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Original Research

Do first trimester maternal serum follistatin like 3 levels predict preeclampsia and/or related adverse pregnancy outcomes?

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Summary

Purpose of Investigation: The aim of this study is to evaluate whether first trimester maternal serum follistatin like 3 (FSTL3) levels can be used to predict preeclampsia and related obstetric complications. *Materials and Methods:* The serum levels of FSTL3, pregnancy associated plasma protein A (PAPP-A), and free β-hCG were determined in the first trimester from a sample of 180 pregnant women. All patients had first- and second-trimester ultrasound evaluations. The pregnancy outcome was defined as 'adverse' if one of the following outcomes were observed: fetal death, preeclampsia, pregnancy-induced hypertension (PIH), delivery of a small infant for gestational age (SGA) or preterm delivery. *Results:* FSTL3 levels were not significantly different for preeclampsia and related adverse obstetric outcomes compared to the control group ($p < 0.05$). Only PAPP-A MoM values were lower in the adverse obstetric outcome group than in the control ($p = 0.040$). There was no significant association among FSTL3 levels and the presence of any complications, according to our ROC curve analyses ($p = 0.846$). *Conclusions:* First trimester FSTL3 levels are not predictive for preeclampsia or adverse pregnancy outcomes.

Key Words: Adverse outcome; First trimester; Follistatin like 3; Preeclampsia; Pregnancy.

Introduction

Preeclampsia is a serious complication affecting 4% to 8% of all pregnancies; it is a leading cause of maternal and perinatal mortality worldwide [1]. The etiology and pathogenesis of preeclampsia are unknown, but some molecular mechanisms may play an important role [2]. Maternal gestational hypertensive disorders and their complications have been proven to be the primary cause of adverse maternal and neonatal outcomes. Today, one of the purposes of prenatal care is to detect incipient preeclampsia and to prevent its progression [3]. First-trimester screenings for preeclampsia have offered physicians a significant opportunity to rethink preventive strategies. The 11–14-week nuchal translucency (NT) screening examination is an ideal point at which to integrate screening for preeclampsia [4]. Maternal historic variables are personal *a priori* risk modifiers, while placental Doppler studies and serum biomarkers can be considered early markers of placental success [4].

Follistatin is a binding protein for transforming growth factor-β (TGF-β) superfamily members, and it regulates their biological activity [5, 6]. Activin and inhibin are glycoproteins belonging to the TGF-β superfamily [7]. Activin regulates embryonic development and tissue homeostasis in adult animals, and follistatin may regulate some paracrine actions of activin within human placenta [8]. Some studies have shown that the placental expression and maternal serum levels of activin A and inhibin A are elevated in preeclamptic women [9, 10]. Maternal serum activin A is increased in some adverse obstetrics outcomes, such as preterm labour, gestational diabetes, hypertensive complications of pregnancy, and particularly preeclampsia [5, 9, 10]. Activin A may play a role in the adaptive responses to these pathologic situations [9]. However, the changes in circulating follistatin remain controversial, and there are contradictory reports in the literature related to maternal serum FSTL3 levels. Some studies show increased levels, whereas others show unchanged levels [5, 9, 11, 12]

The purpose of this study was to evaluate whether FSTL3 levels can be used as a first-trimester predictive serum marker for preeclampsia and related adverse pregnancy outcomes.

Materials and Methods

This is a prospective clinical study to investigate the relationship between maternal serum FSTL3 levels and preeclampsia and/or related adverse obstetric outcomes; it was conducted between February 2014 and February 2016. The hospital's ethics committee approved the study. Furthermore, all procedures performed in studies involving human participants were completed in

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accordance with the ethical standards of the institutional and/or national research committee, and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all participants included in the study.

The first trimester serum-screening test is initially offered to all pregnant patients who come to obstetric outpatient clinics at 11–14 gestational weeks, in accordance with the hospital's policy regarding screening. Those who consent to the screening test are referred to the perinatology clinic, and singleton pregnancies that underwent first-trimester serum screening tests were enrolled in the study consecutively. Exclusion criteria included diabetes mellitus, chronic hypertension, chronic renal disease, and other concurrent medical complications. Gestational diabetes mellitus was excluded after a 75-gram, two-hour glucose tolerance test, which was conducted between 24 and 28 weeks' gestation (n=19). Ten patients' first trimester screening test results revealed them to be at high risk, and they were excluded from the study. Seven patients did not appear for the control examinations, so a total of 180 pregnant patients were enrolled consecutively in the study, but only 144 of them completed it.

