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Tobacco smoking impact on pregnancy-associated plasma protein-A (PAPP-A) levels in the blood serum of women in first trimester of pregnancy.

KEYWORDS: PAPP-A, pregnancy, first trimester, tobacco smoking.

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ABSTRACT *The purpose of this paper was to estimate levels of pregnancy-associated plasma protein-A (PAPP-A) in serum blood of smoking and non-smoking women in the first trimester of pregnancy, taking into account its duration, and also age and body weight of pregnant women.*

Study group consisted of 4473 patients aged 18 to 47 with unifetal pregnancy who underwent first trimester non-invasive screening though blood serum tests. Biochemical parameter levels measurement was conducted through a fully automated immunofluorescence method.

We observed that PAPP-A MoM value for a smoking woman was on average 16.0% lower in comparison with the results of a non-smoking woman of the same weight with the standard error 3.5%.

Smoking cigarettes by pregnant women decreases PAPP-A MoM level by 16.0% on average in comparison with the results of a non-smoking woman of the same weight.

Introduction:
Exposition to tobacco smoke during pregnancy is an important risk factor for the health of both the mother and the fetus. It has a significant effect on a higher rate of miscarriages, sudden fetal deaths, lower birth weight, and respiratory insufficiency after the child's birth [2, 11].

Correlation between smoking by mothers and chilognathopalatoshisis occurrence in children has also been proven [14]. Substances contained in tobacco smoke, such as nicotine, carbon oxide and cyanides, have toxic properties. Their presence in an organism can influence immunological mechanisms which compose an important element determining equilibrium between the bodies of the mother and the fetus [6].

Pregnancy Associated Plasma Protein A (PAPP-A) is a multicellular glycoprotein, containing zinc, built from heterodimer with molecular weight of 500 kDa. This protein is created in syncytiotrophoblasts and plays a role in immunosuppression, causing trophoblasts to be not recognized by the mother's organism as a foreign body [6, 7, 15]. It stimulates growth and development of the fetus' cells through IGF (insulin-like growth factor), released from a complex created with IGFBP (insulin-like growth factor binding protein). In early pregnancy, IGF-II (insulin-like growth factor II) stimulates growth and vitality of the fetus, while IGF-I (insulin-like growth factor I) regulates flow of nutrients in the maternal-fetal unit. Impaired expression of IGF in placenta in initial stages of pregnancy may be a cause of placental insufficiency, which in turn may lead to impaired fetal growth [7, 12, 15].

The purpose of the paper:
The purpose of this paper was to estimate levels of pregnancy-associated plasma protein-A (PAPP-A) in blood serum of smoking and non-smoking women in the first trimester of pregnancy, taking into account pregnancy duration, and also age and body weight of pregnant women.

Methods:
Study group consisted of 4473 women aged 18 to 47 with unifetal pregnancy who underwent first trimester non-invasive screening through blood serum tests referred to Prenatal Genetic Clinic of University Hospital in Bydgoszcz. General characteristics of the group are shown in Table I. Average age was 33.1 with standard deviation of 5.3 years. Gestational age, obtained from CRL (crown rump length) measurements conducted by ultrasonography screening, was between 72 to 97 days with the mean of 89 days.

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Table I
Characteristics of the study group (n = 4473)

	Average	Standard deviation	Median	Range
Age at the time of delivery (years)	33.1	5.3	34.1	(18-47)
Gestational age (days)	89	4	89	(72-97)
Weight (kg)	66.0	12.4	64.0	(40.0-145.0)
PAPP-A	2961	1946	2484	(1-18121)
$\log_{10}$ PAPP-A	3.381	0.297	3.395	(-0.009-4.260)
MoM PAPP-A	1.175	0.727	1.000	(0.006-7.600)

Biochemical parameter levels measurement was conducted through a fully automated immunofluorescence method used in Delfia Xpress (Perkin Elmer) analyzer in Diagnostic and Medical Center „Lipowa" in Bydgoszcz. The blood serum was obtained between 11th and 13.6th week of pregnancy.

The study was approved by the appropriate ethics review board.

