ORIGINAL ARTICLE

Comparative Study of Using (Acvtivin-A levels) as A New Tool in the Diagnosis of Ectopic Pregnancy

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Abstract

Background: ***Ectopic pregnancy (EP) is a significant global health concern. It happens when a blastocyst abnormally implants outside the endometrium of the uterus. It is implanted in the fallopian tube in over ninety-five percent of cases.***

Aim: ***To detect Activin-A early in maternal blood and determine if it can help in the diagnosis of early ectopic pregnancy.***

Patients and Methods: ***This prospective case control research has been performed on 89 cases, subjects were classified into 2 groups: Group 1: 45 cases diagnosed with tubal ectopic pregnancy confirmed by serum β-HCG and transvaginal ultrasound and Group 2: 44 cases with normal pregnancy at the obstetrics and gynecology department, Al-Zahraa University Hospital.***

Results: ***The association of serum Activin A (nanogram per milliliter) with clinical examination and TVS finding (pain, bleeding, presence of Mass, side of Mass and presence of free fluid) of cases with ectopic pregnancy and revealed insignificant positive association among Activin A concentrations and abdominal pain (r-value=0.013, p-value=0.893), bleeding (r=0.073, P=0.633). In contrast, a negative and insignificant association between Activin A and the presence of Mass was observed (r-value =-0.128, P-value =0.403). Conversely, a negative but not statistically significant association was found between Activin A and the presence of free fluid (r=-0.185, P=0.224).***

Conclusion: ***This study concluded that ectopic pregnancy is a frequent and severe condition with a high rate of morbidity and the risk of maternal death. There was no significant association between ectopic pregnancy and Activin-A level. Activin-A was a poor marker for ectopic pregnancy in early diagnosed cases.***

Keywords: EP; Acvtivin-A; maternal blood

1. Introduction

Ectopic pregnancy is a significant global health concern. It happens when a blastocyst abnormally implants outside the endometrium of the uterus. It is implanted in the fallopian tube in over ninety-five percent of cases.[1]

Since 1960, the occurrence of ectopic pregnancy has doubled and now comprises around two percent of 1st -trimester pregnancies. The prevalence of this condition has increased in recent years as a result of the rise in the frequency of pelvic inflammatory disorders, the application of fertility medications, and pelvic operations.[2]

Despite the present reduction in maternal mortality as a result of ectopic pregnancy, it remains one of the most common causes of mortality in the 1st trimester of pregnancy. Consequently, it is crucial to address ectopic pregnancy at an early stage.[3]

In developing countries, ten per cent of females diagnosed with ectopic pregnancy fail to survive because they do not refer to the hospital until the end of their pregnancy. The clinical manifestations of ectopic pregnancy may resemble those of other conditions. This demonstrates the necessity of identifying novel tools for diagnosis.[4]

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Serial measurement of serum B-HCG and transvaginal ultrasound. The most frequent diagnostic methods for EP are concentrations. A diagnosis of the illness fails to be detected in approximately forty percent to fifty percent of the initial cases, regardless of the application of transvaginal Ultrasonography and the measurement of β-hCG concentrations. Transvaginal Ultrasonography may be beneficial in the detection of an intrauterine pregnancy or an adnexal mass. Serum B-HCG concentrations are able to distinguish between a normal intrauterine pregnancy and a nonviable pregnancy; however, they are unable to differentiate between an intrauterine pregnancy that has ceased to grow and an ectopic pregnancy.[5]

Activin A, a dimeric glycoprotein of the TGF-β superfamily, has been identified as a new EP biomarker.[6] Activin-A biological activity is tightly controlled by its binding protein. Activin-A has been shown to be crucial in embryo implantation. As the pregnancy advances, protein concentrations increase due to the placenta's significant contribution to serum activin-A. Consequently, activin-A was suggested as a serum biomarker for the differentiation of viable IUP and ectopic pregnancy .[7]

The aim of this work was to detect of Activin-A early in maternal blood and if it can help in diagnosis of early ectopic pregnancy.

