Global Journal of Current Research Vol. 6 No. 4. 2019. Pp. 172-178

Full Length Research Paper

Association between Serum levels of Maternal Placental proteins and pregnancy outcome in Nulliparous Women

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Article history:
Received: 30-11-2019
Revised: 04-12-2019
Accepted: 12-12-2019
Published: 23-12-2019

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Abstract
Background: *Placental changes mainly reduction of blood flow is associated with adverse pregnancy outcome. Early recognition and prediction of potential adverse pregnancy outcome is of crucial importance. This study designed to determine the use of serum placental proteins as predictors of adverse pregnancy outcome (mainly preterm birth).* ***Subjects and Methods:*** *Nulliparous gravidas with a viable singleton pregnancy at their first trimester were included in the study. Maternal blood samples were collected to determine different potential predictors (e.g., A disintegrin and metalloproteinase domain-containing protein 12 (ADAM12), vascular endothelial growth factor VEGF-A,soluble fms-like tyrosine kinase-1 (sFlt-1), inhibin, β-hCG, and AFP. Primary outcome was preterm birth and secondary outcome includes other complications related to pregnancy.* ***Results:*** *Preterm birth was recorded for 107 (49.3%), and pre-pregnancy BMI was increased in the study (preterm) than control (full term) groups (28.20±4.3 vs 25.3±3.3, respectively). Smoking exposure, chronic hypertension, pregestational diabetes were significantly higher among study than the control group (26.2, 8.4%, 6.5% vs 14.5%, 1.8%, and 0.9% respectively). Finally, there was significant increase of small for gestational age, pregnancy induced hypertension and still birth among study than the control group (31.8%, 21.5%, 13.1% vs 5.5%, 6.4% and 2.7% successively). ADAM12 and sFlt-1 were significantly reduced, while VEGF-A and AFP were significantly increased in the study than the control group in the first trimester. At the second trimester, VEGF-A, inhibin and AFP were significantly higher in the study than the control groups. Vascular endothelial growth factor in the first or second trimester was the most sensitive test for prediction of preterm birth, while sFlt was sensitive only in the first trimester. At the second trimester the AUC did not reach acceptable levels.* ***Conclusion:*** *Maternal serum levels of placental proteins are significantly different early in first and second trimesters in preterm birth pregnancies and other adverse pregnancy outcome. However, these indicators, except VEGF, and SFlt, are poor predictors of adverse pregnancy outcome.*

Keywords: *Nulliparous; Preterm Birth; Vascular Endothelial Growth Factor; Alpha Fetoprotein*

Introduction

The reduction of maternal blood flow into the placenta induces functional and pathological placental changes. These changes are observed in different pregnancy associated disorders (e.g., hypertensive disorders, small for gestational age, and still birth) [(1,2)]. Preterm birth usually complicates these condition [(3,4)]. However, it is not possible to study placenta from biological point of view due to absence of animal models reiterate the human placenta and inability to access human placental tissues during pregnancy. Thus, early detection of placental dysfunction before development of clinical disease conditions associated with pregnancy. The human placenta is unique in its structure as it is mainly composed of a villous tissue, which offers a large surface area for nutrient and gas exchange and placental products are secreted into maternal circulation and intervillous spaces [(5)].

There are many placental proteins, that could be used as novel biomarkers able to predict adverse pregnancy outcomes. Three broad categories of these proteins are in use; angiogenesis indicators [(6-8)], placental implantation and development indicators [(9)] and markers of fetal abnormalities [(10)]. However, the value of placental proteins in prediction of adverse maternal outcome in nulliparous Egyptian women is not sufficiently addressed.

This study designed to determine the use of serum placental proteins as predictors of adverse pregnancy outcome (preterm birth, hypertensive disorders of pregnancy, small for gestational age neonates and stillbirth).

Subjects and methods

In their first trimester of pregnancy, nulliparous gravidas with a viable singleton pregnancy, who attended antenatal care clinic of Damietta General Hospital, were included in the study. Women included at their first visit at 6th to 12th week of pregnancy on the basis of the last menstrual period. Women were evaluated at their first visit after their consent to participate then for the antenatal visit at the end of the second trimester. Blood samples (5- 10 cc of maternal blood) were collected at each visit. Samples were collected in a capillary tube, left for coagulation and then transferred for centrifugation to obtain the serum.

