Kirkuk Journal of Medical Sciences

ORIGINAL ARTICLE

Evaluation of Serum Soluble fms-like Tyrosine Kinase-1 as a Biomarker in Ectopic Pregnancy

Alaa Raeed Khorsheed [1,*] and Aseel Ghazi Rifat [2]

[1] *Gynecology and Obstetrics, Iraqi Board for Medical Specialties in Gynecology and Obstetrics, Iraq*
[2] *Assistant Professor of Gynecology and Obstetrics, University of Kirkuk, College of Medicine, Kirkuk, Iraq*

[*] *Corresponding author email: samsungomer378@gmail.com*

Received: 06 December 2023
Accepted: 12 March 2024
First published online: 01 April 2024

DOI: 10.32894/kjms.2024.145153.1083.

ABSTRACT

Background: An ectopic pregnancy occurs when a blastocyst implants outside the uterine, early diagnosis is crucial for minimizing associated risks. Soluble FMS-like tyrosine kinase1 (sFlt-1) is the soluble variant of the vascular endothelial growth factor receptor1 that forms a barrier against abnormal vascular permeability and abnormal angiogenesis. The study aims to determine the level of (sFlt-1) in ectopic pregnancy and identify whether it can be used as a biomarker to distinguish ectopic from normal intrauterine pregnancy and missed miscarriage.

Methods: A prospective case-control study enrolled 90 pregnant women, with gestational age (4-10 weeks) divided into three groups: 30 women with ectopic pregnancy, 30 missed miscarriages, and 30 women with normal intrauterine pregnancy. (sFlt-1) was measured and statistical analysis of the data was performed to compare the level of biomarker between the studied groups.

Results: The mean level of (sFlt-1) was significantly lower among ectopic pregnancy (0.25 ± 0.07 ng/ml) in comparison to miscarriage and intrauterine pregnancy group. The (sFlt-1) at a level of 0.277 ng/ml was able to distinguish an ectopic pregnancy from normal intrauterine pregnancy and miscarriage with a sensitivity of 90% and a specificity of 70%. The mean serum (sFlt-1) increased with increasing gestational age, especially at more than 8 weeks.

Conclusion: A single measurement of the level (sFLt- 1) level can efficiently discriminate ectopic pregnancy from viable and non-viable intrauterine pregnancy. Serum (sFLt-1) at the cut-off value had the same sensitivity and better specificity than βhCG in the diagnosis of ectopic pregnancy.

Key words: Ectopic pregnancy; Missed miscarriage; Serum FMS like tyrosin kinase 1.

INTRODUCTION

Ectopic pregnancy (EP) originated from the Greek term 'ektopos', which means out of place, in which blastocyst implants are implanted at any site other than the endometrial lining of the uterine cavity [1]. An ectopic pregnancy cannot proceed normally. The fertilized egg can't survive, and the growing tissue may cause life-threatening bleeding if left untreated. Currently, the overall incidence is increasing worldwide due to increasing pelvic inflammatory disease (PID), increased induction of ovulation, and assisted reproductive technology, but the rate of fatality has decreased due to improved diagnostic techniques [2].

More than 95% of EPs are tubal in origin, and 80% are located in the ampullary portion of the fallopian tube [3].

Only about 50% of women diagnosed with EP have identifiable risk factors that include a previous history of EP, a history of pelvic surgery, pelvic inflammatory disease [PID] and chlamydia trachomatis, smoking, intrauterine contraceptive devices, exposure to diethylstilbestrol in utero, assisted reproductive technology, maternal age [4].

With the increasing trends in its incidence, a rapid and non-invasive biomarker to detect this condition early is crucial for improving both survival and conservation of reproductive capacity, thus decreasing the morbidity and mortality associated with the condition [1]. The classic triad of ectopic pregnancy symptoms is amenorrhoea followed by vaginal bleeding and ipsilateral abdominal pain. Free blood in the peritoneal cavity can cause diaphragmatic irritation and shoulder tip pain [5].