2. Patients and methods

This prospective case control research has been performed on 89 cases, subjects were allocated classified into 2 groups: Group 1: 45 cases diagnosed with tubal ectopic pregnancy confirmed by serum β-HCG and transvaginal ultrasound and Group 2: 44 cases with normal pregnancy at the obstetrics and gynecology department, Al-Zahraa University Hospital.

Inclusion Criteria: Age range: from eighteen to forty years, gestational age: five to seven weeks, women with normal intrauterine pregnancy (by ultrasound criteria) in group 1 and women with ectopic pregnancy (diagnosed by B-HCG level above the discriminatory zone and empty uterus by vaginal ultrasound) in group 2.

Exclusion Criteria: Patients refused to enroll in the study, patients with chronic diseases and malignancy and uterine anomaly and Gestational trophoblastic diseases.

Ethical consideration:

The research protocol was submitted for acceptance to the Institutional Review Board at Al-Azhar University. Informed verbal consent was collected from each participant involved in the research. Confidentiality and personal privacy were maintained at all stages of the investigation.

Methods:

Cases have been exposed to: Complete history taking, general investigation, local examination and laboratory investigation.

Assay principle:

This is an enzyme-linked immunosorbent assay (ELISA). The plate was recently treated with Human ACV-A antibody. ACV-A contained in the sample is introduced and attaches to antibodies immobilized on the wells. Subsequently, biotinylated Human ACV-A Antibody was introduced and bound to ACV-A in the sample. Subsequently, Streptavidin HRP has been removed during the washing procedure. The substrate solution was subsequently added, resulting in color development proportional to the quantity of Human ACV-A present. Absorbance was assessed at 450 nanometers, and the reaction was terminated by the addition of an acidic stop solution.

In both categories Activin-A is administered during the fifth to seventh week of gestation. The serum is permitted to coagulate at ambient temperature for a period of between ten and twenty minutes. Centrifuge at a speed of 2000 to 3000 revolutions per minute for a duration of twenty minutes. obtain the supernatant with no sediment.

All reagents, standard solutions, and samples were prepared. The assay was carried out at ambient temperature. We added fifty microliters of the standard to the standard well. Please be advised that we didn't add the antibody to the standard well, as the standard solution contains biotinylated antibody, and the standard wells are not blank control wells. Subsequently, we added forty microliters of the sample to the sample wells, ten microliters of anti-ACV-A antibody to the sample wells, and fifty microliters of streptavidin-HRP to the sample wells. Standard wells (not blank control wells) and a mixed well. The plate has been covered and incubated at thirty-seven degrees Celsius for sixty minutes. The plate had been washed five times with wash buffer after the sealer had been eliminated. We saturated the wells with a minimum of 0.35 milliliter of wash buffer for thirty seconds to one minute for each wash. For automated washing, we aspirate or decant each well and wash it five times with wash buffer. Cover the plate with paper towels or another absorbent material. We added fifty microliters of substrate solution A to each well and subsequently added fifty microliters of substrate solution B to each well. We incubated the plate in the dark at thirty-seven degrees Celsius for ten minutes, after which it was covered with a new sealer. Fifty microliters of stop solution was added to each well, and the

blue color immediately transformed into yellow. The optical density (OD value) of each well was promptly measured ten minutes after the stop solution was added using a microplate reader set to 450 nanometers.

Follow-up and management:

Review the previous blood results and any ultrasound findings. Review the patient and her clinical symptoms. Patients who were hemodynamically unstable required immediate resuscitation and surgical management (via Laparoscopy and laparotomy), undergoing salpingectomy and salpingostomy. The patient, who was hemodynamically stable, received medical treatment.

3. Results

There was no statistically variance among group (1) ectopic cases and group (2) normal pregnancy according to Age, BMI, gravidity and period of pregnancy however, significant variance among both groups according to history of previous caesarian section. There was significant association between ectopic pregnancy and age (P-value less than 0.001) (Table 1).