A disintegrin and metalloproteinase domain-containing protein 12 (ADAM12) was measured by ELISA using human ADAM12 ELISA Kits (R&D Systems, Minneapolis, MN, USA). Moreover, electrochemiluminescence assays were used to determine vascular endothelial growth factor by the human VEGF-A electrochemiluminescence assay kits, and soluble fms-like tyrosine kinase-1 (sFlt-1) was measured by the human Flt-1 electrochemiluminescence assay Kits (Merck Sharp & Dohme, Kenilworth, NJ, USA). Furthermore, inhibin, β-hCG, and AFP were analyzed by a "sandwich-type" immunoassay using a monoclonal antibody on an AutoDELFIA system (PerkinElmer, Waltham, MA).

The primary outcome of the current study was preterm birth (birth between 20 and <37 weeks of gestation). The secondary outcomes were hypertensive disorders of pregnancy, small of gestational age neonates and stillbirth). Hypertensive disorders of pregnancy included preeclampsia, eclampsia and superimposed preeclampsia. Pregnancy associated hypertension was defined as a new onset of hypertension (≥ 140 mmHg systolic or ≥ 90 mmHg of diastolic blood pressure on two different occasions, 6 hours apart, after the end of 20th week of gestation, and before delivery. Small for gestational age neonates was defined if neonate's birthweight < the fifth percentile for gestational age at delivery, as defined previously **(11).**

Stillbirth was defined as a fetal death after the beginning of the 20th week of gestation, with Apgar scores of 0 at first, fifth, and tenth minutes with no other signs of life by direct observation. It may be spontaneous if stillbirth resulting from the preterm labor or premature Prelabor rupture of membranes (PPROM). However, medically indicated preterm labor (PTB)-induced stillbirth was defined as stillbirth from labor induction or cesarean delivery due to maternofetal indication in the absence of PTB or PPROM. Women who developed adverse pregnancy outcome were considered "the study group", while those who did not develop adverse pregnancy outcome were assigned as "the control group".

The data collected included maternal age, pre-pregnancy body mass index (BMI), smoking exposure shortly before pregnancy (at the last 3 months before pregnancy), chronic medical disease before pregnancy (diabetes mellitus, or chronic hypertension). Ultrasound findings of uterine artery indices and cervical length in different visits were also documented.

Statistical analysis: Collected data were presented by their means and standard deviations and groups compared by independent samples "t" test. The difference was statistically significant if p value < 0.05. The predictive power of each protein was measured by plotting Receiver Operation Characteristics (ROC) curve. Area under the curve (AUC) ≥0.75 indicates good predictive power. All analyses were performed by statistical package for social sciences (SPSS) version 13 (SPSS Inc., USA) and MedCalc software.

Results

In the current work preterm birth (the primary outcome) was recorded for 107 out of 217 (49.3%). The pre-pregnancy body mass index (BMI) was significantly increased in study (preterm) than control (full term) groups (28.20±4.3 vs 25.3±3.3, respectively). In addition, the smoking exposure before pregnancy, chronic hypertension, pregestational diabetes were significantly higher among study than the control group (26.2, 8.4%, 6.5% vs 14.5%, 1.8%, and 0.9% respectively). However, the gestational age at the first and second trimester visits was comparable between study and control groups, but at delivery, it was significantly reduced in the study than the control group (35.10±1.06 vs 38.80±0.94 weeks respectively). Finally, there was significant reduction of birth weight in the stud than the control group, while adverse pregnancy outcomes (small for gestational age, pregnancy induced hypertension and still birth) were significantly higher among study than the control group (31.8%, 21.5%, 13.1% vs 5.5%, 6.4% and 2.7% successively) (Table 1).