Ectopic pregnancy should be considered in any patient who presents early in pregnancy with vaginal bleeding or lower abdominal pain in whom intrauterine pregnancy has not yet been established [6]. The diagnosis of ectopic pregnancy in early pregnancy requires superior sensitivity and specificity, as a false negative could lead to serious morbidity and mortality and a false positive could result in the interruption of a potentially desired normal pregnancy [7]. Although surgical intervention such as laparotomy or laparoscopy used to be the mainstay of treatment, earlier detection has allowed a shift towards more conservative nonsurgical management [8].

Soluble FMS-like tyrosine kinase1 (sFLt1) is the soluble variant of the vascular endothelial growth factor (VEGF) receptor1 acts as markers of normal vascularization [9]. (sFLt 1) ensnares VEGF with excessive affinity, inhibiting its mitogenic activity in vascular endothelial cells, and may personate an imperative role in down regulating angiogenesis [10]. In the placenta, sFlt-1 is expressed in trophoblast cells. The trophoblast layer located between umbilical capillaries and maternal blood vessels suggests that sFlt-1 forms a molecular barrier against abnormal vascular permeability and abnormal angiogenesis, trapping VEGF and PlGF [11] Thus, sFlt-1 appears as appealing angiogenic candidate markers for failed pregnancies [9].

Monitoring sFLt-1 levels in the early first trimester, especially during the first 4 to 10 weeks, helps determine the normal development and functioning of the placenta, which is the prerequisite for normal embryonic development [12]. Previous reports suggested that serum measurements of angiogenic factors can be used as markers to identify cases of early pregnancy failure (ectopic or miscarriage) [13].

The aim of this study was to determine the serum level of FMS-like soluble tyrosine kinase in ectopic pregnancy and identify whether it can be used as a biomarker to distinguish ectopic from normal IUP and missed miscarriage, comparing its performance with B.HCG.

MATERIALS AND METHODS

This is a prospective case control study that was conducted in the Department of Obstetrics and Gynecology at Azadi Teaching Hospital / Kirkuk during the period of 9 months from 1 February to the end of October 2021. The study protocol was approved by the Scientific Council of Obstetrics and Gynecology / Iraqi Board for Medical Specializations.

The study included 90 women, between 18-37 years of age, with positive pregnancy test and gestational age between (4-10 wks) based on menstrual date and US scan performed on the day of sample collection.

The included women were divided into three groups: 1. Ectopic group: 30 women with ectopic pregnancy. 2. Miscarriage: 30 women diagnosed with missed miscarriage. 3. Intrauterine pregnancy (IUP): 30 women with viable IUP.

The selection of ectopic cases was made from those admitted to the Obstetrics & Gynecology (OBG) inpatient department with clinical characteristics suspicious of EP. They were followed until the diagnosis of EP was made clinically, surgically, and confirmed by histopathology. The criteria used for the diagnosis of ectopic pregnancy were -the beta HCG levels greater than 1500 IU / L with trans-vaginal ultrasound showing an empty cervical canal and uterine cavity ±other US features of EP (apart from one case of scar site ectopic in which gestational sac present in the anterior lower uterine segment). OR - Serial measurements of B.HCG every 48 hours suggested ectopic pregnancy, when initial level was< 1500

and US characteristics were not conclusive.

The Miscarriage cases were recruited from the outpatient clinic or from those admitted for termination of pregnancy with US diagnosis of missed miscarriage at the same gestational age (GA).

The Intrauterine pregnant women were sequentially included from those attending their first routine hospital reservation visit in a comparable GA with no history of vaginal bleeding and US finding confirming viable intrauterine pregnancy.

Patients who are excluded from the study include those with the followings: A twin or multiple pregnancy, hydatiform mole, systemic diseases: patients with (chronic obstructive pulmonary disease, hypertension, diabetes, cardiovascular disease, chronic kidney disease, and chronic liver disease), using of anticoagulant drugs (eg, heparin) or antioxidant, taking exogenous progesterone and smoking.

