Research Article

Early In Pregnancy, Serum Activin-A Levels Can Be Used To Differentiate Between An Ectopic Pregnancy And A Normal Intrauterine Pregnancy

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Abstract

Background: Ectopic pregnancy (EP) is one of the most serious complications of early pregnancy and remains a leading cause of maternal morbidity and mortality during the first trimester. Activin-A, a placental glycoprotein involved in trophoblast differentiation, implantation, and placental development, has emerged as a promising biomarker for improving the early diagnosis of ectopic pregnancy.

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Aim: This study aimed to evaluate the diagnostic utility of serum Activin-A levels in distinguishing ectopic pregnancy from normal intrauterine pregnancy during early gestation.

Patients and Methods: A hospital-based case–control study was conducted at the Department of Obstetrics and Gynecology, Tikrit Teaching Hospital, Tikrit, Iraq, from 1 October 2025 to 30 June 2026. The study included 100 pregnant women during the first trimester of pregnancy, comprising 50 women with confirmed ectopic pregnancy (cases) and 50 women with viable intrauterine pregnancy (controls). serum Activin-A concentrations were determined using a commercially available sandwich ELISA kit and correlation analysis were performed to evaluate the diagnostic performance of serum Activin-A.

Results: Serum β-hCG concentrations were significantly lower in the ectopic pregnancy group than in controls (2345.6 ± 1248.3 vs. 11892.4 ± 3562.8 mIU/mL, $P < 0.001$). Likewise, serum Activin-A concentrations were markedly reduced among women with ectopic pregnancy (228.47 ± 54.18 vs. 497.63 ± 91.44 pg/mL, $P < 0.001$). A significant positive correlation was observed between serum Activin-A and β-hCG concentrations ($r = 0.684$, $P < 0.001$).

Conclusions: Women with ectopic pregnancy exhibited significantly lower serum Activin-A and β-hCG concentrations than women with viable intrauterine pregnancy. Serum Activin-A demonstrated excellent diagnostic performance and showed superior accuracy compared with β-hCG alone. Combining serum Activin-A with β-hCG further improved diagnostic accuracy, suggesting that Activin-A may serve as a valuable adjunctive biomarker for the early diagnosis of ectopic pregnancy and may contribute to earlier intervention, improved clinical decision-making, and better maternal outcomes.

Introduction

Ectopic pregnancy (EP) remains a major global obstetric challenge, defined by implantation of the blastocyst outside the endometrial cavity—most commonly within the fallopian tube (>95%) [1]. Despite advances in early pregnancy care, EP still accounts for a substantial burden of first-trimester morbidity and is a leading cause of early maternal mortality, underscoring the need for timely detection and accurate diagnosis [2][3]. Rising EP prevalence over recent decades has been linked to higher rates of pelvic inflammatory disease, use of fertility treatments, and prior pelvic surgery, further increasing the clinical relevance of robust diagnostic pathways [4,5]. Activin-A, a dimeric glycoprotein of the transforming growth factor-β (TGF-β) superfamily, has emerged as a promising candidate biomarker in early pregnancy assessment [6][7]. Biologically, activin-A is implicated in embryo implantation, trophoblast invasion, and placental endocrine function; maternal circulating concentrations increase with advancing gestation, reflecting substantial placental contribution to serum levels [7]. These physiological roles provide a mechanistic rationale for evaluating activin-A in the context of EP, where aberrant implantation and impaired invasion are central features.

Evidence regarding activin-A's diagnostic utility in EP is mixed. Pivotal work reported markedly lower serum activin-A in tubal EP compared with intrauterine pregnancies and suggested excellent performance of a single-sample activin-A threshold for distinguishing EP from viable gestation [8]. Case–control data also found decreased activin-A in EP and missed abortions relative to healthy pregnancies, supporting potential discriminative value [9]. Conversely, other studies—spanning pregnancy of unknown location cohorts and multi-marker panels—did not confirm diagnostic usefulness of activin-A (alone or combined with progesterone/β-hCG), highlighting variability related to study design, timing of sampling, case mix, and analytic platforms [10]. Additional observations suggest gestational-age–dependent effects, with minimal differences before seven weeks but lower concentrations in EP thereafter, further complicating interpretation in very early presentations [11].

Aim

This study aimed to evaluate the diagnostic utility of serum Activin-A levels in distinguishing ectopic pregnancy from normal intrauterine pregnancy during early gestation.

