RESEARCH ON VALUES OF SERUM ACTIVIN-A CONCENTRATION IN DIAGNOSIS OF ECTOPIC PREGNANCY

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SUMMARY

Objectives: *To determine values of serum Activin-A and β-hCG concentrations in diagnosis of ectopic pregnancy (EP).* ***Subjects and methods:*** *A cross-sectional study conducted on 351 cases who were followed EP at the National Hospital for Obstetric and Gynaecology, and Military Hospital 103 from 9/2017 - 11/2019.* ***Results:*** *There were 194 cases of EP and 157 cases of intrauterine pregnancy (IUP). Mean serum concentration of Activin-A, β-hCG in EP group (2,440.9 pg/mL; 3,161.5 mUI/mL) was lower compared to IUP group (11,770.8 pg/mL, 39,185.8 mUI/mL); the difference was statistically significant (p < 0.05). The cut-off threshold of Activin-A was 3,233.7 pg/mL; β-hCG was 4921.5 mUI/mL; differential diagnosis of Activin-A and β-hCG between EP and IUP group had sensitivity of 87.1% and 91.7%, respectively; specificity of 27.3% and 71.3%, respectively; the difference was statistically significant (p < 0.05).* ***Conclusion:*** *Concentration of serum Activin-A and β-hCG levels were significant in the differential diagnosis of EP and IUP.*

** Keywords: Activin-A; Ectopic pregnancy; Intrauterine pregnancy.*

INTRODUCTION

Ectopic pregnancy is a leading cause of pregnancy-related maternal mortality in the first trimester. The diagnosis of EP is a challenge for obstetricians and gynecologists due to clinically unclear symptoms. Therefore, it is necessary to have more valuable tests to diagnose and manage promptly, avoid omission or dispensable surgical intervention that cause health and economic damages to patients [1]. A serum test with high sensitivity and specificity in diagnosing EP is a great concern to obstetricians and gynecologists and medical scientists.

Activin-A is a glycoprotein dimeric of TGF-β superfamily (tumor growth factor-β), which plays a role in invasion of cytotrophoblast cells [2]. Florio's study found that serum Activin-A concentration at the cut-off threshold was 0.37 ng/mL with sensitivity of 100% and specificity of 99.6% in the diagnosis of EP [3].

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Rausch found serum Activin-A concentration with sensitivity of 80% and specificity of 72% in diagnosing EP [4]. Daponte also observed that serum Activn-A concentration was significantly lower in EP and spontaneous abortion compared to normal IUP, Activin-A cut-off threshold was 505 pg/mL, differential diagnosis of EP and normal IUP showed sensitivity of 87.9% and specificity of 100% [5]. In contrast, Elito found that serum Activin-A levels could not distinguish between EP and normal IUP [6]. In general, these research results about the role of Activin-A in the diagnosis of EP are still controversial.

Therefore, to assess whether serum Activin-A concentration is really valuable in the diagnosis of EP, it is necessary to have more studies with larger sample sizes and to be conducted in multi-centers before clinical application. Thus, we conducted this study: *To do a research on values of serum Activin-A concentration in diagnosis of EP.*

SUBJECTS AND METHODS

1. Subjects

351 cases were followed EP at the National Hospital of Obstetrics and Gynecology and Military Hospital 103, from September 2017 to November 2019.

* *Selection criteria:* Pregnant women, positive urine and serum hCG test, with or without amenorrhea, lower abdominal pain, abnormal vaginal bleeding, unknown fetal position on vaginal ultrasound.

Patients are followed until the diagnosis of being EP or IUP. In case of EP, patients must be treated surgically whose pathological results are suitable for diagnosis. Once the diagnosis of IUP is established, gestational age ≤ 7 weeks (based on the menstrual period).

* *Exclusion criteria:* EP patients underwent that medical treatment, EPs were located outside the fallopian tube, patients with trophoblast disease, subjects refused to participate in the study.

2. Methods

* *Research design:* Cross-sectional study.

* *Procedures:* Clinical examination, ultrasound, take venous blood (3 - 5 mL) for Activin-A and β-hCG test. Blood samples after collection is stored at 4^0C, centrifuged at a speed of 3,000 - 4,000 rpm, for 10 minutes, serum separation, stored at -80^0C until assay. Quantification of Activin-A in serum by ELISA technique, kit of Human Activin A ELISA, Thermo Scientific. Absorbance is measured with Becman Coulter's DTX 880 at 450/550 nm.

