Serial monitoring of human chorionic gonadotropin and free beta subunit secretion in ectopic pregnancy

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Summary: The diagnosis of ectopic pregnancy (EP) has relied primarily on serial serum sampling for human chorionic gonadotropin (hCG) and timely pelvic ultrasound examinations. We recently described the applicability of free beta hCG, intact hCG ratio (%) to distinguish EP from spontaneous abortion or intrauterine pregnancy and found that 35% of EP were uniquely characterized by ratios >0.10. In the present study, we sought ti determine if this altered pattern of free beta subunit and intact hCG secretion when present persisted as the EP progressed and whether this ratio was influenced by estradiol (E2) and progesterone (P4) secretion. Twelve patients with histologically-confirmed EP and ratios >0.10 were studied longitudinally from initial presentation to surgical intervention. Ratios (%) ranged from .10 to .52 and persisted at levels greater tham 0.10 throughout the period of monitoring in all patients. Intact hCG, free beta hCG and ratios (%) showed no correlation to E2 or P4 concentrations. These data suggest that ratios >0.10 when present persist throughout gestation in EP and may serve as a marker for early diagnosis of EP. Such patterns of hCG secretion in EP may be secondary to altered placental histology with persistence of histologic patterns characteristic of early gestation.

Key words: ectopic pregnancy; beta-unit secretion.

INTRODUCTION

Current clinical practice incorporates both serial monitoring of serum human chorionic gonadotropin (hCG) and pelvic ultrasound examinations to distinguish ectopic pregnancy (EP) or spontaneous abortion (AB) from a normal intrauterine pregnancy (IUP). These techniques however may not be sufficiently specific to distinguish EP from AB [1]. Assays for intact hCG and both its alpha and beta subunits may have utility in the diagnosis of EP.

Recent studies have suggested increased free alpha subunit secretion in EP, gestational neoplasia and AB [2, 3]. We recently described the applicability of the ratio (%) of intact hCG to free beta hCG to distinguish EP from AB or IUP and found

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that 35% of EP were uniquely characterized by ratios >0.10 ([4]). In the present study, we evaluated whether this altered pattern of free beta subunit and intact hCG secretion when present persisted as the EP progressed and whether this ratio was influenced by estradiol (E2) and progesterone (P4) secretion.

MATERIALS AND METHODS

Patient population:

An analysis of serum hCG, beta hCG subunit, E2 and P4 concentrations was made on serum samples obtained between 4 and 9 weeks gestation from patients with histologically-proven EP. All patients were selected from a population presenting for clinical evaluation and management of signs and symptoms of EP. Patients were managed by either salpingectomy, salpingotomy or salpingostomy. All patients had a history of regular menses and a reliable last menstrual period. Serum for assay was drawn at 2- to 3-day intervals in the clinical management of the patient and frozen at –70 degrees C. Samples were assayed for intact hCG and beta hCG. In those patients with ratios >0.10 (N=12), serial samples were then selected at 7-day intervals and assayed for intact hCG, beta hCG, E2 and P4.

