EVALUATION OF SERUM β-hCG AND PAPP-A LEVELS IN PREGNANT WOMEN AT RISK OF DEVELOPING PREECLAMPSIA

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ABSTRACT

Background and aim. *Preeclampsia remains a major problem of modern obstetrics with insufficiently elucidated etiology; early detection would diminish maternal and fetal mortality and morbidity. The aim of this study was to determine the serum values of β-hCG in the first and second trimesters of pregnancy and PAPP-A values in the first trimester of pregnancy in pregnant women with risk factors for preeclampsia, in order to evaluate their relevance in the prediction of this disorder.*

Material and methods. *We performed a prospective longitudinal study on 120 pregnant women divided into two groups according to the evolution of pregnancy: group I - 26 pregnant women who developed preeclampsia and group II - 94 pregnant women who did not develop preeclampsia and had a physiological evolution of pregnancy.*

Results. *Our results indicate the association between high β-hCG levels in the first and second trimesters of pregnancy and the development of PE, β-hCG having the highest predictive power in the second trimester. We also obtained a positive association between low serum levels in PAPP-A in the first trimester and onset of PE. The predictive power of conjugated β-hCG and PAPP-A values in the first trimester of pregnancy was better that any other marker analyzed separately.*

Conclusions. *Increased β-hCG levels in the first and second trimesters of pregnancy and low PAPP-A levels in the second trimester of pregnancy are associated with a higher risk for PE, the study providing only a modest efficiency of the prediction capacity.*

Keywords. Preeclampsia, prediction, β-hCG, PAPP-A.

Introduction

Preeclampsia (PE) remains a major problem of obstetrics, representing one of the main causes of maternal-fetal mortality and morbidity. Consequently, the early detection of patients a high risk that might develop preeclampsia during pregnancy is extremely important. So far, the etiology of PE remains unclear [1,2]. A widely accepted explanation would be the deficient invasion of maternal spiral arteries by the trophoblast [1,2,3,4]. One of the main approaches for the prediction of preeclampsia is related to the placental function and might offer new perspectives for the development of screening tests.

Pregnancy Associated Plasma Protein-A (PAPP-A) and *β-human Chorionic Gonadotrophin* (β-hCG) are secreted by the placenta and are part of the first trimester screening test for Down syndrome [5]. PAPP-A is a large glycosylated protein produced by the trophoblast, which modulates the activity of the insulin-like growth factor (IGF) through the cleavage of binding proteins, with an important role in the trophoblast invasion of the decidua [6,7]. β-hCG is a glycoprotein hormone produced physiologically at the level of trophoblast cells [8].

The aim of our study was to determine the serum values of PAPP-A and β-hCG in the first trimester of pregnancy and subsequently, of β-hCG in the second trimester of pregnancy in pregnant women with risk factors for preeclampsia, in order to evaluate their relevance in the prediction of this disorder.

MATERIAL AND METHOD

We performed a prospective longitudinal study on 120 pregnant women with risk factors for preeclampsia who presented to the "Dominic Stanca" Cluj-Napoca Clinic of Obstetrics and Gynecology in the period January 2011 – July 2013.

The **inclusion criteria** was the presence of at least one

Manuscript received: 05.12.2013

Accepted: 19.12.2013

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of the following risk factors: primiparity; a history of preeclampsia in previous pregnancies; a family history of preeclampsia; pregnancy associated disorders (renal, diabetes mellitus, etc.); a history of obstetric disorders (preeclampsia, fetal hypotrophy, oligoamnios, perinatal mortality, premature detachment of the normally inserted placenta); obesity; young age or age over 40; low socio-economic standard.

The established **exclusion criteria** were as follows: pregnant women with clinically manifest infections; recent treatment with non-steroidal antiinflammatory drugs and corticosteroids (over the past 14 days); autoimmune diseases and chronic inflammatory diseases.

All patients included in the study signed an informed consent and the study was approved by the Ethics Board of the "Iuliu Hatieganu" University of Medicine and Pharmacy Cluj-Napoca.

The study investigated the serum β-hCG and PAPP-A values in the first trimester of pregnancy (weeks 10-14) and subsequently, the serum β-hCG values in the second trimester of pregnancy (weeks 20-24). The samples were analyzed according to the following study protocol: 3 ml blood were collected on anticoagulant, the plasma was separated by centrifugation for 15 min at 1000 xg. The samples were stored in the freezer at -20^{0}C. When processed, the samples were gradually thawed on ice. The samples were processed according to the instructions of the IMMULITE 2000 PAPP-A (Siemens Healthcare, Diagnostics Products Ltd., United Kingdom) and beta-HCG (Nova Tec Immundiagnostica GmbH Dietzenbach, Germany) kits.