Table (2) presents the results of different placental proteins in the maternal circulation in the study and control groups. ADAM12 and sFlt-1 were significantly decreased, while VEGF-A and AFP were significantly increased in the study than the control group in the first trimester. However, inhibition and β-hCG showed non-significant differences between the study and the control groups. At the second trimester, VEGF-A, inhibin and AFP were significantly higher in the study than the control groups. Regarding association between PIH and different placental proteins in maternal circulation, it was significantly associated with significant increase of VEGF-A and AFP. But, there was a significant decrease of ADMA12 and β-hCG unit. However, at the second trimesters there was a significant increase of VEGF-A, inhibin and AFP, while ADAM12 was significantly reduced in the study than the control group (Table 3). In addition, small for gestational age was association with significant reduction of ADAM12 and sFlt-1; while it was associated with a significant increase of VEGF-A, inhibin and AFP at the first trimester. At the second trimester, SGA also significantly associated with a reduction of ADAM12 and sFlt-1. But, with significant increase of VEGF-A, inhibin and AFP (Table 4). The still birth was associated with significant reduction of ADMA12, sFlt-1 and inhibin in the first trimester. But, associated with

significant increase of VEGF-A and AFP. The same situation was recorded at the second trimester (Table 5). Vascular endothelial growth factor in the first or second trimester was the most sensitive test for prediction of preterm birth, while sFlt was sensitive only in the first trimester. At the second trimester the AUC did not reach acceptable levels. Other proteins also did not had enough power of prediction of preterm birth (Table 6).

Table (1): Maternal demographics, chronic medical diseases and adverse outcome among study groups

Variable		Study group (n=107)	Control group (110)	P value
Maternal age (mean±SD)		25.36±3.9	25.96±4.1	0.10
Pre-pregnancy BMI(kg/m^2)		28.20±4.3	25.3±3.3	**<0.001***
Smoking exposure before pregnancy (n,%)		28 (26.2%)	16(14.5%)	**0.025***
Chronic hypertension (n,%)		9(8.4%)	2(1.8%)	**0.05***
Pregestational diabetes (n,%)		7 (6.5%)	1 (0.9%)	**0.034***
Gestational age	First visit	10.19±0.74	10.17±0.80	0.82
	Second visit	19.79±1.07	19.90±1.05	0.46
	At delivery	35.10±1.06	38.80±0.94	**<0.001***
Birth weight (g)		2290.32±523.78	3256.14±403.65	**<0.001***
SGA (n,%)		34 (31.8%)	6 (5.5%)	**<0.001***
PIH		23 (21.5%)	7 (6.4%)	**0.001***
Still birth		14 (13.1%)	3 (2.7%)	**0.005***

Table (2): Comparison between study and control groups regarding different placental proteins in maternal circulation

Timing	Variable	Study group (n=107)		Control group (110)		P value
		Mean	SD	Mean	SD	
First trimester	ADAM12	4.38	0.86	4.65	0.55	**0.007***
	VEGF-A	0.96	0.11	0.78	0.13	**<0.001***
	sFlt-1	780.62	60.18	824.66	26.73	**<0.001***
	Inhibin	327.14	29.12	322.04	24.70	0.17
	β-hCG	19.70	3.14	20.06	2.92	0.37
	AFP	15.76	2.82	13.96	1.79	**<0.001***
Second trimester	ADAM12	9.53	0.87	9.65	0.55	0.24
	VEGF-A	1.12	0.11	1.01	0.15	**<0.001***
	sFlt-1	764.54	70.44	774.66	26.73	0.16
	Inhibin	213.37	36.87	192.04	24.70	**<0.001***
	β-hCG	4.00	.54	3.91	0.54	0.26
	AFP	47.93	5.84	43.96	1.79	**<0.001***

ADAM12: A disintegrin and metalloproteinase domain-containing protein 12; VEGF-A: Vascular endothelial growth factor-A; sFlt-1: Soluble fms-like tyrosine kinase-1: β-hCG: beta subunit of human chorionic gonadotropin; AFP: Alpha-fetoprotein.

Table (3): Association between different placental proteins in maternal circulation and development of pregnancy induced hypertension (PIH)

Timing	Variable	PIH (n=30)		No PIH (n =187)		P value
		Mean	SD	Mean	SD	
First trimester	ADAM12	3.75	0.36	4.64	0.70	**<0.001***
	VEGF-A	1.00	0.13	0.85	0.14	**<0.001***
	sFlt-1	806.97	35.84	802.30	53.34	0.64
	Inhibin	324.57	28.92	324.55	26.79	0.99
	β-hCG	17.90	1.75	20.20	3.07	**<0.001***
	AFP	16.30	1.80	14.61	2.54	**0.001***
Second trimester	ADAM12	8.85	0.51	9.71	0.68	**<0.001***
	VEGF-A	1.16	0.12	1.05	0.14	**<0.001***
	sFlt-1	756.97	35.84	771.71	55.19	0.15
	Inhibin	236.77	37.95	197.07	28.66	**<0.001***
	β-hCG	3.93	0.55	3.96	0.54	0.82
	AFP	48.40	4.93	45.52	4.58	**0.002***