* *Statistical analysis:* Using SPSS version 22.0. Compare mean values: if standard distribution variables, use T-test, otherwise standard distribution, use Mann Whitney test, Kruskal Wallis test. The test has statistical significance when $p < 0.05$.

RESULTS

1. General characteristics of subjects

Table 1:

General characteristics	Maternity condition		Total	p
	EP	IUP		
Age ($\bar{X}$ ± SD)	30.4 ± 5.8	29.8 ± 5.6	30.1 ± 5.7	0.37
Region: Rural (n, %) Urban (n, %)	121 (57.1) 73 (52.5)	91 (24.9) 66 (23.6)	212 (42.8) 139 (47.5)	0.16
Number of children ($\bar{X}$ ± SD)	1.22 ± 0.94	0.94 ± 0.79	1.07 ± 0.88	0.014
History of abortion ($\bar{X}$ ± SD)	0.92 ± 1.2	0.76 ± 1.4	0.85 ± 1.3	0.267
Hisrory of inflammatory gynecological disease (n, %)	61 (17.4)	28 (7 9)	89 (25.3)	0.006
History of prior abdominal incision (n, %)	237 (67.5)	38 (10.8)	118 (33.6)	< 0.001
History of intrauterine device insertion (n, %)	16 (8.2)	8 (5.1)	24 (6.8)	0.424

There was no difference in mean age, region, number of abortions, history of IUD insertion between the two groups. However, there was a statistically significant difference in number of children, history of inflammatory gynecological disease, history of prior abdominal incision between EP and IUP group ($p < 0.05$).

2. Serum Activin-A and β-hCG concentration

Table 2: Mean serum Activin-A and β-hCG concentration.

Markers		n	Mean	SD	95%CI		Min	Max	p
β-hCG (mUI/mL)	EP	194	3,161.5	14,462.3	1,113.6	5,209.5	43.0	193,433.0	0.01
	IUP	157	39,185.8	51,902.0	31,003.7	47,367.9	44.2	255,001.0	
	Total	351	19,275.0	40,469.2	15,026.6	23,523.3	43.0	255,001.0	
Activin-A (pg/mL)	EP	194	2,440.9	6,866.0	1,468.6	3,413.1	7.15	65,049.5	0.04
	IUP	157	11,770.8	75,673.5	-158,7	23,700.4	14.7	920,797.9	
	Total	351	6,614.1	50,989.7	1,261.3	11,966.9	7.15	920,797.9	

Mean serum concentrations of Activin-A and β-hCG in IUP group were higher compared to EP group, the difference was statistically significant ($p < 0.05$).

3. Values of Activin-A and β-hCG in differential diagnosis of EP and normal IUP

Table 3:

Markers	AUC	95%CI	Cut-off	Sensitivity (%)	Specificity (%)	p
Activin-A (pg/mL)	0.56	0.50 - 0.62	3,233.7	87.1	27.3	0.04
β-hCG (mUI/mL)	0.87	0.82 - 0.91	4,921.5	91.7	71.3	< 0.001

The cut-off threshold of Activin-A concentration was 3,233.7 pg/mL, value of differential diagnosis of EP and IUP had sensitivity of 87.1%, specificity of 27.3%, the difference

was statistically significant (p < 0.05). The cut-off threshold of β-hCG concentration was 4921.5 mUI/mL, this value had sensitivity of 91.7%, specificity of 71.3%, the difference was statistically significant (p < 0.05).