The pregnant women were monitored periodically until delivery, by assessing the development of PE or the physiological evolution of pregnancy.

For the diagnosis of PE, we used the classification system proposed by U.S. NMBPEP Working Group 2000 and AGOC: blood pressure values of at least 140/90 mm Hg (on 2 examinations minimum 6 h and maximum 7 days apart), proteinuria higher than 30 mg/dl (in 2 urine samples collected 4-6 h apart). AHT and proteinuria developed after 20 weeks of pregnancy in a pregnant woman that was normotensive prior to pregnancy and normalized until 12 weeks postpartum.

At the end of the study, the patients were divided into two groups depending on the evolution of pregnancy:

Group I - 26 pregnant women who developed preeclampsia: 9, the severe form and 17, the moderate form. Of the 9 pregnant women with the severe form of preeclampsia, 4 also had intrauterine growth retardation (IUGR).

Group II - 94 pregnant women who did not develop preeclampsia and had a physiological evolution of pregnancy.

Statistical analysis

The Mann-Whitney test was used for the comparison of the β-hCG and PAPP-A values between the two groups.

The t test was inadequate in this case because the values recorded for beta-HCG and PAPP-A did not have a normal distribution. The binary logit model was used in order to assess whether β-hCG and PAPP-A were correlated with the probability of developing preeclampsia. We considered Y=1 if the patient developed preeclampsia and Y=0 if the patient did not develop preeclampsia. The t test was used for the study of the statistical significance of the coefficients. We considered a variable as statistically significant if the p-value <0.05. The predictive power of the functions used was assessed based on the ROC (Receiver Operating Characteristics) curve. All statistical processing was performed with the STATA 9.1 software (StataCorp, 4905 Lakeway Drive, College Station, Texas 77845 USA).

Results

The determined serum β-hCG and PAPP-A concentrations are shown in Table I.

Table I. Comparative β-hCG and PAPP-A values (p-value according to the Mann-Whitney test)

Variable	Group	Mean	Median	SD (standard deviation)	p-value
β –HCG	group I	528	539	156	0.029
1st trim.	group II	411	397	214	
ß-HCG					
2nd trim.	group I	446	415	144	0.046
	group II	351	353	167	
PAPP-A	group I	3.30	3.09	1.02	0.058
	group II	4.34	4.16	2.48	

It can be seen that in patients with preeclampsia (group I), the mean and median ß-HCG values were higher than in those of group II, and these differences were statistically significant. PAPP-A values were lower in patients of group I, being significant only at the threshold of 10%.

Predictive significance of β-hCG values in the first trimester

Table II. Results of binary logit regression, with the explanatory variable β-HCG, 1st trimester

	Coefficient	Std. err.	z	p-value
B-HCG				
1st trimester	0.00217	0.00131	1.66	0.096
Constant	-3.02206	0.70899	-4.26	0.000

The correlation of β-hCG values in the first trimester with the subsequent development of preeclampsia during pregnancy was not very statistically significant, but the coefficient had the expected sign, positive, and was significant at the threshold of 10%. Further studies in larger patient samples might clarify this aspect in order to identify the influence of β-hCG in the first trimester on the risk of preeclampsia.

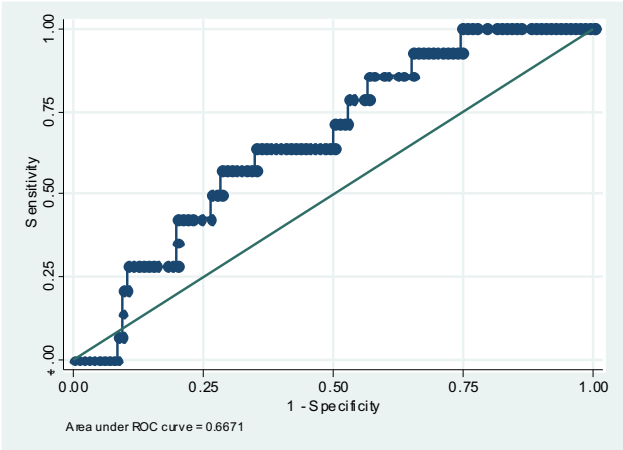

Fig. 1. The ROC curve associated with the binary logit model. Explanatory variable: β-HCG, 1st trimester

The predictive power of β-hCG values in the first trimester was low (AUROC=0.6671) and the development of preeclampsia could not be predicted only based on these values

Predictive significance of β-hCG values in the second trimester

Table III. Results of binary logit regression, with the explanatory variable β-HCG.

