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ORIGINAL ARTICLE

Serum follistatin-like-3 levels in the diagnosis of tubal ectopic pregnancy

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ABSTRACT

Objective: Activin is necessary for maintaining normal pregnancy; follistatin and follistatin-like-3 (FSTL3) are known antagonists of activin. FSTL3 also has a role in the migration and invasion of trophoblasts. We aim to measure the value of serum FSTL3 levels in the diagnosis of tubal ectopic pregnancy.

Material and Methods: Seventy-six pregnant women including 39 women with surgically confirmed tubal ectopic pregnancy and 37 healthy, singleton controls whose gestational ages were between 5 and 8 weeks. Serum FSTL3 levels were measured in both groups through ELISA. Receiver operator characteristic (ROC) curve analysis was performed for the prediction of tubal ectopic pregnancy using serum FSTL3.

Results: Serum FSTL3 levels were significantly lower in the ectopic pregnancy group than in controls ($p<0.001$). ROC analysis showed that the area under the curve in the curve (AUC) was 0.811 (0.713–0.909; 95% confidence interval). The highest value of the sum of sensitivity and specificity in the ROC curve is the point where the FSTL3 level was 2160.7535 (cutoff value) with a sensitivity and specificity of 75.7% and 79.5%, respectively. Furthermore, the subset analysis of patients with prior ectopic pregnancy showed that serum FSTL3 was the highest in patients without any previous ectopic pregnancy ($p<0.001$).

Conclusion: Low levels of serum FSTL3 may be a potential biomarker in the early diagnosis of tubal ectopic pregnancy.

Keywords: Biomarker, follistatin-like-3, transforming growth factor family, tubal ectopic pregnancy.

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INTRODUCTION

Tubal ectopic pregnancy represents 96% of all extrauterine pregnancies.[1] Although there are improvements in diagnostic techniques and management, ectopic pregnancy continues to be a major cause of pregnancy-related mortality and morbidity. Thus, early and accurate diagnosis is crucial in this condition for both preventable mortality and tubal loss due to rupture. Search for sensitive and specific biomarkers continues to facilitate early diagnosis and therefore treatment. The limited specificity and sensitivity of the serum markers that had been investigated and studied raise questions about their reliability.

Members of the transforming growth factor-beta (TGF-β) family, as in growth and embryonic development, also play a role in organ homeostasis and damage/healing response.[2] Activin and myostatin are the structural arms of the TGF-β family that uses cell surface receptors and secondary messengers.[3,4] While activin determines the fate and differentiation of embryonic cells to organs, it also contributes to human organ homeostasis in adulthood.[5] These findings show that tight regulation of activin and myostatin is important in maintaining normal physiology.

Activin and myostatin activity has been regulated by many checkpoints. Extracellular regulators, follistatin-like-3 (FSTL3) and follistatin, are glycoproteins that structurally and functionally bind to and antagonize activin and myostatin.[6] FSTL3 expression has been localized in trophoblast, decidua, and fetal membranes in the first and third trimesters of human pregnancy.[7] FSTL3 protein functions as an extracellular regulator, antagonizes activin (inhibits the ability of activin to bind to its receptor), and bone morphogenetic proteins; therefore regulates their functions extracellularly.[8] FSTL3 expression is highest in the placenta; subsequently in the testes, pancreas, and heart follow, it has also been shown in ovaries and kidneys.[9] Most of the circulating follistatin is found to be related to activin, while FSTL3 is complexed with myostatin in humans and mice.[10]

Activin and follistatin are found in human endometrial and tubal epithelium and are the main regulators of endometrial and tubal physiology throughout the menstrual cycle. Pathological expression of activin and follistatin has been observed in tissue and serum samples of ectopic pregnancy cases.[11]

The platelets have a role in thrombosis and inflammation. In the ectopic pregnancy site, there is increased platelet aggregation, therefore, the number of platelets in circulation and mean platelet volume (MPV) decreases.[12]

Ectopic pregnancy may be linked to impaired tubal peristalsis or increased tubal receptivity. Therefore, in light of these findings, we reasoned that serum FSTL3 levels might be a novel, valuable biomarker in the diagnosis of tubal ectopic pregnancy. We aimed to demonstrate the possible diagnostic accuracy and differences in serum levels in intrauterine and tubal ectopic pregnancies and the correlation of serum FSTL3 with MPV values.