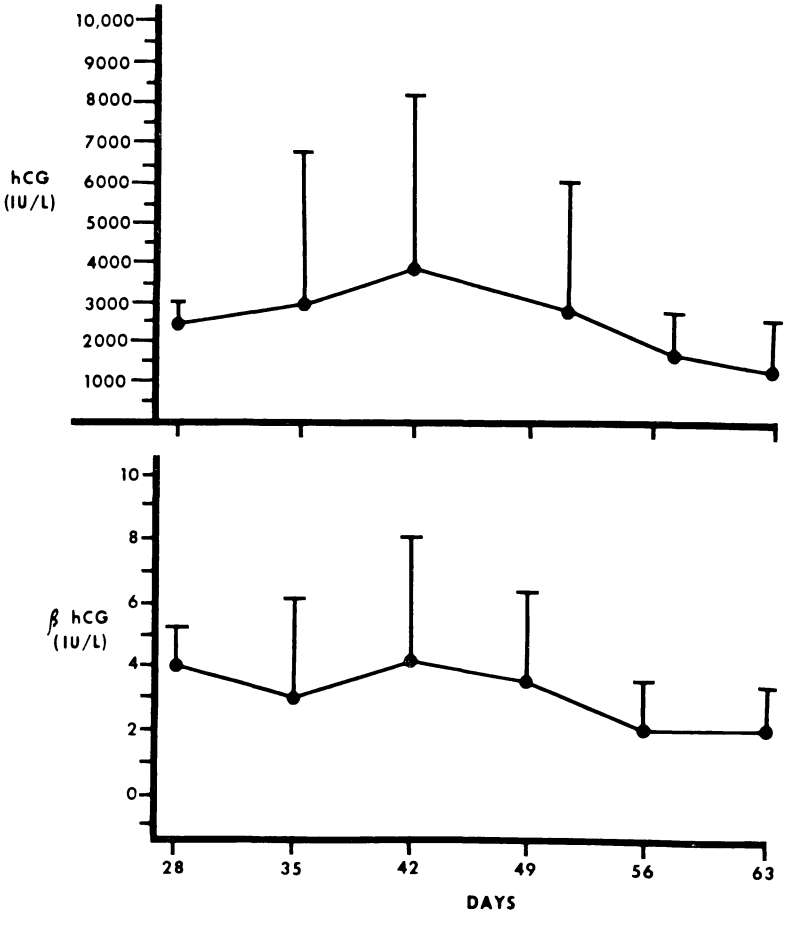

Fig. 1. — Intact hCG and beta hCG concentrations in ectopic pregnancy from 28 to 63 days.

IMMUNOASSAYS

Human Chorionic Gonadotropin Assays

Free serum beta hCG levels were measured in duplicate by an immunoradiometric kit procedure using monoclonal antibodies against beta hCG purchased from Bioclone Australia (Sydney, New South Wales, Australia). Dimer (intact) hCG was measured using a modified commercial immunoradiometric assay marketed by Hybritect, Inc. (San Diego, CA) as described previously (6).

Assay standards were calibrated against the first International Reference Preparation (IRP) for dimer hCG (IRP 75/537) and beta hCG (IRP 75/551). The results are given in terms of micrograms and international units, where 1 IU for hCG immunoassay was defined as the activity contained in 1 μp pure IRP standard preparation. All immunoassays had a minimum sensitivity of 0.5 μg/L. The intraassay coefficients of variation were 2.8% and 2.5% and the interassay coefficients of variation were 3.9% and 4.5% for beta hCG and dimer hCG respectively. In the dimer hCG assays, LH, FSH and TSH cross-reacted 1.3%, 1.1% and 0.01% respectively, but beta hCG did not significantly cross-react in the hCG assay.

Steroid assays

Serum levels of E2 and P4 were measured by standard radioimmunoassay techniques in routine batch analysis. The sensitivity limits for these assays were 20 pg/ml and 0.20 ng/ml respectively. Intraassay and interassay coefficients of variation were 8% and 16% for E2 and 7% and 12% for P4 respectively.

Statistical analysis

All data are presented as the mean ± standard deviation (SD). Statistical comparisons were made using a repeated measure analysis of variation (ANOVA) for 12 patients and five repeated measures (28, 35, 42, 56 and 63 days during observation). A Bonferroni T comparison was made to determine significance at $p=0.05$. ANOVA was used to compare serum concentrations of intact hCG, beta hCG, E2 and P4. Correlation of intact hCG to free subunits and to E2 and P4 concentrations was made using a Pearson correlation coefficient.

RESULTS

Twelve patients with intact hCG/beta hCG ratios >0.10 were monitored from 28 days to 63 days from last menstrual period.

Intact (dimer) hCG concentrations ranged from a low of 1224.5±1407.85 IU/L (mean ± standard deviation [SD]) at 63 days to a high of 3506.08+4530.94 IU/L at 42 days (Figure 1). Beta hCG concentrations exhibited a similar pattern of secretion and were positively correlated to intact in hCG secretion ($r=0.95$) (Figure 1). Concentrations ranged from 2.4±1.69 IU/L at 63 days to 4.68±4.28 IU/L at 42 days. Overall, ratios of intact hCG to beta hCG ranged from 0.10 to 0.52 and once established persisted at levels greater than 0.10 throughout the period of observation in all patients. Mean ratios ranged from a low of 0.17±0.07 at 35 days to a high of 0.34±0.25 at 63 days (Figure 2). Ratios varied by less than 25% during the interval from 28 to 56 days.

Mean E2 and P4 concentrations showed no significant changes throughout the 5-

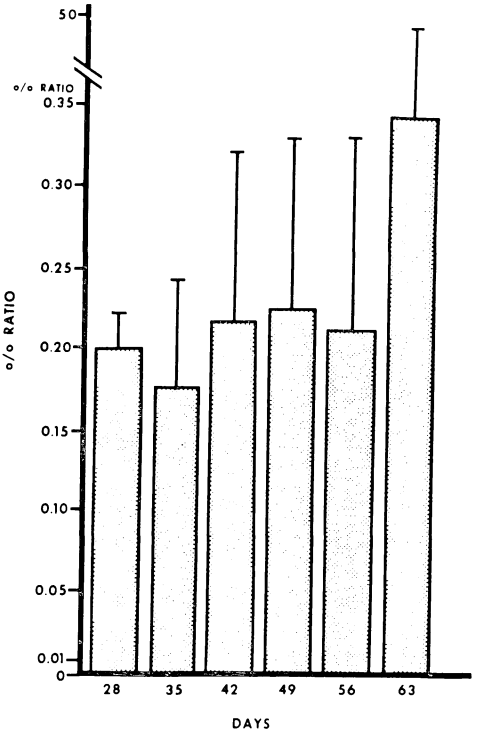

Fig. 2. — Ratio (%) of beta hCG/hCG concentrations.

week observation period and ranged from 154.5±114.0 pg/ml to 180.63±161.05 pg/ml and 3.47±0.14 ng/ml to 11.6± 5.36 ng/ml respectively (Figure 3). These concentrations were consistently lower for any given gestational age and showed no correlation to intact hCG, free beta hCG and ratios of intact to beta hCG.