Informed consent is obtained from all participants after discussing with them the nature of the study. A questionnaire had been applied to collect the needed information, including age, parity, and LMP. A general examination was performed that included vital signs and body mass index, abdominal and pelvic examinations were performed. Five ml of venous blood were drawn; in addition to routine investigations, the studied groups were investigated for Beta hCG level and sFLt-1. Serum sFlt-1 was measured using an enzyme-linked immune sorbent assay (ELISA) based on double antibody sandwich technology.

The Statistical Package for Social Sciences (SPSS) software version 23 was used for data entry and analysis. In the descriptive statistics for sociodemographic characteristics, the means, standard deviations, min, max values were used for continuous data. Numbers and percentage values were used for countable data AVONA test used. In analyzing the differences between the groups, analysis of the ROC curve was considered to find the cutoff level of Serum sFlt-1. Pearson correlation has been used to find the correlation between 2 continuous variables. $P<0.05$ was used as the threshold for statistical significance.

RESULTS

The total number of participants in this study was 90, 30 had ectopic pregnancy, 30 had missed miscarriage, and 30 were normal IUP, Of the 30 cases of ectopic pregnancy, all were tubal ectopic except for one case diagnosed as scar site ectopic.

The distribution of the study participants by demographic and clinical characteristics and the comparison in the mean of the age of the participants and the body mass index (BMI) are shown in **(Table 1)**.

The age of the study participants ranged from 18 to 37 years, the mean age in the ectopic, miscarriage and intrauterine pregnancy (IUP) groups was 26.6 ± 4.4 years, 26.5 ± 4.7 years, and 26.03 ± 4.6 years, respectively, and there were no significant differences in the mean age between all groups (P=0.856).

Although the mean body mass index in intrauterine pregnancy (25.73) was higher than in other groups, (23.71 and 22.68) for ectopic and miscarriage, respectively, there were no statistically significant differences between the groups (P=0.011). The highest proportion of participants in the three groups (ectopics, miscarriage and intrauterine pregnancy) were multiparous with parity between 1-3 (66.7%, 70% and 70%respectivly) and more than half of the participants in the three groups had no history of previous miscarriage (60%, 56.7% and 53.3%respectivly).

The gestational age of the participants ranged from (4-10 weeks) with the majority of the participants between 6-8 weeks (66.6%, 70% and 63.3% in ectopic, miscarriage, and intrauterine pregnancy, respectively) (P=0.281).There were no statistical differences between the groups regarding their parity, history of miscarriage, and gestational age, as shown in **(Table 1)**.

The mean, maximum, and minimum βhCG level in all groups is shown in **(Table 2)**. The mean βhCG was significantly higher among the intrauterine pregnancy group (24851.07 IU/L) compared to other groups (P=0.005). Although the mean of βhCG was higher among the miscarriage group (9783.67 IU/L) than that of ectopic pregnancy (6270.87 IU/L) there were no statistically significant differences between the two groups (P=0.079).

The mean, maximum, and minimum levels of sFLt-1 between the studied groups are shown in **(Table 2)**. The mean biomarker level in the ectopic pregnancy group was 0.25 ± 0.07 ng/ml, in the miscarriage group was 0.33± 0.08ng/ml, and in the IUP group it was 1.07 ± 0.63ng/ml, There was a significantly lower mean of sFLt-1 between the EP group compared to the miscarriage and the intrauterine pregnancy group (P=0.001), Also, there was significantly lower sFL-1 among miscarriage compared to the IUP group (P=0.001).

The receiver operating characteristic (ROC) curve analysis was constructed for the serum βhCG biomarker as a predictor of EP, the cutoff point of serum βhCG $\leq$ 4100 IU/L was able to distinguish an ectopic pregnancy from normal pregnancy and miscarriage with a sensitivity of 90% and a specificity of 56% as a large significant area under the ROC curve was found AUC= 81.7% **(Figure 1)**.