Table 1. Comparison among both examined groups according to demographics and clinical characteristics

		ECTOPIC PREGNANCY GROUP (1) N=45	CONTROL GROUP (2) N=44	P-VALUE
AGE (YEARS)	<20	1(2.2%)	6(13.6%)	0.006*
	20-30	22(48.9%)	29(65.9%)	
	>30	22(48.9%)	9(20.5%)	
	Range	19–40	18-40	<0.001*
	Mean ± S. D	30.44 ±5.27	25.75±5.37	
BMI (KG / M²)	18.5-25	16(36.4%)	11(26.8%)	0.178
	25.1-<30	17(38.6%)	24(58.5%)	
	≥30	11(25%)	6(14.6%)	
	Range	20.44-50.4	20.31-33.69	0.436
	Mean ± S. D	27.91 ± 6.4	27.08 ± 3.2	
GRAVITY	PG N(%)	4(8.9%)	10(22.7%)	0.04*
	≤3 N(%)	31(68.9%)	19(43.2%)	
	>3 N(%)	10(22.2%)	15(34.1%)	
	Range	1-5	1-6	0.675
	Mean ± S. D	± 1.05 2.73	± 1.35 2.84	
HISTORY OF PREVIOUS CESAREAN SECTION	Absent N(%)	6(13.3%)	23(52.3%)	<0.001**
	Present N(%)	39(86.7%)	21(47.7%)	
DURATION OF PREGNANCY (WEEKS)	Range	5-7	5-7	0.162
	Mean ± S. D	5.96±1.1	5.75±0.84	

Table 2 showed that 88.9 % of cases with ectopic pregnancy have pain, 15.6% have bleeding, and 97.8% have a mass; the mass is on the right side in 25 cases (55.6%) while it was left in 19 cases (42.2%). Additionally, free fluid presented in 48.9% of cases with ectopic pregnancy.

Table 2. Comparison among both groups according to clinical examination and TVS finding

		ECTOPIC PREGNANCY GROUP NUMBER=FORTY-FIVE	CONTROL GROUP NUMBER=FORTY-FOUR	P-VALUE
ABDOMINAL PAIN	N	40	o	<0.001
	%	88.9%	0%	
BLEEDING	N	7	0	0.012*
	%	15.6%	0%	
PRESENCE OF MASS	N	44	0	<0.001**
	%	97.8%	0%	
RIGHT SIDE OF MASS	N	25	0	<0.001**
	%	55.6%	0%	
LEFT SIDE OF MASS	N	19	0	<0.001**
	%	42.2%	0%	
PRESENCE OF FREE FLUID	N	22	0	<0.001**
	%	48.9%	0%	

Table 3 demonstrated that statistically insignificant variance was discovered in the mean level of Activin A within ectopic pregnancy patients and control participants (0.7676±0.268 versus 0.7443±0.2514 (nanogram per milliliter)), (P=0.628).

Table 3. Comparison of serum Activin A concentrations among controls group and ectopic pregnancy group:

		ECTOPIC PREGNANCY GROUP NUMBER=FORTY- FIVE	CONTROL GROUP NUMBER=FORTY-FOUR	P-VALUE
ACTIVIN A (NG/ML)	Range	0.34–1.53	0.20-1.46	0.628
	Mean ± S.D	0.7676±0.268	0.7443±0.2514	

The majority of cases with ectopic pregnancy (73.3%) were managed with Laparoscopy and, while 24.4% underwent laparotomy and the remaining 2.2% were managed medically (Table 4).

Table 4. Treatment of cases presented with ectopic pregnancy

		ECTOPIC PREGNANCY GROUP NUMBER=45
LAPAROSCOPY (SALPINGECTOMY AND SALPINGOSTOMY)	N	33
	%	73.3%
LAPAROTOMY(SALPINGECTOMY)	N	11
	%	24.4%
MEDICAL TREATMENT	N	1
	%	2.2%

Table 5 showed that the correlation of Activin A with age, BMI, with ectopic pregnancy and showed negative non-significant correlation between Activin A levels and age, duration of pregnancy). Conversely, insignificant positive association has been found among Activin A and BMI, gravidity, number of previous CS (r -value=0.200, P-value =0.187, r -value =-0.105, P-value =0.494, r -value =-0.105, P-value =0.494 and r -value =-0.172, P-value =0.259; respectively).

