm birth, whereas obesity did not.[18]

With these factors in consideration, the weight of each newborn was obtained; there were a few aspects to be noted. The average weight of the newborn from mothers with secondary or higher education qualifications were significantly higher than that of those with lower educational
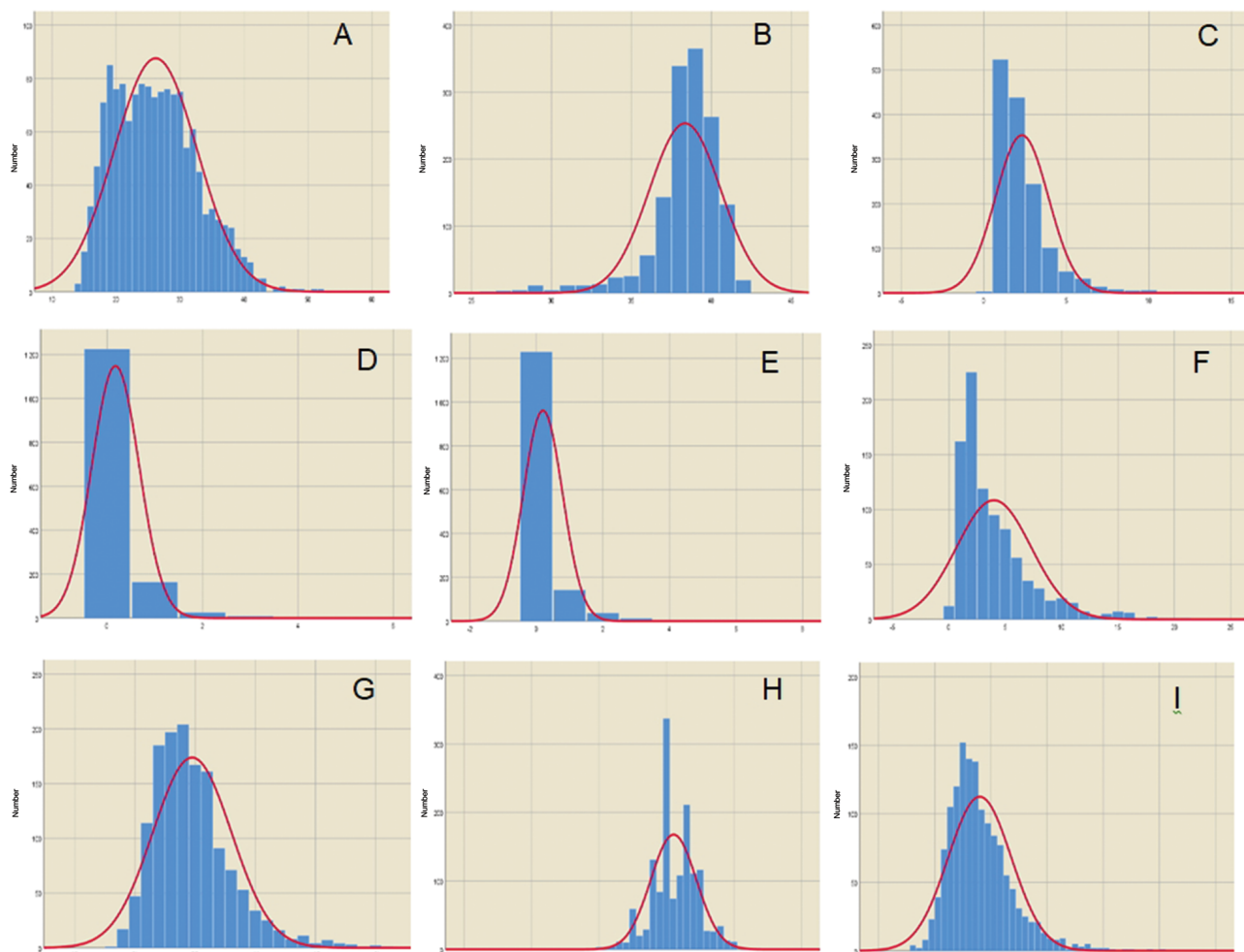

