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Short Communication

Serum activin B concentration as predictive biomarker for ectopic pregnancy

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ABSTRACT

We evaluated the diagnostic accuracy of activin B in discriminating tubal ectopic pregnancy (tEP) from intrauterine miscarriages (IUM), and normal viable intrauterine pregnancy (IUP). We included 28 women with tEP, 31 women with IUM, and 29 normal IUP, confirmed both by clinical examination and ultrasonography. Serum activin B concentration was measured at the time of admission using the ELISA kit. The median serum activin B concentration was found to be significantly decreased in both tEP ($p = 0.004$) and IUM ($p = 0.022$) compared to normal IUP. When compared between tEP and IUM, activin B concentrations did not differ significantly. ROC analysis of activin B and free β-hCG demonstrated AUC of 0.722 and 0.805, respectively to discriminate tEP from viable IUP. The model including both activin B and free β-hCG improved the discriminating potential with greater AUC (0.824), and specificity (93%) than individual one. To discriminate tEP from IUM, activin B, free β-hCG and combination of both performed poorly. We conclude that serum activin B concentration is lower in tubal ectopic pregnancy, and can discriminate it from normal pregnancy with moderate accuracy. It also shows improved diagnostic potential along with free β-hCG, but cannot distinguish tEP from IUM reliably.

1. Introduction

Ectopic pregnancy, a disorder of early pregnancy, is associated with high morbidity and mortality, predominantly due to delay in diagnosis. This delay stems from the fact that the current modality of diagnosis, that is transvaginal ultrasonography and serial free beta human gonadotropin (free β-hCG) estimations is not entirely reliable [1]. These fallacies of the available diagnostic tests have led to the search of reliable biomarkers for diagnosing ectopic pregnancy, to prevent life threatening complications from abdominal bleeding, and to minimize the need of surgical interventions. Various biomarkers have been investigated in the diagnosis of ectopic pregnancy as single and multiple markers, which include markers of normal implantation, abnormal corpus luteum and inflammatory markers [2]. Activins, dimeric proteins of transforming growth factor-beta (TGF-b) family, first isolated from follicular fluid, have demonstrated growth factor like actions [3]. They are produced by many organs including pituitary gland, gonads, placenta, adrenal, and spleen. These molecules bind to their transmembrane receptors and bring about downstream phosphorylation of different proteins to alter gene expression, leading to increased decidualization of the endometrium during pregnancy [4]. Consequently low serum activin concentration has been associated with ectopic pregnancy [4–8]. Recent studies have mainly focussed on the role of serum activin A as a potential marker of ectopic pregnancy [3], and limited literature is available regarding the role of activin B, in spite of the strong experimental evidence of the differential effect of activin B on the decidua [5]. We hypothesize that activin B concentration is low in tubal ectopic pregnancy (tEP) than normal intrauterine pregnancy (IUP) and intrauterine miscarriage (IUM), and therefore can be useful in its diagnosis. Hence, we evaluated the role of serum activin B in discriminating and predicting tEP from normal IUP and IUM.

2. Material and methods

a. Study design: This is a study of diagnostic accuracy. This prospective case–control study was carried out in JIPMER hospital, Puducherry in the Department of Biochemistry in collaboration with the Department of Obstetrics and Gynecology between January 2011 to December 2012.
b. Subjects: After seeking approval of the Institute's ethics committee and written informed consent from the subjects, patients who presented to the hospital with bleeding per vaginum, abdominal pain and positive free β-hCG, were recruited as subjects. To further confirm the diagnosis of the patients for subsequent management, transvaginal ultrasonography scan was done by a group of

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obstetricians trained in the management of early pregnancy failure. Women who demonstrated the evidence of embryo in the fallopian tube and absence of intrauterine gestational sac on ultrasound, were included in the case group called tEP (n = 28). Diagnosis of tEP was further confirmed in all cases by diagnostic laparoscopy. All women in this group presented with impending rupture. Women who had ultrasonography evidence of intrauterine pregnancy and absence of vital signs of embryo at admission were included in the control group called IUM (n = 31). We also included another control group that consists of women with normal intrauterine pregnancy (IUP) (n = 29). Clinical conditions which can affect the serum concentrations of activin B such as hydatiforme mole, history of missed abortion, subjects on medications based on progesterone, non-tubal EP were excluded from this study.

c. Blood sampling: Five milliliter of their venous blood samples was collected at the time of admission. The freshly separated serum was stored at −70 °C until use.
d. Methods: Serum activin B concentrations were analyzed using the commercially available ELISA kit (USCN Life Science Inc., USA). The assay was specific for human serum activin B using activin beta homodimer antibody, with the lower limit of detection being 6.4 pg/ml. Capture antibody was a monoclonal antibody and detection antibody used was a polyclonal antibody derived from a rabbit. The inter-assay and intra-assay CV were <12% and <10% respectively. Total duration of assay was 5 h. Briefly, all reagents, samples and standards were prepared and 100 μL was added to each well, which was precoated with antibody specific to activin B. After an incubation of 2 h at 37 °C, 100 μL of biotin-conjugated antibody specific to activin B was added. Following an incubation of 1 h at 37 °C, 100 μL of avidin conjugated to Horseradish Peroxidase (HRP), and subsequently TMB substrate was added. This was followed by an incubation of 15–25 min (37 °C). Lastly, 50 μL stop solution was added and the plate was read at 450 nm. Serum free β-hCG was measured by the principle of chemiluminescence in Siemens' Advia Centaur (Siemens diagnostics, Germany).
e. Statistical analysis: Statistical analysis was performed using the SPSS version 16 (SPSS, Chicago, IL, USA). P < 0.05 was considered as statistically significant. Results were compared using non-parametric Kruskal–Wallis tests to identify differences in the concentrations of activin B among the three groups. The performance of serum activin B as a biomarker for ectopic pregnancy was evaluated by Receiver operator curve analysis (ROC) using MedCalc®, Belgium.