Table (4): Association between different placental proteins in maternal circulation and small of gestational age (SGA)

Timing	Variable	SGA (n=40)		No SGA (n =177)		P value
		Mean	SD	Mean	SD	
First trimester	ADAM12	3.77	0.49	4.69	0.67	***<0.001****
	VEGF-A	1.02	0.13	0.83	0.13	***<0.001****
	sFlt-1	711.23	47.75	823.67	19.30	***<0.001****
	Inhibin	355.15	40.93	317.64	16.26	***<0.001****
	β-hCG	20.02	3.09	19.85	3.02	0.75
	AFP	18.77	1.64	13.96	1.70	***<0.001****
Second trimester	ADAM12	9.05	0.76	9.71	0.66	***<0.001****
	VEGF-A	1.20	0.13	1.03	0.13	***<0.001****
	sFlt-1	719.73	92.49	780.96	29.53	***<0.001****
	Inhibin	240.60	37.02	193.96	25.10	***<0.001****
	β-hCG	4.03	0.51	3.94	0.55	0.31
	AFP	53.65	4.56	44.18	2.48	***<0.001****

Table (5): Association between different placental proteins in maternal circulation and still birth

Timing	Variable	Still birth (n=17)		No SGA (n =200)		P value
		Mean	SD	Mean	SD	
First trimester	ADAM12	3.78	0.45	4.58	0.72	***<0.001****
	VEGF-A	1.05	0.15	0.85	0.14	***<0.001****
	sFlt-1	767.35	74.42	805.97	47.82	***0.003****
	Inhibin	355.00	42.09	321.96	23.76	***<0.001****
	β-hCG	19.88	3.52	19.89	2.99	0.99
	AFP	16.53	2.40	14.71	2.48	***0.004****
Second trimester	ADAM12	8.79	0.45	9.66	0.70	***<0.001****
	VEGF-A	1.19	0.15	1.05	0.14	***<0.001****
	sFlt-1	746.65	76.93	771.63	50.37	***0.05****
	Inhibin	253.65	29.51	198.22	29.50	***<0.001****
	β-hCG	3.83	0.51	3.96	0.54	0.32
	AFP	51.41	6.20	45.46	4.29	***<0.001****

ADAM12: A disintegrin and metalloproteinase domain-containing protein 12; VEGF-A: Vascular endothelial growth factor-A; sFlt-1: Soluble fms-like tyrosine kinase-1: β-hCG: beta subunit of human chorionic gonadotropin; AFP: Alpha-fetoprotein.

Table (6): Sensitivity and Specificity of Different placental proteins in maternal circulation for preterm birth

Test Result Variable(s)		AUC	Std. Error	Asymptotic 95% Confidence Interval		Cutoff	Sensitivity	Specificity
				Lower Bound	Upper Bound			
First trimester	VEGF	**0.868**	0.025	0.819	0.916	0.8	**95.3**	67.3
	Inhibin	0.537	0.039	0.460	0.614	> 325	30.8	77.3
	AFP	0.687	0.036	0.615	0.758	>15	48.6	85.5
	ADAM12	0.611	0.041	0.531	0.691	4	48.6	92.7
	sFlt	**0.761**	0.032	0.699	0.823	≤ 829	**88.8**	53.6
Second trimester	VEGF	**0.755**	0.034	0.689	0.822	0.98	**93.5**	54.5
	Inhibin	0.655	0.038	0.581	0.729	> 215	41.1	90.9
	AFP	0.697	0.036	0.626	0.768	> 45	50.5	85.5
	ADAM12	0.543	0.042	0.461	0.625	8.9	42.1	93.6
	sFlt	0.559	0.040	0.480	0.638	≤ 769	44.9	73.6