	Coefficient	Std. err.	z	p-value
β-hCG 2nd trimester	0.00352	0.00168	2.10	0.036
Constant	-3.43777	0.78893	-4.36	0.000

The β-hCG value in the second trimester was not statistically significant at the threshold of 5%, and the coefficient had the expected sign, positive. Consequently, a higher β-hCG value increases the probability of preeclampsia.

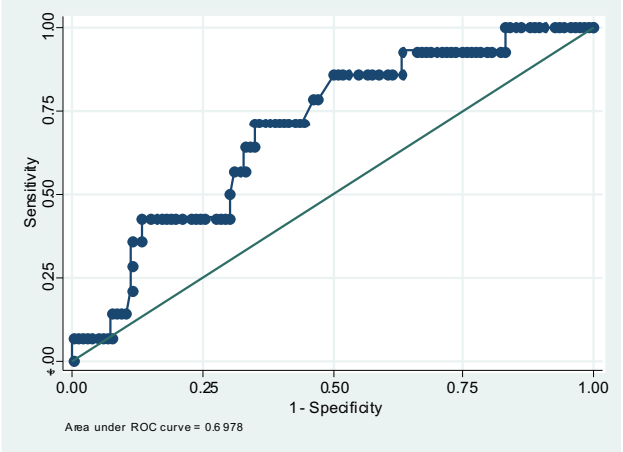

Fig. 2. The ROC curve associated with the binary logit model. Explanatory variable: β-HCG, 2nd trimester

The predictive power of β-hCG values in the second trimester was higher (AUROC=0.6978) than that of values in the first trimester. However, the development of preeclampsia could not be predicted only based on β-hCG values in the second trimester because predictability was rather low.

Predictive significance of PAPP-A values

Table IV. Results of binary logit regression, with the explanatory variable PAPP-A

	Coefficient	Std. err.	z	p-value
PAPP-A	-0.37489	0.20918	-1.79	0.073
Constant	-0.62978	0.76480	-0.82	0.410

The PAPP-A value was statistically significant at the threshold of 10%, but not significant at the threshold of 5%. The coefficient was negative, indicating lower PAPP-A values for patients who subsequently developed preeclampsia.

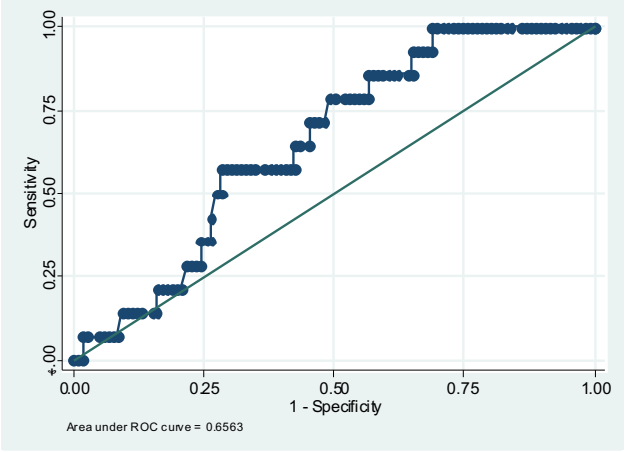

Fig. 3. The ROC curve associated with the binary logit model. Explanatory variable: PAPP-A

The predictive power of PAPP-A values was moderate (AUROC=0.6563). The risk of preeclampsia could be assessed only based on PAPP-A values, because predictability was not of the highest level. However, lower PAPP-A values in the first trimester of pregnancy may give a signal, which should be correlated with the values of other markers.

Predictive significance of β-hCG and PAPP-A in the first trimester

Table V. Results of binary logit regression, with the explanatory variables PAPP-A and β-HCG

	Coefficient	Std. err.	z	p-value
β-HCG_1	0.00231	0.00134	1.73	0.084
PAPP-A	-0.22160	0.19605	-1.13	0.258
Constant	-2.21509	0.96815	-2.29	0.022

The coefficients of the variable had approximately the same significance as in simple regressions. The signs of the coefficients were also maintained, suggesting the increased probability of preeclampsia for higher β-HCG values in the first trimester and lower PAPP-A values.