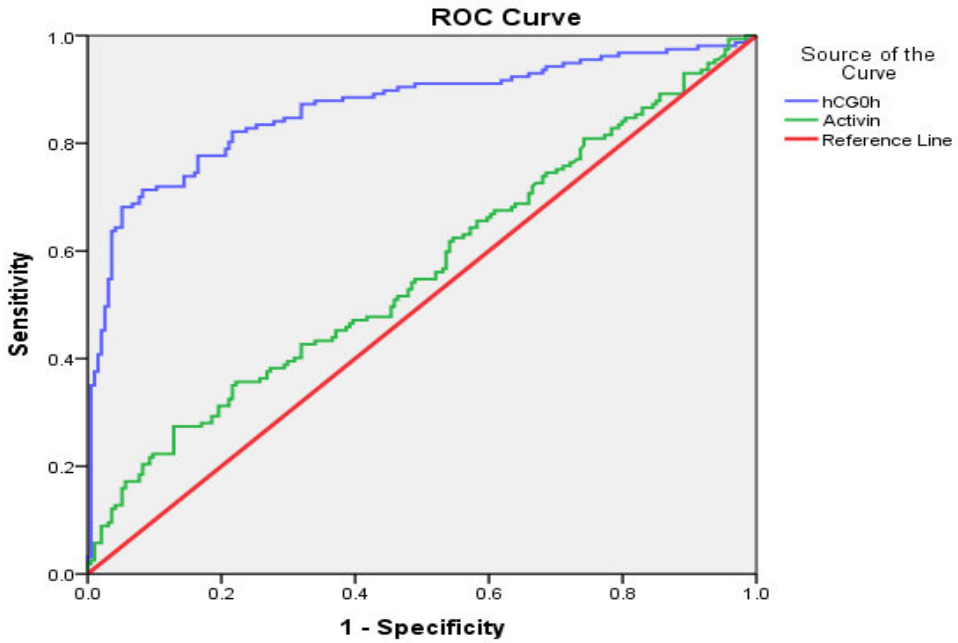

Figure 1: Receiver operating characteristics (ROC) curves for Activin-A and β-hCG in differential diagnosis EP and normal IUP.

DISCUSSION

In normal pregnancy, the change of serum Activin-A concentration is remarkable, the adjustment of increase and decrease is caused by the development of cytotrophoblast and syncytiotrophoblast. Activin-A levels in pregnant women are higher than in non-pregnant women and increase throughout gestational period until about 28 weeks. However, in the early stage of pregnancy, the expression of Activin-A is low because the invasion of trophoblast is not enough strong. It is reported that normal Activin-A levels in pregnancy increases 69 times during pregnancy (from 700 ± 200 pg/mL at week 6 - 7 to at 90,000 ± 54,000 pg/mL at week 38 - 39) [7].

According to current studies, Activin-A and β-hCG are actually low in EP compared to normal IUP. In 2010, Florio identified that Activin-A was an important protein in pregnancy, increased during pregnancy. Activin-A was originally secreted by the endometrial stromal cell, which was transformed into decidua membrane and prepared for embryo implantation, after implantation, placenta was the main source of Activin-A [3]. In this study, the concentration of β-hCG in the IUP group was higher than that of

EP group, the difference was statistically significant ($p < 0.05$). Our results were similar to studies by Al Maini (2019): The concentration of β-hCG in the IUP group was higher than in the EP group and spontaneous abortion ($p < 0.05$). Because when the fetus implanted in the normal uterus, the placenta, fetus and uterus grew dramatically, cytotrophoblast and syncytiotrophoblast which produced β-hCG increased, in contrast in EP, spontaneous abortion, the poor function of blastocyst cells led to low β-hCG level [8].

For Activin-A, Activin-A concentration in normal uterine pregnancy was higher than in EP group, the difference was statistically significant ($p < 0.05$). Our results were consistent with the studies by Rausch, Al-Maini... Activin-A can distinguish IUP with EP, with cut-off threshold of 3,233.7 pg/mL, EP diagnosis had sensitivity of 87.1%, specificity of 27.3%. Compared with β-hCG, the sensitivity and specificity for differential diagnosis of EP and IUP were lower (sensitivity: 91.7%; specificity: 71.3%).

Table 4: Comparison of diagnostic value of Activin-A in EP with other studies.

Study		Threshold cut-off	Sensitivity (%)	Specificity (%)	p
Florio (2007) [3]	Activin-A	370.0	99.6	100.0	< 0.01
	β-hCG	658.0	75.0	71.9	< 0.01
Daponte (2013) [5]	Activin-A	504.0	97.9	87.9	< 0.01
Al-Maini (2019) [8]	Activin-A	298.0	99.0	99.0	< 0.01
Our study (2020)	Activin-A	3,233.7	87.1	27.3	0.04
	β-hCG	4,921.5	91.7	71.3	< 0.01

CONCLUSION

Concentration of serum Activin-A and β-hCG were significant in the differential diagnosis between EP and IUP.

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