Figure 1. Distribution of the studied quantitative indicators. The vertical axes expresses the number of the patients distributed of the following indicators at the horizontal axes: **A)** age (years) (*p*<0.001); **B)** birth in a completed gestational week (*p*<0.001); **C)** sequence of pregnancy (*p*<0.001); **D)** previous miscarriages (*p*<0.001); **E)** previous interruptions (*p*<0.001); **F)** inter-delivery period (*p*<0.001); **G)** weight of the patient (kg) (*p*<0.001); **H)** height of the patient (m) (*p*<0.001); **I)** Body mass index (kg/m^2) (*p*<0.001).

qualifications. In terms of ethnicity, the percentage of women of Bulgarian ethnicity was 53.9%, followed by those of Roma with 39.1% and other ethnicities accounting for another 0.7%. The average value of newborn weight from the Bulgarian ethnic group was significantly higher than those of the other ethnic groups. Analysis of the studied quantitative indicators showed that with the highest coefficient of variation (299.0%) is from previous induced abortions, followed by previous spontaneous abortions (281.7%).

Analysing the results, it is evident that preterm birth is multi-factorial in etiology. By simply considering only one or few aspects of the history of the patient, we cannot have an accurate indication of preterm labour in pregnant women. Transvaginal cervical length measurements along with pIGFBP-1 testing and a thorough history of the patient are all seen to have a combined effect in predicting preterm birth more accurately.

DISCUSSION

Premature deliveries are the leading cause of fetal morbidity in the 21st century. This has led to many investigations studying preterm labour. Some of these investigations were made in an attempt to predict preterm labour easily; others wanted to determine the ability of a pregnant woman carrying out a full-term pregnancy during its early stages. It was found in such studies that measuring the cervical length can help in detecting patients at the highest risk of preterm labour. Alongside this, a newer generation of clinical biomarkers were found in the CVF. Examples of the discovered biomarkers include fFN along with pIGFBP-1. It was found that the quantity of these biomarkers can also contribute to identifying the risk of preterm labour.

A study of 60 000 singleton pregnancies was performed in which the cervical length of all patients was measured. The study established that potential preterm births could be identified by effective obstetric history taking and measuring the patient's cervical length. It found that the detection rate for extreme births was 80% for a 10% screen-positive rate. Fetal Medicine Foundation then implemented the findings as a routine procedure in antenatal care, followed using progesterone.[19]

A protocol was put forward by the Society of Maternal-Fetal Medicine to routinely predict preterm labour by carrying a thorough history of the patient's previous pregnancies along with transvaginal and cervical measurements in the second trimester.[20] Those who carry out this procedure are trained practitioners and follow correct guidelines. Transvaginal and cervical measurements can be obtained via transvaginal ultrasounds as recommended by the American College of Obstetricians and Gynecologists (ACOG).[21] The ACOG suggests that this should be done routinely and before confirming the need for further treatment, testing for biomarkers can be performed if necessary.

In 2018, a study was conducted in the city of Pleven in Bulgaria, where 139 babies were found to be born prior to the 37th week of gestation. The premature babies were monitored post birth and 11.5% of them had not survived due to severe complications. Premature babies who survived but had long term health complications due to their early birth made up 20.1%. A majority of 68.4% of the premature babies were healthy at the time; however, it was not possible to predict if they would suffer from complications in the future. These findings were the result of a lack of protocols being in place that would have allowed obstetricians to predict and prevent premature births.