3. Results

The study groups were matched for age and weeks of pregnancy. The median values with interquartile range of serum activin B and free β-hCG are depicted in Table 1. Activin B concentration was significantly lower in woman with tEP compared to patients with normal IUP (P = 0.004), and in women with intrauterine miscarriages compared to IUP (P = 0.022). No statistically significant difference was observed between intrauterine miscarriage and tEP. ROC analysis of activin B and free β-hCG demonstrated AUC of 0.722 and 0.805 respectively to discriminate tEP from viable IUP demonstrating moderate sensitivity and specificity (Table 2). The model including both the activin B and free β-hCG improved the discriminating potential with greater AUC (0.824), and specificity (93%) than individual one (Fig 1 and Table 2). To discriminate tEP from intrauterine miscarriage, activin B, free β-hCG, and combination of both performed poor with p values of 0.65, 0.16 and 0.39 respectively. Correlation analysis between free β-hCG and activin B in different study groups was r = 0.081(p = 0.675) in IUP, r = 0.192 (p = 0.333) in tEP and r = 0.146(p = 0.441)in IUM, indicating weak correlation, which was statistically insignificant.

4. Discussion

Reliable serum biomarkers for tubal ectopic pregnancy are in immense need for this potentially life threatening condition. This study addressed the above issue using single point measurement of serum activin B. Activins, a dimer of TGF-β family of proteins, functions to promote normal decidualization of the endometrium during pregnancy [4]. Since tEP is associated with poor decidualization, serum activin concentrations are expected to be low in this condition. Though the functional aspect of activin A on endometrium has been described in detail, molecular action of activin B is yet to be explored. It has also been established in the previous studies that activin A and activin B function separately in the tissues [9]. Therefore, it would be prudent to study the diagnostic accuracy of activin B as a biomarker in discriminating tEP from normal IUP and IUM.

Our study hypothesizes that activin B concentration is lower in tEP than normal pregnancy and IUM, and hence can be used as a diagnostic tool. We observed a lower concentration of activin B in women with tEP compared to those with normal IUP supporting our hypothesis. This result may be explained by the fact that activin B is involved in decidualization of endometrium in pregnancy, and in ectopic pregnancy this process is hindered causing decreased local and systemic concentration of activin B. Our results are in concordance with Horne et al., who found decreased expression of activin B in endometrium along with decreased serum concentration of activin B with normal concentration of progesterone in 11 women with tEP [5]. The same study also demonstrated a positive association of activin B with decidualization of endometrium, which in turn reinforce its functional significance in the endometrial development. We observed the mean value of activin B in tEP to be 4 pg/ml, which is lower than the concentration reported in Horne et al. study. This difference in results can be attributed either due to difference in gestational age of women in two studies(between 6 and 8 weeks in the Horne et al. study & 4–6 weeks in our study),or the use of different ELISA kits for the estimation of serum activin B (activin B ELISA that incorporates the use of monoclonal antibody 46 A/F ,as both capture and detection antibody with a sodium dodecyl sulfate and heat pretreatment of samples in the study by Horne et al., whereas the commercial ELISA kit (USCN Life Science Inc., USA) using monoclonal capture antibody and polyclonal detection antibody, with no sample pretreatment in our study). It has been reported that

Table 1
General characteristics, serum concentration of free β-hCG and activin B in tubal ectopic pregnancy, Intrauterine miscarriages and normal viable intrauterine pregnancy.

Parameters	Tubal ectopic pregnancy (n = 28)	Intrauterine miscarriages (n = 31)	Normal pregnancy (n = 29)
Maternal age (yrs)	24.3 ± 3.2	24.2 ± 4	25 ± 5
Gestational age (wks)	4.6 ± 1.5	5.2 ± 1.2	5.4 ± 2.1
Free β-hCG (IU/L)	11,415 ± 19,724*	20,115 ± 28,424*	56,213 ± 52,530
(Min–max)	(20–33,520)	(0.02–130,875)	(200–200,000)
Activin B (pg/mL)	4.0(0.0–17.6)*	5.9(0.0–21.0)*	37.5 (6.4–55.6)
(Min–max)	(6.4–104.5)	(6.4–1816.7)	(6.4–364.1)

Variables are expressed in mean ± standard deviation except for Activin B which is expressed in Median with interquartile ranges.
* Indicates p value < 0.05 in comparison to Normal Pregnancy. Normally distributed data was compared by one way ANOVA with post hoc Tukey test and non-normal data with Kruskal–Wallis test followed by Bonferroni correction.