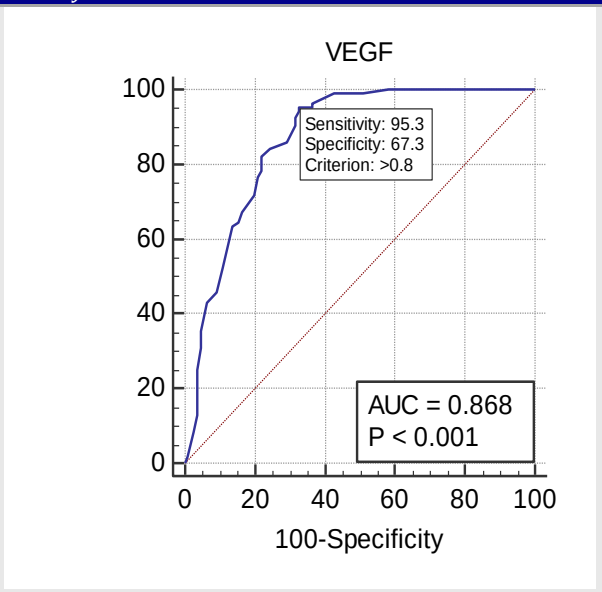

Fig (1): ROC curve for VEGF in the first trimester

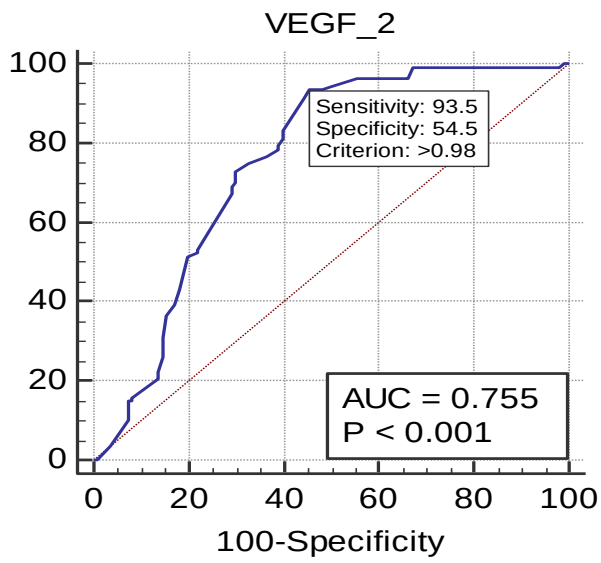

Fig (2): ROC curve for VEGF in the second trimester

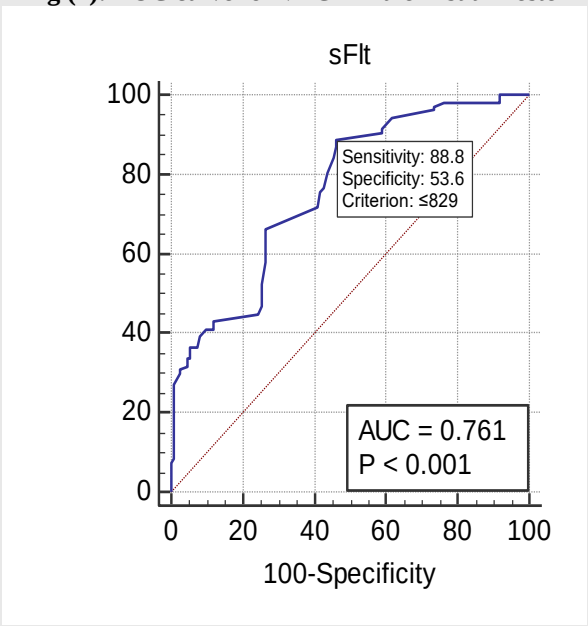

Fig (3): ROC curve for sFlt in the first trimestrer

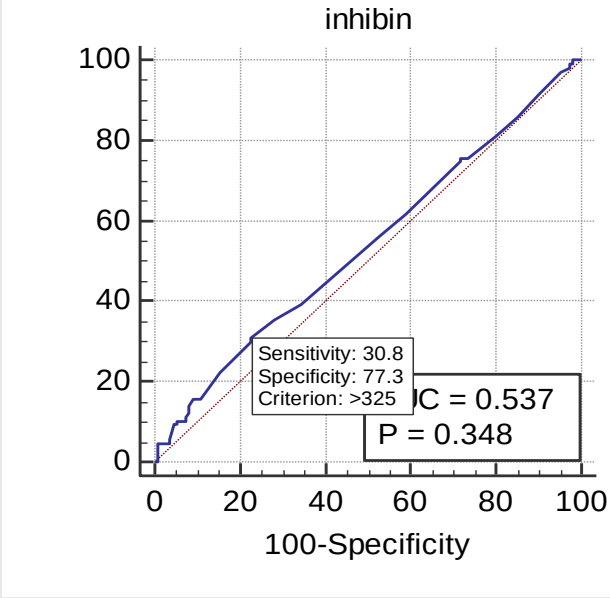

Fig (4): ROC curve for inhibin in the first trimester

Discussion

The current work results showed that, women with preterm birth were significantly older, exposed to smoking, had higher chronic hypertension and pregestational diabetes mellitus. Their babies had significant reduction of birth weight, had significantly higher rates of small for gestational age, pregnancy induced hypertension and still birth. This is line with previous studies indicated that, the risk of preterm delivery was higher in smokers than in non-smokers. A meta-analysis included 20 prospective studies reported that the risk of preterm delivery in smokers was increased by a factor of 1.3 [(12)]. Another population-based study of 1219159 singleton pregnancies reported that in smokers the risk of both spontaneous and iatrogenic delivery before 37 weeks was increased [(13)]. It has been suggested that cigarette smoke components may increase the risk of preterm pre-labor membrane rupture through impairment of immune function and promotion of inflammatory mechanisms [(14)].

In addition, results of the current work revealed that, preterm delivery was associated with significant decrease of ADAM12 and sFlt-1, but significant increase of VEGF-A and AFP in the first trimester. However, in the second trimester, there was significant increase of VEGF-A, inhibin and AFP in the preterm than the control groups. Additionally, and regardless of preterm delivery, women who developed PIH, small for gestational age, or still birth had significant changes of different placental proteins. However, VEGF and sFlt at the first trimester were able to predict preterm delivery. But, at the second trimester VEGF was the only biomarker able to predict

preterm birth (area under the curve >0.75). These results are in line with previous studies indicating that, maternal placental proteins are associated with adverse pregnancy outcome [15-17]. Others indicated that, clinical maternal factors are the only predictors of importance to predict adverse pregnancy outcome. Placental maternal proteins, however, only marginally improved the predictive values [6].