MATERIAL AND METHODS

Study Population and Inclusion Criteria

The present study was designed as a case–control study, conducted between June and December 2017 at the Health Sciences University Zeynep Kamil Women and Children's Health Training and Research Hospital. This study was performed in line with the principles of the Declaration of Helsinki and approved by the Institutional Review Board (Decision number: 94/2017). All participants gave their informed consent to participate in the study.

A total of 76 women were included in the study. Thirty-nine patients with surgical and histopathological proof of tubal ectopic pregnancy constituted the study group; and 37 healthy, singleton first trimester pregnancies constituted the control group. Patients who enrolled in the study group were, older than 18 years, ectopic pregnancy cases and pathologically confirmed as an ectopic pregnancy. Healthy, viable, singleton pregnancies presented in the outpatient clinic for routine antenatal visit, with gestational age (GA) 5–8 weeks, without a history of progesterone use or threatened abortion in the current pregnancy were enrolled in the control group. The GA was determined according to the 1st day after the last menstrual period (LMP) for both groups and was confirmed by sonographic crown-rump length measurement in the control group. Diagnosis of ectopic pregnancy was suspected with clinical signs and symptoms, transvaginal ultrasound scan, and quantitative serum beta-human chorionic gonadotropin (beta-HCG) levels, confirmed after laparoscopic surgery and histopathologic examination of specimens. Diagnosis of intrauterine healthy pregnancy was made after an ultrasound scan revealed an embryo with cardiac activity within the uterus.

Exclusion criteria were patients who received medical treatment (methotrexate) and had ruptured ectopic pregnancy or tubal abortion (visually confirmed in surgery by authors) multiple pregnancies, fetal congenital anomalies, abortus imminens, incomplete abortion, and maternal chronic diseases.

Data Collection

Detailed obstetric and gynecological history was obtained from women who consented to participate in the study. Physical examination findings, age, body mass index (BMI), gravida, parity, LMP, number of living children, previous pregnancy history, and presence of chronic disease were questioned in detail. The complete blood count of both groups and serum beta-HCG values of the study group were recorded. A 5 cc of venous blood was taken into one additional biochemistry tube for both groups and it was centrifuged for 10 min at 3500 rpm within 30 min until the serum was separated. The serum part was taken into the Eppendorf tube and frozen at –80°C, stored in optimal conditions until the day of analysis. Serum samples were analyzed for levels of FSTL3 by Human FLRG/FSTL PicoKine ELISA Kit (Boster Biological Technology Co., CA, USA).

Statistical Analysis

Data were analyzed by IBM SPSS for Windows version 23.0. Parametric tests were used to analyze normally distributed data, and non-parametric tests were used for non-normally distributed data. Mean±standard deviation and median (min–max) for descriptive statistics of continuous variables; numbers and percentages for categorical variables were used for expression. The significance of the difference between groups for categorical variables was evaluated with the Chi-square test and/or Fisher's exact test. In group comparisons, Mann–Whitney U-test (paired groups) and Kruskal–Wallis

Table 1: Clinical characteristics and demographic data of the study and control groups

	Control group (n=37)	Study group (n=39)	p
Age (years)	26.0 (18–35)	28.0 (21–35)	0.181
BMI (kg/m^2)	24.3 (19.4–36.8)	24.5 (19.7–30.1)	0.938
GA (weeks)	7.3 (5.6–8)	6.25 (5–7.6)	0.181
Number of miscarriages; median (min–max)	0 (0–2)	0 (0–6)	**0.030**
Number of ectopic pregnancies; median (min–max)	0 (0–0)	1 (0–2)	**<0.001**
0; n (%)	37 (100%)	3 (7.7%)	
1; n (%)	–	34 (87.2%)	
2; n (%)	–	2 (5.1%)	

The data presented as median (min–max) and percentage. BMI: Body mass index; GA: Gestational age; Min: Minimum; Max: Maximum.