Statistical analysis was conducted using program Statistica v.10.0. For comparison of distribution of continuous variables Kruskal-Wallis test was used, together with a multiple comparison test. Since PAPP-A variable has an approximately log-normal distribution, we analyze differences in distribution of $\log_{10}$PAPP-A. Prior to conducting correlation and regression analysis, outliers for $\log_{10}$PAPP-A were removed by making use of the Grubbs test. In order to adjust difference of $\log_{10}$PAPP-A values for smoking and non-smoking women for potential confounding factors multiple regression. Differences were considered as significant for p value lower than 0.05 [3, 5].

Results and discussion:

In the analyzed group there were 4119 (92.1%) non-smoking patients, 288 (6.4%) smokers and 66 (1.5%) patients who quit smoking during pregnancy. There were no significant differences between these groups in distribution of age, weight, and gestational age. Results are presented in Table II. Nevertheless, we observed significant differences for PAPP-A levels, $\log_{10}$PAPP-A, and MoM PAPP-A. Multiple comparison test proved the results of smoking and non-smoking groups to be substantially different. Median values for variables were substantially lower in the group of smoking women (p<0.001).

Table II
Comparison of smoking, non-smoking, and the group that stopped smoking during pregnancy*

	Non-smoking (n=4119)	Smoking (n=288)	Stopped smoking during pregnancy (n=66)	p value for Kruskal-Wallis test
Age at the time of delivery (years)	34.0 (24.0-40.6)	34.6 (20.8-42.0)	33.5 (24.7-40.9)	0.77
Gestational age (days)	89 (81-96)	89 (81-96)	88 (81-95)	0.35
Weight (kg)	64.0 (50.0-90.0)	63.0 (50.5-92.0)	63.0 (52.7-92.8)	0.84
PAPP-A	2518 (812-6683)	2069 (684-5718)	2383 (677-5609)	<0.001
$\log_{10}$ PAPP-A	3.401 (2.910-3.825)	3.316 (2.835-3.757)	3377 (2831-3749)	<0.001
MoM PAPP-A	1.013 (0.352-2.580)	0.823 (0.280-2.343)	0.878 (0.321-2.020)	<0.001

**- table contains median and percentile values (P_5-P_{95})*

It was found that $\log_{10}$ PAPP-A variable was significantly correlated with gestational age (R=0.38, p<0.001), patient's weight (R=-0.33, p<0.001), and age at the moment of delivery (R= 0.07, p<0.001). A positive correlation between $\log_{10}$ PAPP-A and gestational age means that when gestational age increases, $\log_{10}$ PAPP-A value tents to increase. A negative correlation between $\log_{10}$ PAPP-A and weight indicates that the women who had higher weight had a tendency to obtain low $\log_{10}$ PAPP-A value, and vice versa. Correlation of $\log_{10}$ PAPP-A with age was very low, it was however still statistically significant.

Differences between average $\log_{10}$ PAPP-A values for groups of smoking and non-smoking women with adjustment for gestation age, weight, and patients' age were studied using multiple regression. Smoking was considered as a dichotomous variable, with 0 value being assigned to non-smokers and 1 to smokers. Results of multiple regression are shown in Table III. The simultaneous adjustment for weight and age was not allowed, since the correlation between age of patients and weight was much higher than the correlation between age of patients and $\log_{10}$ PAPP-A. Not to compromise the assumptions of a multiple regression, two models were considered. In both models $\log_{10}$ PAPP-A difference between smoking and non-smoking women were statistically significant and higher than original difference. Partial correlations were higher than correlations in models with one variable. Coefficient of determination allows to assess the goodness of fit of the regression equations. It indicates that the model which with body weight, gestational age, and smoking gives better fit than the model with patients' age, gestational age

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and smoking. The first model explains 28% of the total variation in log_{10} PAPP-A, while the second one explains only 17%.

The results shown that PAPP-A level was on average 16.0% ((1-$10^{-0.076}$)×100%) lower in smoking group than in non-smoking group with same values for body mass and gestational age with the standard error of 3.5%. Moreover, we observed that 1 kg difference of weight with the same level of the other variables gave on average 1.8% ((1-$10^{-0.008}$)×100%) lower PAPP-A level with the standard error of 0.1%. We also verified that PAPP-A level rise on average 6.3% (($10^{0.027}$-1)×100%) in every day of pregnancy. In this case the standard deviation was 0.2%. It should be stressed that cited estimates can only be referred to the studied range of variable values.