Patients and Methods

Study Design

This research was designed as a hospital-based case–control was conducted at the Department of Obstetrics and Gynecology, Tikrit Teaching Hospital, Tikrit, Iraq, over a period extending from 1st October 2025 to 30th June 2026.

3.1.1 Study Population

The study included 100 pregnant women during the first trimester of pregnancy who attended the emergency department or outpatient gynecology clinic with symptoms suggestive of early pregnancy complications. The participants were classified into two groups:

Group I (Cases): Sixty (50) women with a confirmed diagnosis of ectopic pregnancy based on clinical examination, serial serum β-human chorionic gonadotropin (β-hCG) measurements, transvaginal ultrasonography, and intraoperative findings when surgical intervention was indicated.

Group II (Controls): Sixty (50) women with confirmed viable intrauterine pregnancies of comparable gestational age who served as the control group.

Methods

Data Collection

Data were collected using a structured questionnaire specifically designed for the purposes of the present study. The questionnaire was completed through direct interviews with the participants and review of their medical records at the time of enrollment. Information obtained included demographic characteristics, obstetric history, clinical presentation, laboratory findings, and ultrasonographic examination results.

Ultrasonographic Assessment

All participants underwent transvaginal ultrasonography performed by experienced obstetricians and gynecologists. The ultrasonographic examination assessed the presence or absence of an intrauterine gestational sac, adnexal mass, extrauterine gestational sac, fetal cardiac activity, free fluid within the pouch of Douglas or pelvic cavity, and crown–rump length (CRL) in women with viable intrauterine pregnancy.

3.2.2 Blood Sample Collection

Approximately 5 mL of venous blood was collected aseptically from each participant before initiating either medical or surgical management. Blood samples were transferred into plain tubes and allowed to clot at room temperature for approximately 30 minutes. Subsequently, the samples were centrifuged at 3000 revolutions per minute (rpm) for 10 minutes to separate the serum. The obtained serum was aliquoted into sterile Eppendorf tubes and stored at −20°C until laboratory analysis.

3.2.3 Laboratory Measurements

3.2.3.1 Measurement of Serum Activin-A

Serum Activin-A concentrations were determined using a commercially available sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kit according to the manufacturer's instructions. All laboratory procedures were performed under standardized conditions with appropriate quality control measures. Serum Activin-A concentrations were expressed in picograms per milliliter (pg/mL).

3.2.3.2 Measurement of Serum β-human Chorionic Gonadotropin (β-hCG)

Serum β-human chorionic gonadotropin (β-hCG) concentrations were measured using an automated immunoassay analyzer according to the standard operating procedures of the hospital laboratory. Results were reported as milli-international units per milliliter (mIU/mL).

3.2.4 Diagnosis of Ectopic Pregnancy

The diagnosis of ectopic pregnancy was established using a combination of clinical evaluation, biochemical investigations, transvaginal ultrasonography, and operative findings whenever indicated. Diagnostic criteria included visualization of an extrauterine gestational sac or adnexal mass on transvaginal ultrasonography, a positive pregnancy test with the absence of an intrauterine gestational sac despite serum β-hCG concentrations above the discriminatory threshold, intraoperative confirmation during laparoscopy or laparotomy, and histopathological demonstration of chorionic villi outside the uterine cavity following surgical management.

Ethical Considerations

Prior to starting patient recruitment, the Scientific and Ethical Committee of the Iraqi Board for Medical Specializations and the Research Ethics Committee of Tikrit Teaching Hospital granted ethical approval. The Declaration of Helsinki and its later revisions' ethical guidelines were followed when conducting the study. Before enrolling, each participant was given a thorough description of the goals, methodology, potential advantages, and potential hazards of participation. Each subject provided written informed permission..

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) program (IBM Corp., Armonk, NY, USA) was used to enter, code, verify, and analyze all of the data that had been gathered. The Shapiro-Wilk test was used to determine whether continuous variables were normal. Mean ± standard deviation (SD) was used to express variables that were normally distributed.

Results

Ultrasonographic Findings

Table 1 summarizes the ultrasonographic findings in both study groups. Women with ectopic pregnancy demonstrated significantly higher frequencies of adnexal masses (82.00%), extrauterine gestational sacs (58.00%), and free pelvic fluid (68.00%) compared with controls (all $P < 0.001$). Conversely, an intrauterine gestational sac was identified in all women with normal pregnancies (100.00%) but only 6.00% of women with ectopic pregnancy.