Results of the current work are in line with Odibo *et al.* [18] who reported that, significant differences were seen in mean levels of PAPP-A and ADAM12 in cases with preterm birth, pregnancy induced hypertension (preeclampsia) and small for gestational age (SGA) with placental histological pathological lesions when compared with controls. In addition, Goetzinger *et al.* [19] concluded that, first-trimester ADAM12 is predictive of preterm birth.

In agreement with the current work, Puntachai *et al.* [20] who reported that, serum levels of alpha-fetoprotein are associated with adverse pregnancy outcome such as preterm birth, PIH and small for gestation age. However, the area under the curve is < 0.75 in the prediction of three conditions, indicating poor predictive power. In addition, Bredaki *et al.* [21] in a case-control study on maternal serum AFP and preeclampsia revealed that AFP was elevated in both the first and the second trimesters in pregnancies that developed preeclampsia. Nunthapiwat *et al.* [22] reported that, high AFP and β-hCG levels were significantly associated with higher rates of preterm, early preterm and very early preterm delivery. The predictive models had low diagnostic performance in predicting preterm birth with the areas under the curve of 0.688 and 0.534, 0.599 for AFP and β-hCG levels. These results are comparable to the current work.

Andraweera *et al.* [23] conducted a review to summarizes knowledge of the roles of the VEGF family in early placentation, role in maternal plasma angiogenic proteins and adverse pregnancy outcome compared to normal pregnancy. PlGF and sFLT- in late first or second trimester predict early-onset PIH with a high sensitivity and specificity. However, VEGF family do not have sufficient power to accurately predict late-onset PIH, small-for-gestational age or preterm birth.

Alleman *et al.* [24] study revealed that, maternal serum markers for screening and prediction of adverse pregnancy outcome remained significant predictors of preterm birth, even after controlling for maternal characteristics. The best predictive model included second-trimester alpha-fetoprotein and inhibin A.

Conclusions

Maternal serum levels of placental proteins are significantly different early in first and second trimesters in women who later have preterm birth and other adverse pregnancy outcome (e.g., small for gestational age, pregnancy induced hypertension and still birth). However, these indicators, except VEGF, and SFlt, are poor predictors of adverse pregnancy outcome. These results must be explained in caution due to small sample size of included women.

Conflict of Interest: **None**

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