Table III
The results of multiple regression for the dependent variable log_{10} PAPP-A and selected independent variables (n=4401)*

	Model 1			Model 2		
	Regression coefficients	Partial correlations	p value	Regression coefficients	Partial correlations	p value
Age at the time of delivery (years)				-0.004	-0.08	<0.001
Gestational age (days)	0.026	0.41	<0.001	26	0.39	<0.001
Smoking (0-smoking 1-not smoking)	-0.078	-0.08	<0.001	-0.080	-0.07	<0.001
Weight (kg)	-0.008	-0.37	<0.001			
Intercept	1.611		<0.001	1.237		<0.001
Coefficient of determination R^2	0.27			0.16		
p value for model	<0.001			<0.001		

*Legend: *- for all regression coefficients in both models p<0.001*

Evaluation of the influence of smoking on PAPP-A MoM value with adjustment for patients' weight is shown in Table IV. Results allow us to make the following prediction. PAPP-A MoM value for a smoking woman was on average 16.0% ((1-$10^{-0.076}$)×100%) lower than for a non-smoking woman with the same weight, whereas 1kg increase in weight caused on average 1.8% ((1-$10^{-0.008}$)×100%) decrease of PAPP-A MoM value with the standard error of 0.1%.

Table IV
The results of multiple regression for log_{10} PAPP-A MoM and selected variables (n = 4400)*

	Regression coefficients	Standard error	Partial correlations
Smoking (1-smoking 0-not smoking)	-0.076	0.015	-0.37
Weight (kg)	-0.008	0.0003	-0.07
Intercept	0.518	0.02	
Coefficient of determination R^2	0.14		
p value for the model	<0.001		

**- for all regression coefficients in both models p<0.001*

Discussion:

PAPP-A concentration in maternal serum reflects PAPP-A level in tissues and bioavailability of IGF factors, a consequence of which may be a disturbance of fetal and/or placental development [6, 7]. Tobacco smoking inhibits syncytiotrophoblast cell apoptosis, which in turn hinders regular exchange of maternal-fetal circulation [7, 16].

The difference in average values of log_{10}PAPP-A between groups of smoking and non-smoking women equaled -0.067 and was statistically significant. Average log_{10}PAPP-A value turned out to be considerably lower in smoking women (p<0.001). Lack of significant differences for the group which stopped smoking during pregnancy is probably due to inhomogeneity of this group. The moment of smoking cessation, having most probably an influence on PAPP-A levels, was not controlled in the study. Due to this fact, this group was not included in correlation analysis and multiple regression models.

PAPP-A levels studies were first conducted by Spencer et al., de Graaf et al. and Tul et al. According to their studies, PAPP-A levels in smoking women were 13%-18% lower than in non-smoking women. MoM PAPP-A in studied groups was respectively 1.00 and 1.07 for non-smoking women, and 0.85 and 0.81 for smoking women [4, 8, 18]. Lower PAPP-A values in smoking women was confirmed by Adrawi et al., Miron et al., Gajewska et al. [2, 7, 17]. In their studies, concentration of this protein in smokers in first trimester of pregnancy was between 0.80 and 0.86 MoM in comparison with 1.00-1.04 MoM in non-smoking women [2, 4, 10].

In the studies conducted by Spencer et al., Ardavi et al. and Kagan et al. it was not unequivocally proven that a connection between PAPP-A level and the amount of smoked cigarettes existed [2, 9, 17]. Spencer et al. noticed lower values of the studied protein irrespectively of the amount of smoked cigarettes [17]. Ardavi et al. observed lower PAPP-A levels with a higher amount of smoked cigarettes [2].

Due to the fact that during genetic risk assessment of giving birth to a child with aneuploidy on the basis of first trimester tests (PAPP-A and B-hCG) risk from maternal age is also added, women older than 35 have a higher probability of obtaining a false positive result than younger women [12, 13]. Age influence on PAPP-A levels in study material was low, it was however statistically significant, while the age itself was correlated with body weight of the women. In many author's studies we can observe a decrease of PAPP-A concentration with the increase of female body weight. It is connected with a higher volume of distribution. It is therefore necessary to introduce corrections to PAPP-A levels measurements according to body mass and smoking [13, 19].

Conclusions:

1. Smoking cigarettes by pregnant women decreases PAPP-A MoM level by 16,0% on average in compari-

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