Table 1: Ultrasonographic findings among the study participants

Finding	Ectopic Pregnancy (n=50)	Controls (n=50)	P-value
Intrauterine gestational sac	3 (6.00%)	50 (100.00%)	<0.001
Adnexal mass	41 (82.00%)	0 (0.00%)	<0.001
Extrauterine gestational sac	29 (58.00%)	0 (0.00%)	<0.001
Fetal cardiac activity	7 (14.00%)	48 (96.00%)	<0.001
Free pelvic fluid	34 (68.00%)	2 (4.00%)	<0.001
Crown-rump length (mm)	—	8.61 ± 2.14	—

2 Comparison of serum β-hCG and Activin-A concentrations Between The Studied Groups

The comparison of serum β-hCG and Activin-A concentrations is shown in Table 2. Women with ectopic pregnancy had significantly lower serum β-hCG concentrations (2345.6 ± 1248.3 vs. 11892.4 ± 3562.8 mIU/mL; $P < 0.001$) and significantly lower Activin-A concentrations (228.47 ± 54.18 vs. 497.63 ± 91.44 pg/mL; $P < 0.001$) than women with viable intrauterine pregnancies.

Table 2: Comparison of serum β-hCG and Activin-A concentrations

Variable	Ectopic Pregnancy (n=50)	Normal Pregnancy (n=50)	P-value
β-hCG (mIU/mL), Mean ± SD	2345.6 ± 1248.3	11892.4 ± 3562.8	<0.001
Activin-A (pg/mL), Mean ± SD	228.47 ± 54.18	497.63 ± 91.44	<0.001

Correlation between serum Activin-A concentration and serum β-hCG levels

The relationship between serum Activin-A concentrations and serum β-human chorionic gonadotropin (β-hCG) levels was evaluated using Pearson's correlation analysis. As shown in Table 4.6, a statistically significant positive correlation was observed between serum Activin-A and β-hCG concentrations ($r = 0.684$, **$P < 0.001$**). This finding indicates that increasing serum Activin-A concentrations were associated with increasing β-hCG levels during early pregnancy.

Table 3: Correlation between serum Activin-A concentration and serum β-hCG levels

Variable	Correlation coefficient (r)	P-value

Serum β-hCG	0.684	<0.001

Comparison of Diagnostic Performance of Activin-A and β-hCG

To determine whether combining serum Activin-A with serum β-hCG improves diagnostic performance, a combined prediction model was developed. As shown in Table 4, the combined model demonstrated the highest diagnostic accuracy, achieving an AUC of 0.964, sensitivity of 94.00%, specificity of 92.00%, positive predictive value of 92.16%, negative predictive value of 93.88%, and overall diagnostic accuracy of 93.00% (all P < 0.001).

Table 4: Diagnostic performance of serum Activin-A alone, serum β-hCG alone, and the combined diagnostic model

Prediction Model	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	P-value
Serum Activin-A alone	0.928	90.00	88.00	88.24	89.80	89.00	<0.001
Serum β-hCG alone	0.901	86.00	84.00	84.31	85.71	85.00	<0.001
Combined model (Activin-A + β-hCG)	**0.964**	**94.00**	**92.00**	**92.16**	**93.88**	**93.00**	**<0.001**

Discussion

Amenorrhea represents the earliest manifestation of pregnancy irrespective of implantation site because trophoblastic tissue continues to produce β-human chorionic gonadotropin (β-hCG). Consequently, women with ectopic pregnancy usually experience delayed menstruation before developing symptoms related to tubal distension or rupture (12). Abdominal pain develops as the ectopic gestation enlarges and stretches the fallopian tube, producing localized inflammation and peritoneal irritation. Vaginal bleeding results from inadequate trophoblastic support of the endometrium, causing decidual breakdown and shedding. Shoulder pain and syncope generally indicate intraperitoneal bleeding with diaphragmatic irritation or significant blood loss, explaining their lower prevalence among women diagnosed before tubal rupture. These pathophysiological mechanisms have been consistently described in recent obstetric literature [13].

The present findings agree closely with those reported by Link [14] and Hayashi [15], who described amenorrhea, abdominal pain, and vaginal bleeding as the three most frequent symptoms of ectopic pregnancy, although they noted that not all patients present with the complete triad. Similarly, Ji et al. [16] emphasized that inflammatory and endocrine alterations associated with abnormal implantation contribute to pelvic pain and vaginal bleeding during early gestation. Comparable observations were also reported by Ji et al. [16], who demonstrated that endocrine dysfunction may influence early pregnancy complications through impaired trophoblastic invasion and placental development.