Fasting blood samples for FSTL3 levels were taken from the patients at the time of the first trimester screening test. All blood samples were centrifuged at 4000 g for ten minutes to clarify the serum. The serum assays were analysed in the hospital's biochemistry laboratory, and the risk was calculated via the immunochemiluminometric method. The PAPP-A assay had a sensitivity of 0.025 mIU/ml. The free β-hCG assay had a sensitivity of 1 ng/ml. For FSTL3 measurements, serum was collected and stored at -80°C until the assay was conducted. Serum FSTL3 concentrations were measured using a sensitive and specific ELISA kit. The assay had a sensitivity of 20 pg/ml.

All patients had first- and second-trimester ultrasound evaluations and Doppler velocimetry studies at 11–14 and 22–24 weeks of gestation. A single experienced ultrasonographer using an ultrasound machine with an abdominal convex probe (2-7 MHz, RAB6-D) performed each sonogram. The uterine artery Doppler waveform was visually analysed for the presence or absence of a diastolic notch. All pregnant women were followed up regularly during pregnancy.

Fetal death, preeclampsia, pregnancy-induced hypertension [(PIH) pregnancy onset hypertension without associated proteinuria], delivery of a small infant for gestational age [(SGA) baby's weight <10th percentile according to gestational age] and preterm delivery (delivery before 37 weeks' gestation) were defined as 'adverse pregnancy outcomes'. Preeclampsia was diagnosed according to criteria recommended by the American Congress of Obstetricians and Gynaecologists: blood pressure greater than 140/90 mmHg after 20 weeks of gestation and proteinuria ≥300 mg / 24 hours not resulting from chronic renal disease.

Data analyses were performed using SPSS Statistics version 22.0 software. Whether the distributions of continuous variables were normal or not was determined using the Kolmogorov Smirnov test. Data are shown as mean ± standard deviation or number of cases and percentages, where applicable. The mean differences between groups were compared using the Student's *t*-test, and the Mann Whitney U test was applied for the comparisons of not normally distributed data. Categorical data were analysed by Continuity (Yates) corrected Chi-square or Fisher's exact test, where appropriate. Whether the FSTL3 measurements had an effect on the prediction of preeclampsia or related obstetric complications was statistically significant or not was evaluated by the receiver operating characteristic (ROC) curve analyses. A *p*-value less than 0.05 was considered statistically significant.

Table 1. — *Clinical and biochemical characteristics of all patients.*

	Min–Max	Mean±SD
Maternal age	18–40	29.15±4.90
Birth week	21–42	38.49±2.31
Birth weight (grams)	550–4900	3297.52±533.74
PAPP-A (MoM)	0.06–4.07	0.93±0.59
Free β-hCG (MoM)	0.17–3.96	1.11±0.72
Follistatin like 3 levels (pg/ml)	0.448–27.12	11.1±4.72
Fetal sex	n	%
Male	73	50.7
Female	70	48.6
Unidentified sex (ex fetus)	1	0.7
Parity		
Nulliparous	33	22.9
Primiparous / multiparous	111	77.1

Results

The clinical and biochemical characteristics of all patients are presented in Table 1. The clinical characteristics of the pregnant women who developed hypertension (PIH and preeclamptic patients) (n=16) and those who did not (n=128) during follow up are shown in Table 2. There were no significant differences for smoking, parity, fetal sex, birth week, or birth weight between two groups ($p = 0.722$, $p = 0.762$, $p = 1.00$, $p = 0.101$, and $p = 0.074$, respectively). There were no significant differences between the two groups for biochemical markers or Doppler results either (Table 2).