Table 1. Distribution of study participants according to demographic and clinical characteristics

Variables	Category	Ectopic	Miscarriage	IUP	P-value
Age	Mean SD	26.6 4.4	26.53 4.7	26.03 4.6	0.856*
BMI	Mean SD	23.71 3.21	22.68 3.11	25.73 4.2	**0.011***
Parity	0 1-3 4+	5 (16.7%) 20 (66.7%) 5 (16.7%)	6 (20%) 21 (70%) 3 (10%)	7 (23.3%) 21 (70%) 2 (6.7%)	0.756**
Miscarriage	0 1-2 3+ < 6 weeks	18 (60%) 9 (30%) 3 (10%) 8 (26.6%)	17 (56.7%) 11 (36.7%) 2 (6.7%) 5 (16.7%)	16 (53.3%) 14 (46.7%) 0 (0%) 8 (26.7%)	0.431**
G.A.	6 – 8 weeks > 8 weeks	20 (66.6%) 2 (6.6%)	21 (70%) 4 (13.3%)	19 (63.3%) 3 (10%)	0.281**

* ANOVA test, ** Chi-square test, BMI=body mass index, IUP=intrauterine pregnancy, G.A.=gestational age, SD=standard deviation

Table 2. Comparing the mean of βhCG level between study groups

Variables		Ectopic	Miscarriage	IUP	P-value
βhCG (IU/L)	Mean SD	6270.87 11586.80	9783.67 6398.15	24851.07 30787.21	**0.005* 0.079****
sFLt-1	Mean SD	0.25 0.07	0.33* 0.08	1.07** 0.63	**0.001* 0.001****

AVONA test* IUP compared to others, ** Miscarriage compared to ectopic, βhCG=beta-human chorionic gonadotropin, sFLt-1=soluble fms-like tyrosine kinase-1, IUP=intrauterine pregnancy

The level sFLt-1 level of ≤ 0.277 ng/ml was able to distinguish an ectopic pregnancy from a normal pregnancy or a miscarriage with a sensitivity of 90% and a specificity of 70% with a large area under the ROC curve (AUC=88.7%) **(Figure 2)**.

Comparison between ROC curve analyses for the diagnostic precision of EP biomarkers showed higher specificity for sFLt-1 (70%) compared to serum βhCG (56%) with both having nearly the same sensitivity **(Figure 3)**.

As shown in **(Table 3)** generally the mean sFLt-1 was increasing with increasing G.A., but this change was only significant at more than 8 weeks, as there was a significantly higher level of mean s.FLt-1 in G.A more than 8 weeks compared to the means at earlier G.A. (P=0.007).

The mean levels of sFLt-1 were compared between patients with ruptured and unruptured ectopic pregnancies. The analysis included 29 patients in total. Among them, 10 patients experienced ruptures, while 19 did not. The mean sFLt-1 level for those with ruptured ectopic pregnancies was 0.252 (SD=0.061), and for those without rupture, it was 0.259 (SD=0.085). The statistical comparison, conducted using an ANOVA test, revealed no significant difference in sFLt-1 levels between the ruptured and unruptured groups (P=0.821).