Table 5. Association between Activin A levels and the demographic characters of the two studied groups:

PARAMETERS	ACTIVIN A (NG/ML)	
	Pearson's correlation (r)	P-value
AGE (YEARS)	-0.072	0.640
BMI (KG/M2)	0.200	0.187
GRAVIDITY	0.105	0.494
HISTORY OF PREVIOUS SURGERY	0.139	0.364
NUMBER OF PREVIOUS CS	0.172	0.259
DURATION (WEEKS)	-0.082	0.590

Table 6 showed that non-significant positive correlation was found between Activin A and history of previous surgery (r -value =0.139, P-value =0.364).

Table 6. Association among Activin A levels and History of previous surgery in cases with ectopic pregnancy.

PARAMETERS	ACTIVIN A (NG/ML)	
	Spearman's rho correlation	P-value
HISTORY OF PREVIOUS SURGERY	0.139	0.364

Table 7 showed that the association of serum Activin A (nanogram per milliliter) with clinical examination and TVS finding (pain, bleeding, presence of Mass, side of mass and presence of free fluid) of cases with ectopic pregnancy and revealed insignificant positive association among Activin A concentrations and abdominal pain (r -value=0.013, p-value =0.893), bleeding (r=0.073, P=0.633). In contrast, negative insignificant association among Activin A and presence of mass was observed (r-value =-0.128, P-value =0.403). Conversely, negative association but not statically significant was found between Activin A and presence of free fluid (r=-0.185, P=0.224).

Table 7. Association of Activin A (nanogram per milliliter) with clinical examination and TVS finding

LOCAL EXAMINATION AND TVS FINDING	ACTIVIN A (NG/ML)	
	Spearman's rho correlation	Pvalue
ABDOMINAL PAIN	0.013	0.893
BLEEDING	0.073	0.633
PRESENCE OF MASS	-0.128	0.403
SIDE OF MASS	-0.005	0.974
PRESENCE OF FREE FLUID	-0.185	0.224

4. Discussion

There was no statistically significant variance among group (1) normal and group (2) ectopic cases according to Age, BMI, gravidity and period of pregnancy; however, A significant variance was discovered among both groups according to history of previous cesarean section. There was a significant association between ectopic pregnancy and age (P< 0.001)

In contrary to our results Rueangket and Rittiluechai,[8] (their study was a retrospective research involved 347 pregnant females presenting with 1st trimester complications vaginal bleeding and abdominal pain) reported that the mean age of their research population

was 30.1 ± 6.2 years with statistically insignificant variance between ectopic pregnancy and non- ectopic pregnancy regarding the age groups more than thirty-five and equal or more than thirty-five years.

In agreement with our findings, Tahmina et al.,[9] (the study was a retrospective cohort study, conducted on the medical reports for all females with ectopic pregnancy from 2009 to 2015) also reported that the most frequent risk factors for EP were prior pelvic operation (37.5%) and prior abortion (36.1%).

There were 88.9 % of cases with ectopic pregnancy, there was pain, 15.6% had bleeding, and 97.8% had a mass; the Mass was on the right side in 25 cases (55.6%), while it was on the left side in 19 cases (42.2%). Additionally, free fluid was present in 48.9% of cases with ectopic pregnancy.

Rueangket and Rittiluechai[8] also reported that, according to clinical presentation and evaluation, abdominal pain was experienced by 87.4% of cases in the ectopic pregnancy group, while abnormal vaginal hemorrhage was observed by 74.3% of them. Abdominal tenderness was observed in 69.1% of cases with ectopic pregnancy. In comparison to cases without ectopic pregnancy, they additionally discovered that complex adnexal Mass and free fluid in the cul-de-sac were more prevalent in ectopic pregnancy cases at the initial ultrasound, with 87.4% and 62.3 versus 17.3%, respectively.

The majority of cases with ectopic pregnancy (73.3%) were treated with Laparoscopy, whereas 24.4% underwent laparotomy, and the remaining 2.2% were managed medically.