The frequency of adverse obstetric outcomes in this study population was as follows: preterm labour (n=6, 4.19%), SGA baby (n=12, 8.39%), in utero fetal death (n=5, 3.49%), preeclampsia/eclampsia (n=13, 9.09%), PIH (n=3, 2.09%), gestational thrombocytopenia (n=2, 1.39%), and cholestasis of pregnancy (n=1, 0.69%).

When preeclamptic patients (n=13) were compared with non-preeclamptic ones (n=130), their PAPP-A MoM ($p = 0.137$), free β-hCG MoM ($p = 0.226$), FSTL3 levels ($p = 0.781$), and the presence of uterine artery end diastolic notch were not significantly different between the two groups (Table 3, Figure 1). When patients with adverse pregnancy outcomes (preterm delivery, SGA infant, in utero fetal death) were compared with all other patients for biochemical markers, only in the preterm delivery patients group was the PAPP-A MoM level significantly lower than all other patients ($p = 0.026$, Table 4).

Table 5 shows the biochemical and Doppler findings comparison among all patients with adverse pregnancy outcomes (n=36), and patients without adverse obstetric outcomes (n= 107). Some patients had two or more adverse obstetric outcomes. The PAPP-A MoM value was significantly lower, and the presence of a second trimester uterine

Table 2. — *Comparison of clinical factors and biochemical and Doppler findings among patients who developed hypertension in pregnancy (preeclampsia+PIH) and those who did not.*

	Patients with hypertension (HT) (n=16) n (%)	Patients without hypertension (HT) (n=128) n (%)	
*Smoking			
Patients with HT	3 (18.8)	20 (15.6)	0.722
Patients without HT	13 (81.2)	108 (84.4)	
**Fetal sex			
Male	8 (50)	65 (50.8)	1.000
Female	8 (50)	62 (48.4)	
*Parity			
Nulliparous	4 (25)	29 (22.7)	0.762
Primaparous/multiparous	12 (75)	99 (77.3)	
*11–14 weeks Doppler			
Notch (+)	4 (25)	31 (24.2)	1.000
Notch (-)	12 (75)	97 (75.8)	
*22–24 weeks Doppler			
Notch (+)	2 (12.5)	7 (5.5)	0.265
Notch (-)	14 (87.5)	120 (94.5)	
*** Birth week (mean±SD)	37.31±2.96	38.64±2.18	0.101
***Birth weight (gr) (mean±SD)	3072.81±497.67	3325.83±533.24	0.074
****PAPP-A MoM (mean±SD)	0.86±0.6 (0.8)	0.94±0.59 (0.79)	0.515
****Free β-hCG MoM (mean±SD)	0.99±0.77 (0.7)	1.12±0.71 (0.96)	0.210
***Follistatin like 3 levels (pg/ml) (mean±SD)	11.39±5.54	11.2±4.84 0.884	

**Fisher's Exact Test, **Continuity (Yates) düzeltmesi, ***Student t-Test, ****Mann-Whitney U Test.*

Table 3. — *Comparison of biochemical and Doppler findings among patients who developed preeclampsia in pregnancy and those who did not.*

	Patients with preeclampsia (n=13) mean±SD	Patients without preeclampsia (n=131) mean±SD	
*PAPP-A MoM	0.69±0.41 (0.76)	0.95±0.6 (0.79)	0.137
*Free β-hCG MoM	0.9±0.57 (0.77)	1.13±0.73 (0.95)	0.226
**Follistatin like 3 levels (pg/ml)	10.85±5.55	11.25±4.86	0.781
	n (%)	n (%)	
***11–14 weeks Doppler			
Notch (+)	4 (30.8)	31 (23.7)	0.518
Notch (-)	9 (69.2)	100 (76.3)	
***22–24 weeks Doppler			
Notch (+)	2 (15.4)	7 (5.4)	0.191
Notch (-)	11 (84.6)	123 (94.6)	