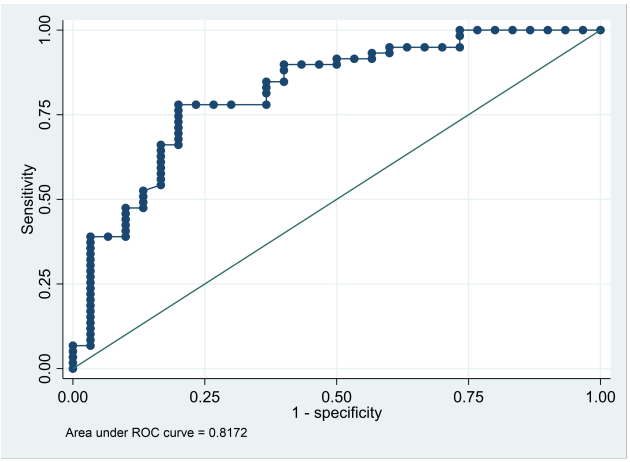

Figure 1. Receiver Operating Characteristics Curve Analysis of βhCG

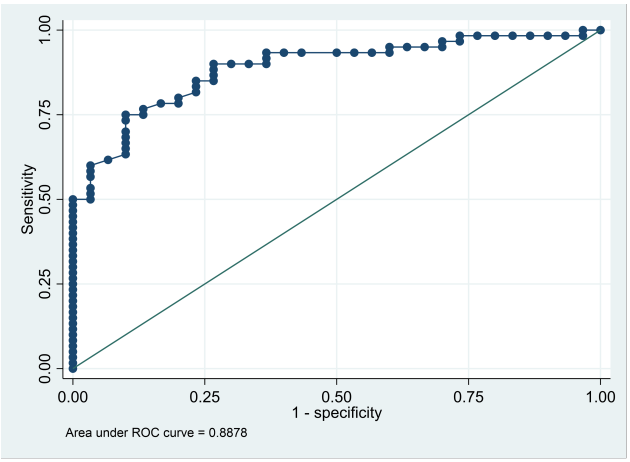

Figure 2. Receiver operating characteristic curve analysis of sFLt-1

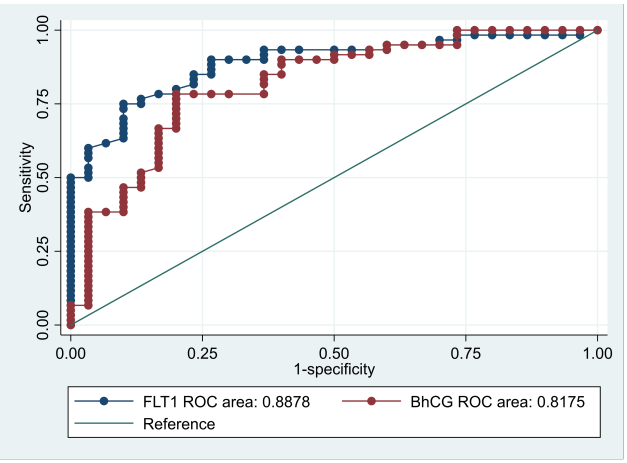

Figure 3. Comparison of Receiver Operating Characteristics Curve Analysis of the Biomarkers

Table 3. Comparing the mean of βhCG level between study groups

G.A.(Weeks)	Mean	SD	P-value
< 6 weeks	0.41	0.23	**0.012**
6 – 8 weeks	0.51	0.36	**0.007**
> 8 weeks	1.35	1.2	

* AVONA test, G.A.=gestationa age, SD=standard deviationl

DISCUSSION

Motherhood- An eternal, comprehensive, and natural dream that every woman has. This dream may not always be pleasant and can involve nightmares, one of these is ectopic pregnancy [14].

The early diagnosis of an ectopic pregnancy is one of the greatest challenges for an obstetrician, and its importance lies in the fact that women can receive a conservative treatment that can definitely have a beneficial effect on their reproductive capacity [15]. Although ultrasound is currently the standard of care used to diagnose ectopic pregnancy, it often requires multiple examinations and weeks to make a definitive diagnosis, for this. Serum biomarkers are currently being investigated as a solution to the need for a rapid and accurate test for ectopic pregnancy [16].

Human chorionic gonadotropin is a glycoprotein produced by syncytiotrophoblast and can be detected in serum as early as 8 days after the surge of luteinizing hormone (LH). Since the thresholds of increase in βhCG that distinguish normal and abnormal pregnancies in the early stages of pregnancy have evolved with time, βhCG values below the discriminatory zone combined with the absence of an intrauterine sac need serial measurement and the diagnosis must be supported by the US finding [17].