Table 2: Serum FSTL3 values of the study and control groups

	Control group (n=37)	Study group (n=39)	p
FSTL3 (ng/dL) (Mean±SD)	3252.277±2196.144	1897.601±1277.049	<0.001

FSTL3: Follistatin-like-3; SD: Standard deviation.

test (more than 2 groups) were used. The correlation of continuous variables was evaluated with the Spearman correlation test, the correlation coefficient was considered as weak for 0–0.3, medium for 0.3–0.7, and as strong above 0.7. Receiver operator characteristic (ROC) curve analysis was performed to calculate the power of continuous variables in predicting pathology, the point where the sum of sensitivity and specificity value was highest from the points in the ROC analysis was determined as the threshold value, false-negative and false-positive results were calculated at this threshold value. p<0.05 was considered statistically significant.

RESULTS

The age, BMI, GA, and obstetric history of both groups are shown in Table 1. The number of prior ectopic pregnancy was significantly higher in the ectopic pregnancy group.

Serum FSTL3 levels were significantly lower in the study group (p<0.001) (Table 2). Figure 1 has shown the distribution of FSTL3 in the control and study groups by the box plot graph. In Figure 2, ROC curve analysis of serum FSTL3 level in the diagnosis of ectopic pregnancy was shown. The area under the curve in the ROC curve was 0.811 (0.713–0.909; 95% confidence interval). The highest value of the sum of sensitivity and specificity in the ROC curve is the point where the FSTL3 level was 2160.7535 (cutoff value) with a sensitivity and specificity of 75.7% and 79.5%, respectively (Table 3).

Spearman correlation analysis of serum FSTL3 levels with age, GA, BMI, and MPV is shown in Table 4. There were positive correlations between GA and FSTL3 levels (p=0.043, r=0.233) and also between MPV and FSTL3 levels (p<0.001, r=0.467). In the subgroup analysis, the mean serum beta-hCG level of ectopic pregnancy group was 3088±2429, and there was no significant correlation between beta-HCG and FSTL3 levels in the ectopic pregnancy group (r:0.0313, p:0.8478).

Table 3: Distribution of serum FSTL3 levels among groups based on cutoff value

	High FSTL3 (≥2160.7535)	Low FSTL3 (<2160.7535)	Total
Control group	28	9	37
	75.7%	24.3%	100%
Study group	8	31	39
	20.5%	79.5%	100%
Total	36	40	76
	47.4%	52.6%	100%
	Result	**95% CI**	
Positive likelihood ratio	3.27	1.81–5.90	
Negative likelihood ratio	0.27	0.14–0.51	

FSTL3: Follistatin-like-3; CI: Confidence interval.

We compared serum FSTL3 levels according to obstetric history, and median FSTL3 was the highest in patients without prior ectopic pregnancy (p<0.001) (Table 5).

Table 4: Correlation analysis of FSTL3 with maternal clinical characteristics

	FSTL3 (ng/dL)	
	r*	p
Age (years)	-0.123	0.289
GA (weeks)	0.233	0.043
BMI (kg/m^2)	0.079	0.498
MPV (fL)	0.467	<0.001

*r: Spearman correlation coefficient; FSTL3: Follistatin-like-3; GA: Gestational age; BMI: Body mass index; MPV: Mean platelet volume.

Table 5: Comparison of serum FSTL3 levels with a history of ectopic pregnancy

Number of past ectopic pregnancies	FSTL3 (ng/dL)	
	n	Median (min–max)
0	40	2550.987 (1182.235–12,641.372)
1	34	1689.332 (606.314–6794.95)
2	2	1951.171 (398.613–3503.729)

FSTL3; Follistatin-like-3; Min: Minimum; Max: Maximum.