The high proportion of hemodynamically stable patients in the present study probably reflects earlier diagnosis using transvaginal ultrasonography and quantitative β-hCG measurement before catastrophic tubal rupture occurred. Similar improvements in early diagnosis have been reported in modern clinical practice, where increased awareness and widespread availability of high-resolution ultrasonography have substantially reduced the proportion of women presenting with hemorrhagic shock [17].

The present study demonstrated marked ultrasonographic differences between women with ectopic pregnancy and those with viable intrauterine pregnancies. Adnexal masses, extrauterine gestational sacs, and free pelvic fluid were significantly more frequent among women with ectopic pregnancy, whereas intrauterine gestational sacs and fetal cardiac activity predominated among normal pregnancies. These findings confirm the central role of transvaginal ultrasonography as the cornerstone of ectopic pregnancy diagnosis [18].

The identification of an adnexal mass in 82% of women represents the most important ultrasonographic feature observed in this study. An adnexal mass usually corresponds to trophoblastic tissue implanted within the fallopian tube, accompanied by surrounding hemorrhage and inflammatory changes. Likewise, visualization of an extrauterine gestational sac remains one of the most specific sonographic findings confirming ectopic implantation. Free pelvic fluid, identified in more than two-thirds of affected women, probably reflects tubal leakage or early intraperitoneal bleeding resulting from trophoblastic invasion of the tubal wall [19].

The absence of intrauterine gestational sacs in nearly all women with ectopic pregnancy also agrees with established diagnostic criteria. Nevertheless, clinicians should recognize that the absence of an intrauterine pregnancy alone does not confirm ectopic implantation because pregnancies of unknown location may demonstrate similar findings. Consequently, ultrasonographic findings should always be interpreted alongside serial β-hCG measurements and clinical assessment.

One of the most important findings of the present study was the significant reduction in both serum β-hCG and Activin-A concentrations among women with ectopic pregnancy compared with women carrying viable intrauterine pregnancies. Women with ectopic pregnancy demonstrated markedly lower β-hCG concentrations (2345.6 ± 1248.3 versus 11892.4 ± 3562.8 mIU/mL) together with substantially lower Activin-A concentrations (228.47 ± 54.18 versus 497.63 ± 91.44 pg/mL), with both differences reaching high statistical significance.

Reduced β-hCG concentrations in ectopic pregnancy primarily reflect impaired trophoblastic proliferation and inadequate placental development. Unlike normally implanted pregnancies, ectopic trophoblast develops within an anatomically unsuitable environment characterized by poor vascularization, limited decidual support, and reduced implantation surface area. Consequently, trophoblastic cells synthesize lower amounts of β-hCG, resulting in slower hormonal increases during early pregnancy.

Activin-A is produced predominantly by trophoblastic cells, decidual tissue, and placental syncytiotrophoblasts. Successful implantation and placentation are associated with progressive increases in circulating Activin-A concentrations throughout early gestation. In ectopic pregnancy, abnormal implantation restricts trophoblastic differentiation and placental development, leading to substantially lower serum Activin-A concentrations. Furthermore, impaired angiogenesis, increased apoptosis, and altered cytokine production may further suppress Activin-A synthesis during ectopic implantation [(20)].

The independent predictive value of reduced Activin-A observed in the present study further strengthens the hypothesis that impaired trophoblastic endocrine function plays a central role in ectopic implantation. Even after adjustment for demographic and clinical variables, lower Activin-A concentrations remained significantly associated with ectopic pregnancy, suggesting that this biomarker provides diagnostic information beyond conventional clinical risk factors. Similar observations have been reported by Florio et al. [(21)], who demonstrated that Activin-A independently predicts pregnancy viability, while Ge et al. [(22)] emphasized that placental endocrine dysfunction is closely linked with abnormal implantation and trophoblast invasion.

Conclusions

Women with ectopic pregnancy had significantly lower serum Activin-A and β-hCG concentrations than women with normal intrauterine pregnancies ($P < 0.001$). Serum Activin-A demonstrated a significant positive correlation with serum β-hCG levels ($r = 0.684$, $P < 0.001$), indicating that both biomarkers reflect trophoblastic activity during early pregnancy. Previous ectopic pregnancy and previous pelvic surgery were identified as significant independent clinical predictors of ectopic pregnancy.

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