This article therefore aims to highlight the importance of measuring the cervical length, testing for the level of fFN and pIGFBP-1 and the implementation of their routine use in the region of Pleven. Though this study has a comparatively small cohort group, there is plenty of evidence that supports routine cervical measurement taking as well as checking for fFN and pIGFBP-1 levels to prevent preterm labour. Appropriate scanning for these pregnant women can be of excellent value as this will reduce the number of premature babies being born; this would also mean the health complications associated with premature births can be prevented. The mother also benefits from this as studies show preterm mothers do not get to spend as much time with their newborn as mothers who carry to full term.[22] This affects the bond between mother and child as well the mental wellbeing of the mother, which can have long lasting effects.

CONCLUSIONS

In the world of modern medicine, preterm births should not be a health problem without timely prediction and careful management. Obstetrical investigations that are available at present, and potential new methods of screening that can prevent preterm labour are vital. This is because fewer premature births would mean it is less likely that neonates suffer health complications; consequently, reducing the work pressure on medical staff and the burden on the health care system. Moreover, it ensures the well-being of the mother, since mothers of preterm babies are more likely to suffer from ill-health and negative feelings towards their baby. As agreed, by multiple studies, the more markers used to screen, the higher the specificity, and the better the prognosis and, as a result the better the outcome of the pregnancy.

Although a perfect protocol for the prediction of preterm labour is not yet in place, the options available now need to be utilized efficiently. Screenings that are known to predict preterm labour with high sensitivity and specificity are fFN and pIGFBP-1, when combined with measurements of the cervical length. The ultimate aim is to identify women who are about to deliver preterm, with a high degree of accuracy. It is also to postpone their labour for as long as necessary, ensuring that the woman is transferred to a unit capable of providing effective preterm neonatal care. In conclusion, implementing these screening methods into routine obstetrical consultations with pregnant women would decrease the rate of preterm births, fetal morbidity, and mortality.

Acknowledgments

Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Прогнозирование преждевременных родов с помощью pIGFBP-1 и биометрии шейки матки

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Резюме

Введение: Ошеломляющие 30% смертей новорожденных связаны с преждевременными родами. Наиболее частой причиной перинатальной заболеваемости и смертности во всём мире являются преждевременные роды, также называемые преждевременными. Следовательно, способность прогнозировать преждевременные роды приведёт к значительному снижению смертности.

Вероятность прогнозирования преждевременных родов можно измерить по длине шейки матки; однако существуют и другие диагностические процедуры, в которых для более точного диагноза используется содержимое цервико-вагинальной жидкости (ЦВЖ). Последнее также помогает в обнаружении других аномалий, связанных с беременностью. Фосфорилированный белок-1, связывающий инсулиноподобный фактор роста (pIGFBP-1), является примером клинического биомаркера, который часто тестируется, поскольку он оказывается индикатором для прогнозирования преждевременных родов.

Цель: Таким образом, в этом исследовании наша цель состояла в том, чтобы оценить точность pIGFBP-1 как маркера преждевременных родов в сочетании с биометрией шейки матки.

Материалы и методы: Проведено поперечное исследование 32 беременных. Пациентки были разделены на 2 группы, в которых группу А составили пациентки с риском преждевременных родов, а группу Б – женщины с нормальным и неосложненным течением беременности. Все участники были протестированы на pIGFBP-1 вместе со сбором данных трансвагинальных измерений длины их шейки матки.

Результаты: У всех участников исследования мы обнаружили умеренную связь между экспрессией pIGFBP-1 в ЦВФ и началом преждевременных родов. У восьми пациенток с симптомами были положительные результаты, и, как и предполагалось, в течение 14 дней у 6 из этих пациенток начались преждевременные роды.

Заключение: Надлежащее сканирование беременных женщин может иметь большое значение, поскольку оно может уменьшить число рождений недоношенных детей; это также означает, что осложнения для здоровья, связанные с преждевременными родами, можно предотвратить.

Ключевые слова

цервико-вагинальная жидкость, фибронектин плода, преждевременные роды, фосфорилированный белок-1, связывающий инсулиноподобный фактор роста

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