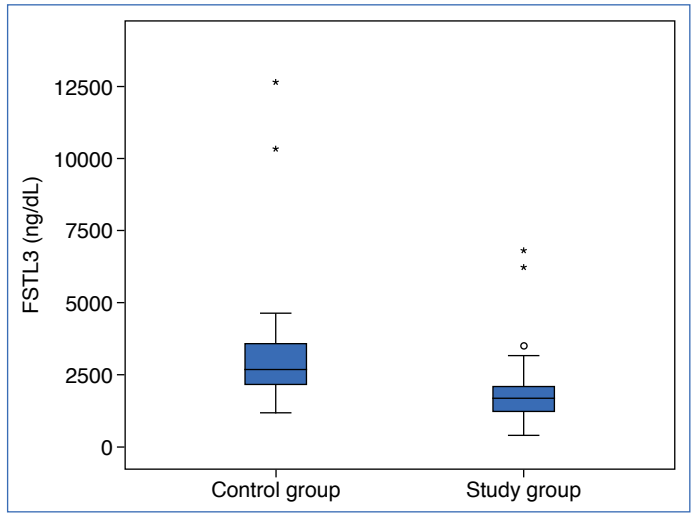

Figure 1: Comparison of serum FSTL3 levels between the study and control groups.

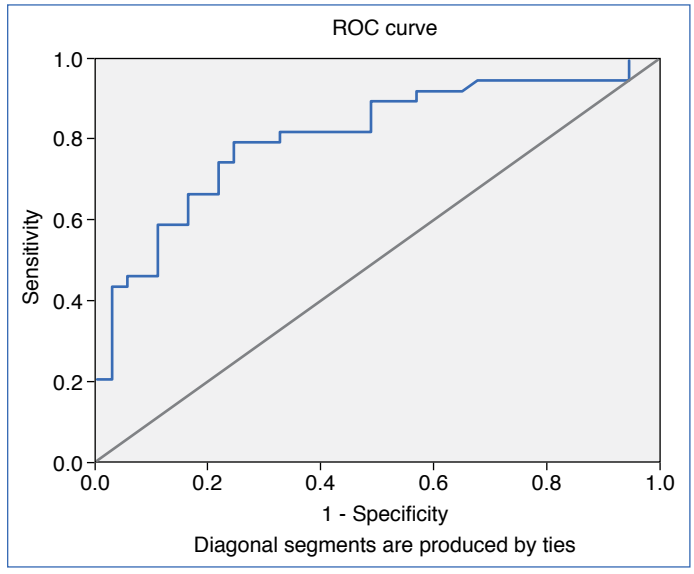

Figure 2: ROC curve of FSTL3 in ectopic pregnancy.

DISCUSSION

Tubal ectopic pregnancies account for 96% of all ectopic pregnancies.[1] Improvement in diagnostic methods increased its early detection, still, it continues to be responsible for 4% of pregnancy-related deaths.[13] Suspicion should be raised if there is no evidence of intrauterine pregnancy with transvaginal ultrasound scan, when serum beta-HCG levels are above discriminatory level and/or presence of complex adnexal mass with or without free fluid in the abdomen on a transvaginal ultrasound scan. It can only be confirmed when an embryo with or without cardiac activity is visible in an extrauterine gestational sac on a transvaginal ultrasound scan and/or direct visualization during surgery with histopathologic examination of the specimen. Since surgical management is reserved for hemodynamically unstable patients, and not all cases demonstrate definitive ultrasound characteristics; this pregnancy of unknown location time frame can be stressful for patients, as well as could lead to delayed diagnosis.

Several biomarkers for early detection of ectopic pregnancy were studied. Markers of trophoblast function (hyperglycosylated hCG, activin-A, pregnancy-associated plasma protein-A, pregnancy-specific beta-glycoprotein 1, human placental lactogen, a disintegrin and metalloprotease-12, placental mRNA, and microRNA's), markers of corpus luteal function (progesterone and inhibin-A), markers of angiogenesis (vascular endothelial growth factor and placental-like growth factor), and markers of endometrial function (leukemia inhibitory factor, glycodelin, mucin-1, and adrenomedullin) have all once been proposed as promising biomarkers, however, none have been able to consistently predict ectopic pregnancy.[14]

Activin A measurement has been proposed for the diagnosis of missed abortion or ectopic pregnancy.[15,16] Activin A induces the release of FSTL3 and the decreased levels of activin A are found to be associated with pregnancy loss.[17] It was previously reported that FSTL3 has a role in the invasion and migration of trophoblasts.[18] Since lower serum activin A levels may predict unsuccessful pregnancies; and keeping FSTL3's antagonist effect on activin in mind, this indirectly suggests a similar predictive value for FSTL3.[19] Serum FSTL3 levels were significantly lower in tubal ectopic pregnancies in our study (p<0.001). This finding is novel and leads us to suggest that impaired release of FSTL3 is associated with defective trophoblast implantation.