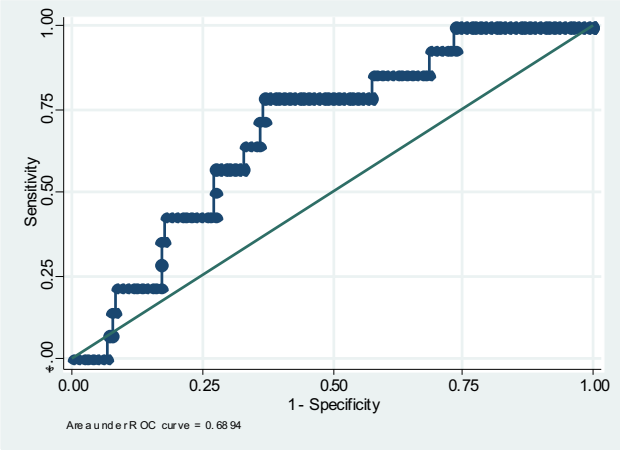

Fig. 4. The ROC curve associated with the binary logit model. Explanatory variables: β-hCG and PAPP-A

The predictive power based on the conjugated β-hCG and PAPP-A values (AUROC=0.6894) was better than that of any marker analyzed separately. However, it is far from being satisfactory, with a proportion of correct predictions of approximately 70%. The high β-hCG values and low PAPP-A values in the first trimester may give a signal of increased preeclampsia risk, but they cannot represent a reliable prognostic basis.

Discussion

Our study investigated the serum levels of β-hCG and PAPP-A in the first trimester of pregnancy and of β-hCG in the second trimester in 120 Caucasian pregnant women presenting risk factors of preeclampsia. 94 of the patients had a physiological evolution of pregnancy, 9 developed the severe form of PE, and 17 the moderate form. Of the pregnant women with the severe form of PE, 4 cases were associated with IUGR. The high percentage of patients who subsequently developed PE (21.6%) is explained by the fact that the study included only pregnant women with at least one risk factor for preeclampsia.

Our results indicate the association between high β-hCG levels in the first and second trimesters of pregnancy and the development of PE, β-hCG having the highest predictive power in the second trimester. β-HCG, being a product of trophoblast cells, might be a marker for the evaluation of placental turnover. Literature studies reveal the fact that high β-hCG levels in the second trimester are associated with adverse effects on the fetus, for example the development of PE [9].

In accordance with the results obtained by us there is the ONG study (2000), which reports a significant association between β-hCG in the first trimester and the subsequent development of PE [10]. In contrast, Spencer demonstrated that serum β-hCG levels in the first trimester in patients who subsequently developed PE were reduced or similar to those of uncomplicated pregnancies [11]. Also, Dugoff (2004) evidenced no association between β-hCG values and the development of PE [12]. Goetzinger published a study according to which high β-hCG levels in the first trimester were associated with an increased risk of IUGR [13].

In agreement with our results, other multicenter studies showed that pregnant women with low PAPP-A levels in the first trimester of pregnancy were predisposed to the development of PE or other complications during the course of pregnancy [14]. A pattern of increase in the risk of PE with the decrease in PAPP-A levels was seen. These results were confirmed by other authors, who detected low, statistically significant PAPP-A values in pregnancies with PE compared to those without PE [15].

Individually, low PAPP-A levels have a low sensitivity in the prediction of PE [16]. Spencer supports the need for the association with other parameters, including uterine artery Doppler, for the improvement of the capacity of prediction of PE. The alteration of placental development phenomena in the second trimester seems to influence the subsequent development of PE. The low PAPP-A level between 10-14 weeks of gestation can be considered as a marker of deficient placental development and of a small size placenta (ONG study 2000) [17].

The paracrine effect of IGF controls trophoblast invasion at decidual level. Given that PAPP-A is a protease for the IGF-binding protein, the decrease of PAPP-A is associated with increased IGF-BP levels. Consequently, serum IGF levels are low, which leads to deficient trophoblast invasion and increases the risk of complications in pregnancy (including PE). This hypothesis provides biological plausibility regarding the association of low PAPP-A levels and the subsequent development of PE in pregnancy (Kinirs 1999, Clemmmons 1998, Irwin 1999 and Dugoff 2004) [17,18,19,20].

For a better and early prediction of PE, we associated the first trimester markers; the predictive power of conjugated β-hCG and PAPP-A values was better, without being statistically satisfactory at the threshold of 5%.

As a conclusion, our data suggest the fact that increased β-hCG levels in the first and second trimesters of pregnancy and low PAPP-A levels in the second trimester of pregnancy are associated with a higher risk for PE, the study providing only a modest efficiency of the prediction capacity. Further investigations of additional factors that might improve the prediction rate are justified in order to develop more accurate methods for the identification of a population at risk and to initiate an adequate monitoring protocol.

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