**Mann-Whitney U Test, **Student t Test, ***Fisher's Exact Test .*

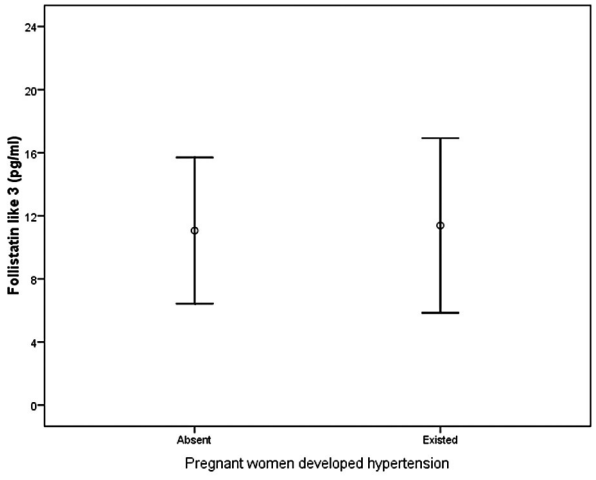

Figure 1. — Comparison of follistatin like 3 levels among patients who developed hypertension (preeclampsia+PIH) in pregnancy and those who did not. The circle in the middle of each whisker indicates the arithmetic mean, while the whiskers above and below the circle mark the (+) SD and (–) SD levels, respectively.

Table 4. — *Comparison of biochemical markers among patients with adverse obstetric outcomes (preterm labour, SGA infant, intrauterine fetal death) and patients without any adverse obstetric outcomes.*

	Patients with preterm labour (n=6) mean±SD	Patients with preterm labour (n=138) mean±SD	
*PAPP-A (MoM)	0.52±0.21 (0.49)	0.95±0.6 (0.82)	0.026
*Free β-hCG (MoM)	0.88±0.64 (0.58)	1.12±0.72 (0.95)	0.340
**Follistatin like 3 levels (pg/ml)	10.99±6.33	11.23±4.86	0.910
	Patients with SGA infant (n=12) mean±SD	Patients without SGA infant (n=132) mean±SD	
*PAPP-A (MoM)	0.81±0.45 (0.73)	0.94±0.6 (0.81)	0.454
*Free β-hCG (MoM)	1.11±0.5 (1.18)	1.11±0.74 (0.94)	0.472
**Follistatin like 3 levels (pg/ml)	11.9±5.91 (10.91)	11.16±4.83 (11)	0.614
	Patients with intrauterine foetal death (n=5) mean±SD	Patients without intrauterine foetal death (n=139) mean±SD	
*PAPP-A (MoM)	0.99±0.48 (0.9)	0.93±0.6 (0.78)	0.530
*Free β-hCG (MoM)	1.46±0.97 (1.21)	1.1±0.71 (0.95)	0.383
**Follistatin like 3 levels (pg/ml)	11.5±8.25 (7.85)	11.21±4.79 (10.99)	0.898

*Mann-Whitney U Test, **Student t-Test.

Table 5. — *Comparison of biochemical and Doppler findings among patients who developed any complications during pregnancy and those who did not.*

	Patients with any complications (n=36) mean±SD	Patients without complications (n=108) mean±SD	
*PAPP-A (MoM)	0.79±0.51 (0.84)	0.98±0.61 (0.67)	0.040
*Free β-hCG (MoM)	1.07±0.70 (0.95)	1.12±0.72 (0.86)	0.626
**Follistatin like 3 levels (pg/ml)	11.58±5.73	11.10±4.62	0.610
	n (%)	n (%)	
***11–14 weeks Doppler			
Notch +	11 (30.6)	24 (22.2)	0.432
Notch -	25 (69.4)	84 (77.8)	
*** 22–24 weeks Doppler			
Notch +	5 (14.3)	4 (3.7)	0.040
Notch -	30 (85.7)	104 (96.3)	

*Mann-Whitney U Test, **Student t-Test, ***Fisher's Exact Test.

Table 6. — *Results of the ROC analyses*

Outcomes	Area under the curve	95% confidence interval		*p*-value
		Lower Boundary	*Upper Boundary*	
Hypertension	0.507	0.346	0.669	0.924
Preeclampsia	0.522	0.346	0.698	0.794
Preterm labour	0.516	0.262	0.770	0.897
SGA infant	0.510	0.338	0.682	0.908
Intrauterine fetal death	0.538	0.172	0.904	0.772
Any complication	0.511	0.394	0.628	0.846

notch was more frequent in the patients with adverse pregnancy outcomes than in those without adverse obstetric outcomes ($p = 0.040$).