Another hypothesis of the impaired embryo transfer within the fallopian tube is the relationship between progesterone and activin. Activin decreases progesterone in human luteinized cells. Progesterone is known to slow down the contractions of the longitudinal muscular layer of human fallopian tubes and decrease tubal ciliary function which could affect embryo transport.[20,21] Activin interacts both with FSTL3 and progesterone, and future studies need to search this intricate relation as FSLT3 levels may be linked to progesterone levels.[22]

Although most of the studies investigated the diagnostic value of serum biomarkers, limited information available in terms of correlation between levels of biomarkers and GA. Birkhahn et al.[23] examined the relationship between serum smooth muscle heavy chain myosin levels and GA in ectopic pregnancy, interestingly, a positive correlation was found in non-ectopic pregnancy. Failure to find a significant relationship with ectopic pregnancy may be due to an unpredictable growth rate. In addition, Güvendağ et al.[24] looked into the relationship of carcinoembryonic antigen 125 (CA125), estradiol, and progesterone with the size of ectopic pregnancy and found that the levels of biomarkers have positively correlated with size. This study supports our findings; there was a positive correlation of FSTL3 levels with GA ($p<0.001$, $r=0.446$), which was expected because naturally as GA increases and size will be expected to grow. However, since this phenomenon applies to both ectopic and intrauterine pregnancies, its value in differential diagnosis of ectopic pregnancy is controversial.

MPV levels decrease in tubal ectopic pregnancy, this was attributed to possible high inflammation at the implantation site.[25] Similar to available data in the literature, we found a positive correlation between FSTL3 levels and MPV levels in the ectopic pregnancy group.[26]

We found lower levels of FSTL3 in patients with previous ectopic pregnancies, however, this finding could not reach a statistical significance level. This result suggests that lower FSTL3 in fallopian tubes may provide a basis for the development of ectopic pregnancy. However, it might also indicate that tubal damage caused by previous ectopic pregnancy may result in lower FSTL3 levels in those with a history of previous ectopic pregnancy. Subgroup analysis of the study group showed no significant difference between those who have a previous ectopic pregnancy and those not ($p=0.712$). This could be attributed to our low sample size in prior ectopic pregnancy group. This brings up the question of the relationship between serum markers and number of ectopic pregnancies which has not been addressed in the literature.

Our study is not without limitations. FSTL3 levels have not been compared with previously studied markers such as progesterone. Furthermore, we only compared serum FSTL3 levels of tubal ectopic and viable intrauterine pregnancies. Determination of serum FSTL3 levels in non-viable intrauterine pregnancies would more accurately estimate the success of the marker in differential diagnosis of ectopic pregnancy.

CONCLUSION

To the best of our knowledge, this is the first prospective study focused on the value of serum FSTL3 levels in tubal ectopic pregnancy diagnosis. FSTL3 stands out as a promising biomarker for early prediction. To be able to compare with other diagnostic methods used in ectopic pregnancy, more studies with larger sample sizes are needed.

Statement

Ethics Committee Approval: The Zeynep Kamil Maternity and Children's Training and Research Hospital Clinical Research Ethics Committee granted approval for this study (date: 05.05.2017, number: 94).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – ATT, IA; Design – ATT, IA, İD; Supervision – IA; Resource – HB; Materials – MA, BY; Data Collection and/or Processing – IA; Analysis and/or Interpretation – ATT, IA; Literature Search – IA; Writing – IA; Critical Reviews – IA.

Conflict of Interest: The authors have no conflict of interest to declare.

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