Serum soluble FMS-like tyrosine kinase-1 has been studied extensively for diagnosis and prediction of many gynecological conditions like preeclampsia, preterm birth, growth restriction, stillbirth, and miscarriage [16, 18, 19]. However, studies on its role in ectopic pregnancy are still spared [10]

In the current study, the mean βhCG was significantly higher among IUP group (24851.07 IU/L) in comparison to ectopic pregnancy (6270.87 IU/L) and miscarriage (9783.67 IU/L), although the mean βhCG level was higher among miscarriage group than that in ectopic pregnancy there was no statistically significant differences between the two groups .

This result agreed with the work of Martinez-Ruiz et al. [7] comparing βhCG levels between the EP and the miscarriage group, which did not show statistically significant differences between the two types of abnormal pregnancy. This indicates that although βhCG is beneficial in discriminating ectopic from IUP, it is not so efficient in discriminating it from miscarriage. Furthermore, in this study the βhCG level of < 4100 IU/L was able to distinguish an ectopic pregnancy with a sensitivity of 90% and a specificity of 56%.

In this study, there was a significantly lower mean level of sFLt-1 level among the EP group (0.25 ± 0.07 ng/ml) in comparison to both IUP pregnancy (1.07 ± 0.63 ng/ml) and miscarriage (0.33±0.08ng/ml). This finding shed light on the importance of sFLt-1 in the diagnosis of EP, as well as in discriminating it from miscarriage, when βhCG fails. The mechanism behind the reduction in the level of sFLt-1 in EP may be related to increased consumption due to the subsequent binding of the sFLt1 receptor to the excessively expressed VEGF, or a decrease in sFLt-1 production or both. This was in line with other studies finding that showed the level of sFLt-1 level was significantly lower in ectopic pregnancy and miscarriage compared to normal pregnancy, such as Daponte et al. [9] study in which the mean value of sFLt-1 was significantly lower in EP (178.16 ±76.03 pg/ml) and miscarriage (399.42 ± 337.54 pg/ml) compared to women with viable IUP (1390.32 ± 655.37pg/ml) and in the Selvarajan et al. [10] study that reported that the sFLt-1 level is significantly lower in the ectopic group (419 pg/ml) compared to IUP(898 pg/ml). The differences in the mean sFLt-1 reported in our study with studies of Selvarjan et al. and Daponate et al. [9, 10]. may be related to different units used for the measurement of sFLt-1.

Furthermore, Martínez-Ruiz et al. [7] study that investigated the role of sFLt-1 as a diagnostic biomarker for ectopic pregnancy and missed miscarriage (recruited those pregnant who suffered a failed early pregnancy with levels of βhCG between 800 and 3500 IU/L.) showed that sFlt-1 could be a useful marker to differentiate between an EP or a missed miscarriage when βhCG levels are similar in both groups. This

gives more strength to the sFLt-1 to play an essential role in differentiation between ectopic pregnancy from miscarriage especially when βhCG is below the discriminatory level.

Moreover, receiver operating characteristic curve (ROC) analysis of sFLt-1 showed that the sFLt-1 at level of $\leq$ 0.277 ng/ml was able to distinguish an ectopic pregnancy from miscarriage and IUP with a sensitivity of 90% and a specificity of 70%. This was in line with Selvarajan et al. study [10], which showed that sFLt-1 was able to distinguish an EP from a normal pregnancy at a cut-off of 623 pg/ml with a good sensitivity of 98.6% and specificity of 90.7% however, the cutoff value reported was higher than our results Similarly Daponte et al study [17], sFlt-1 showed a sensitivity of 62.5% and a specificity of 92.1% for sFLt-1 in the diagnosis EP from a miscarriage at the threshold value of 228.08 pg/ml.