ROC curve analyses evaluated whether the FSTL3 measurements were effective for the prediction of preeclampsia or related obstetric complications. There were no statistically significant associations between FSTL3 levels and the presence of hypertension ($p = 0.924$), preeclampsia ($p = 0.794$), preterm labour ($p = 0.897$), SGA infant ($p = 0.908$), and/or intrauterine fetal death ($p = 0.772$) (Table 6).

In addition to the above, there was no statistically significant association among FSTL3 levels and the presence of any complications ($p = 0.846$). Consequently, it was determined that FSTL3 levels have no effect on the prediction of preeclampsia or related obstetric complications, according to the ROC curve analyses.

Discussion

The etiology and pathology of pre-eclampsia seem to be multifactorial. Literature findings show that the disorganisation of the activin pathway is involved in preeclampsia [11]. It has been reported that maternal serum levels of activin A and inhibin A are elevated in preeclamptic women [5, 7, 9]. However, the changes in circulating follistatin are controversial.

Pryor-Koishi *et al.* reported that the placental expression of FSTL3 via messenger RNA and protein, and the mater-

nal serum concentration of FSTL3 were significantly elevated in cases of preeclampsia, compared with normal pregnancy [13]. Guo *et al.* detected the significant elevation of myostatin, a ligand of FSTL3 and a member of the TGF-β superfamily, in the circulation and placentas of preeclamptic women [11]. The localisation of FSTL3 to walls of decidual and placental blood vessels is consistent with the role of FSTL3 in the vascular remodelling of vessels perfusing the intervillus space [14, 15]. The increased circulating concentration of FSTL3 across gestation in women who subsequently developed preeclampsia is consistent with the finding that FSTL3 mRNA is increased in primary human trophoblasts-cultured hypoxic conditions [16]. Women with elevated FSTL3 at mid-gestation had greater than three-fold odds of developing preeclampsia [17].

Some publications have described different findings regarding follistatin; for example, D'Antona *et al.* found that the maternal serum follistatin level was not significantly different between preeclamptic and normotensive pregnancies [9]. In the present study, the authors also found normal first trimester FSTL3 levels in patients with preeclampsia or related adverse obstetric outcomes. In the literature, the elevation of FSTL3 was demonstrated in the second trimester of pregnancy as a precursor to developing preeclampsia [16, 17]. It was recognised that human placenta secretes significant amounts of inhibin A, activin A, and follistatin throughout gestation, resulting in a progressive increase of these proteins in maternal circulation up to week 36 [9, 16]. The physiological increase of follistatin during the third trimester is preserved in these patients, regardless of hypertensive syndrome and in spite of placental distress. Follistatin may be a mechanism that protects the mother from the widespread actions of activin [18]. The present results reported here must be interpreted cautiously, because we measured follistatin only in first trimester as a means of preeclampsia prediction. Furthermore, a precise quantification of total follistatin concentrations is still unachievable by current immunoassays. In addition, according to weeks, FSTL3 levels cut-offs have yet to be determined in the literature. When the FSTL3 values are standardised, these values may be interpreted more accurately.

In agreement with the literature, the present data showed that pregnancies that subsequently developed an adverse outcome showed a significantly higher prevalence of bilateral notch, compared with pregnancies with normal outcomes [19–21].

Maternal serum PAPP-A has been shown to be relatively low in the first trimester of pregnancies complicated with SGA and/or preeclampsia [22, 23]. In accordance with the literature, the present results showed that maternal serum PAPP-A values were low in the first trimester of pregnancies complicated with adverse obstetric outcomes.

In summary, the present authors have shown that maternal serum FSTL3 levels during the first trimester of pregnancy are not predictive of preeclampsia and/or related complications. FSTL3 does not appear to be a good candidate for use among the first trimester markers in routine evaluations. Further studies with FSTL3 level measurements at each trimester of pregnancy are suggested. The results of this study are not decisive, and additional studies are needed to delineate the predictive role of FSTL3 in preeclampsia.

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