Ganta et al.[10] research, out of a total of forty cases with ectopic pregnancies, twenty-three reported rupture. Most of them, twenty, were treated with Laparoscopy, whereas three underwent laparotomy. Sixteen of the seventeen undisturbed ectopic pregnancies were medically treated with intramuscular methotrexate, whereas one was a cervical pregnancy that was treated with suction evacuation.

A statistically significant variance was discovered within the mean level of Activin A in ectopic pregnancy cases and control participants (0.7676±0.268 versus 0.7443±0.2514 (nanogram per milliliter)), (P-value=0.628).

Our results come in line with two investigations which demonstrated there isn't value for serum activin-A in the diagnosis of EP for each group given the gestational age wasn't stated by Kirk et al.[11] and Warrick et al.[12] (the study was a prospective interventional study conducted on 363 females with pregnancy of unidentified location).

The correlation of Activin A with age, BMI, and ectopic pregnancy showed a negative non-significant correlation between Activin A levels and age, duration of pregnancy. Conversely, insignificant positive association has been found among Activin A and BMI, gravidity, number of previous CS (r -value=0.200, P-value =0.187, r-value =-0.105, P-value =0.494, r-value =-0.105, P-value =0.494 and r -value =-0.172, P-value =0.259; respectively).

Insignificant positive correlation was found between Activin A and history of previous surgery (r-value =0.139, P-value =0.364).

This initial research in this point of research was conducted via Florio et al.,[13] and in 536 cases with pregnancy of unidentified location (PUL) to differentiate among viable normal IUP, miscarriage, and ectopic pregnancy with a specificity of 99.6% and sensitivity of 100% at a cut-off value of 370 picogram per milliliter, it was shown that a single measurement of serum activin-A served as a highly sensitive and specific indicator. Subsequently, the same team of researchers stated that serum levels of activin-A were significantly reduced in thirty cases diagnosed with tubal ectopic pregnancy in comparison to the control group. The cut-off concentration of 0.43 nano-grams per milliliter was able to obtain a specificity of 100% and sensitivity of 96.7% for the diagnosis of ectopic pregnancy.

Additionally, Daponte and his colleuges[14] (The study was a case control research that included sixty cases with failed early pregnancy who presented with vaginal bleeding, mild or abdominal pain. The results showed that the concentrations of Activin A were significantly decreased in females with ectopic pregnancy (mean 277± 94, median 265 picogram per milliliter) than in cases with IUP (mean of 843± 338, median of 788 picogram per milliliter).

The association of serum Activin A (nanogram per milliliter) with clinical examination and TVS finding (pain, bleeding, presence of Mass, side of Mass and presence of free fluid) of cases with ectopic pregnancy and revealed insignificant positive association among Activin A concentrations and abdominal pain (r -value=0.013, p-value =0.893), bleeding (r -value =0.073, P-value =0.633). In contrast, a negative and insignificant association between Activin A and the presence of Mass was observed (r-value =-0.128, P-value =0.403). Conversely, a negative but not statistically significant association was found between Activin A and the presence of free fluid (r=-0.185, P=0.224).

Burlev and his co-workers,[15] concluded that the concentration of activin A in the blood during spontaneous ectopic pregnancy until seven weeks is insignificantly different from that of uterine pregnancy. However, they stated that the concentration of activin A was statistically

significantly lower at ectopic pregnancy following seven weeks. Consequently, they suggested that the concentration of activin A in the blood be utilized for the processes of cytotrophoblast invasion and placentation under dynamic supervision following seven weeks of pregnancy.

4. Conclusion

Ectopic pregnancy is a frequent and severe condition with a high rate of morbidity and the risk of maternal death. Numerous people have no established risk factors and no ectopic pregnancy indicators. The first examination must be performed with an ED patient with first-trimester bleeding or pain, using Ultrasonography (formal or ED-based). There was no significant association between ectopic pregnancy and Activin-A level. Activin-A was a poor marker for ectopic pregnancy in early diagnosed cases.

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