According to the result of this study sFLt-1 at a cutoff level had better specificity and similar sensitivity for diagnosis of EP compared with βhCG 90%, 70%, vs 90%, 56% respectively.

Additionally in this study, the mean sFLt-1 was increased with increasing gestational age especially at more than 8 weeks, as there was a significantly higher level of sFLt-1 in pregnant women at this age compared to earlier pregnancy. This is an expected finding due to increasing trophoblastic cell mass, the major source of this biomarker, with advancing pregnancy. This finding was in line with the Selvarajan et al study [10].

Further analysis for ectopic pregnancy and sFLt-1 level based on presence of ruptured ectopic at time of surgery, find that the mean sFLt-1 level showed no significant difference between ruptured and non-ruptured ectopic. This may indicate that sFLt-1 can be regarded as an early diagnostic marker for ectopic pregnancy changing even before rupture occurs; unfortunately, we did not find research comparing sFLt-1 between ruptured and unruptured ectopic pregnancy.

CONCLUSION

A single sFLt-1 measurement can efficiently differentiate ectopic pregnancy from viable and non-viable intrauterine pregnancies at the same gestational age. Serum sFLt-1, at a specific cutoff, demonstrates equal sensitivity and superior specificity to βhCG in diagnosing ectopic pregnancy. Additionally, changes in sFLt-1 levels precede tubal rupture, suggesting a promising role in early tubal pregnancy diagnosis.

RECOMMENDATIONS

We recommend incorporating serum sFLt-1 level estimation into the diagnostic workup for ectopic pregnancy, particularly in emergency settings, due to its rapid, noninvasive, and cost-effective nature. Further prospective studies with larger participant sizes are crucial for comprehensive investigation and validation.

ETHICAL DECLARATIONS

- **Acknowledgements**

  None.

- **Ethics Approval and Consent to Participate**

  This study was approved by the Local Scientific Council of the Iraqi Board of Medical Specialization of Obstetrics & Gynecology, the concept of the study was discussed and verbal consent was taken from each participant.

- **Consent for Publication**

  Non.

- **Availability of Data and Material**

  The datasets are available from the corresponding author upon reasonable request.

- **Competing Interests**

  The authors declare that there is no conflict of interest.

- **Funding**

  Self funded.

- **Authors' Contributions**

  All stated authors contributed significantly, directly, and intellectually to the work and consented it to be published.

REFERENCES

[1] Patel TL, Chouhan G. Clinical study on ectopic pregnancy. International Journal of Reproduction, Contraception, Obstetrics and Gynecology 2023;12(2):382–387.

[2] Lawani OL, Anozie OB, Ezeonu PO. Ectopic pregnancy: a life-threatening gynecological emergency. International journal of women's health 2013;p. 515–521. https://doi.org/10.2147%2FIJWH.S49672.

[3] Zhou CC, Ahmad S, Mi T, Xia L, Abbasi S, Hewett PW, et al. Angiotensin II induces soluble fms-

Like tyrosine kinase-1 release via calcineurin signaling pathway in pregnancy. Circulation research 2007;100(1):88–95. https://doi.org/10.1161/01.RES.0000254703.11154.18.

[4] Jalal S. Antichlamydial Antibodies In Women With Ectopic Pregnancy. Kirkuk Journal of Medical Sciences 2022;10(1):105–118. https://doi.org/10.32894/kjms.2022.174201.

[5] Girardi G, Lingo JJ, Fleming SD, Regal JF. Essential role of complement in pregnancy: from implantation to parturition and beyond. Frontiers in immunology 2020;11:560852.

[6] Hendriks E, Rosenberg R, Prine L. Ectopic pregnancy: diagnosis and management. American family physician 2020;101(10):599–606.

[7] Martínez-Ruiz A, Sarabia-Meseguer MD, Pérez-Fornieles J, Vílchez JA, Tovar-Zapata I, Noguera-Velasco JA. Placental growth factor, soluble fms-like tyrosine kinase 1 and progesterone as diagnostic biomarkers for ectopic pregnancy and missed abortion. Clinical biochemistry 2014;47(9):844–847. https://doi.org/10.1016/j.clinbiochem.2014.03.013.

[8] Kirsch JD, Scoutt LM. Imaging of ectopic pregnancy. Applied Radiology 2010;39(3):10.

[9] Daponte A, Pournaras S, Polyzos NP, Tsezou A, Skentou H, Anastasiadou F, et al. Soluble FMS-like tyrosine kinase-1 (sFlt-1) and serum placental growth factor (PlGF) as biomarkers for ectopic pregnancy and missed abortion. The Journal of Clinical Endocrinology & Metabolism 2011;96(9):E1444–E1451. https://doi.org/10.1210/jc.2011-0037.

[10] Selvarajan S, Ramalingam J, Vijayaraghavan J, Bobby Z. Serum soluble FMS-like tyrosine kinase-1 in ectopic pregnancy. Journal of Laboratory Physicians 2019;11(04):335–339. https://doi.org/10.4103/JLP.JLP_168_18.

[11] Shibuya M. Vascular endothelial growth factor and its receptor system: physiological functions in angiogenesis and pathological roles in various diseases. The Journal of Biochemistry 2013;153(1):13–19. https://doi.org/10.1093/jb/mvs136.

[12] SELVARAJAN S, RAMALINGAM J, VIJAYARAGHAVAN J, BOBBY Z. Evaluation of Soluble Fms-like Tyrosine Kinase-1 in First Trimester of Pregnancy: A Cross-Sectional Study. Journal of Clinical & Diagnostic Research 2019;13(12). https://doi.org/10.7860/JCDR/2019/42533.13351.

[13] Daponte A, Pournaras S, Zintzaras E, Kallitsaris A, Lialios G, Maniatis A, et al. The value of a single combined measurement of VEGF, glycodelin, progesterone, PAPP-A, HPL and LIF for differentiating between ectopic and abnormal intrauterine pregnancy. Human reproduction 2005;20(11):3163–3166. https://doi.org/10.1093/humrep/dei218.

[14] Lee R, Dupuis C, Chen B, Smith A, Kim YH. Diagnosing ectopic pregnancy in the emergency setting. Ultrasonography 2018;37(1):78. https://doi.org/10.14366%2Fusg.17044.

[15] Hoffman BL, Schorge JO, Bradshaw KD, Halvorson LM, Schaffer JI, Corton MM. Williams gynecology, vol. 25. McGraw-Hill Education New York; 2016.

[16] Andersen LB, Dechend R, Karumanchi SA, Nielsen J, Joergensen JS, Jensen TK, et al. Early pregnancy angiogenic markers and spontaneous abortion: an Odense Child Cohort study. American journal of obstetrics and gynecology 2016;215(5):594–e1. https://doi.org/10.1016/j.ajog.2016.06.007.

[17] Nwabuobi C, Arlier S, Schatz F, Guzeloglu-Kayisli O, Lockwood CJ, Kayisli UA. hCG: biological functions and clinical applications. International journal of molecular sciences 2017;18(10):2037. https://doi.org/10.3390/ijms18102037.

[18] Thadhani R, Mutter WP, Wolf M, Levine RJ, Taylor RN, Sukhatme VP, et al. First trimester placental growth factor and soluble fms-like tyrosine kinase 1 and risk for preeclampsia. The Journal of Clinical Endocrinology & Metabolism 2004;89(2):770–775. https://doi.org/10.1210/jc.2003-031244.

[19] Smith GC, Crossley JA, Aitken DA, Jenkins N, Lyall F, Cameron AD, et al. Circulating angiogenic factors in early pregnancy and the risk of preeclampsia, intrauterine growth restriction, spontaneous preterm birth, and stillbirth. Obstetrics & Gynecology 2007;109(6):1316–1324. https://doi.org/10.1097/01.AOG.0000265804.09161.0d.