DISCUSSION

Previous data suggest that the absolute secretion of intact hCG and its beta subunit is decreased in EP and a comparison of the relative secretion of each (i.e., ratio of beta hCG/hCG) revealed characteristic patterns for IUP, AB and EP ([4]). These data suggest that although EP are characterized by a wide range of ratios from 0.015 to 0.176, 35% of EP are characterized by ratios >0.10, a finding noted only in EP. Data of the present study confirm and extend this concept and suggest that once established, this altered ratio of >0.10 persists throughout the ectopic gestation and is not influenced by the secretion of E2 or P4. These ratios may be secondary to an increased pro-

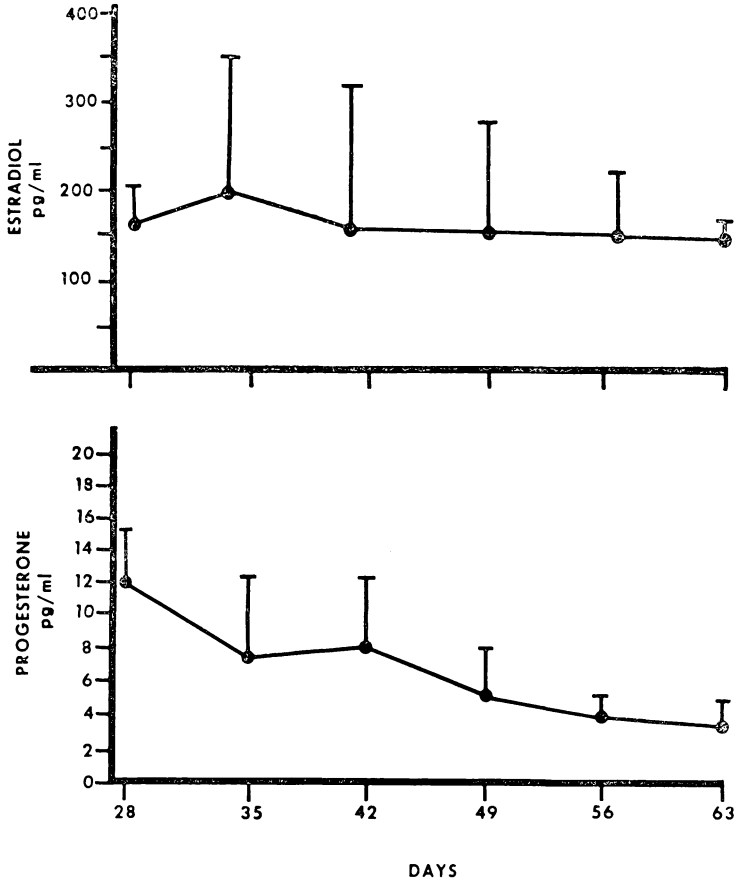

Fig. 3. — Serum concentration of estradiol and progesterone in ectopic pregnancy from 28 to 63 days.

duction of beta hCG or decreased production of intact hCG providing a ratio in these cases unique to EP.

Altered beta hCG secretion suggests that subtle alterations in hCG production and secretion may occur and persist in EP ([4, 5]).

Data of the present study suggest that perturbations in the specific, sequential production of hCG may be associated with abnormal events of pregnancy providing an attractive hypothesis explaining these events. The primary abnormalities in EP may be histologic with abnormal placentation an initial and persistent event reflected in subsequent abnormal secretory patterns of both intact hCG and free beta subunit noted clinically. The presence and persistence of this pattern of hCG secretion may reflect a failure of normal, progressive trophoblastic maturation and differentiation of cytotrophoblasts into syncytiotrophoblasts ([6]).

Prior analyses of these events in gestational trophoblastic neoplasia suggest that such a correlation between trophoblastic histology and hCG heterogeneity does exists ([7]).

The lower serum concentrations of E2, P4 and hCG noted in this study are consistent with data of prior reports ([3, 8]). The lower levels of steroid hormones demonstrated no correlation to either intact hCG or free beta subunit. This altered secretory capacity may reflect a defect in corpus luteum function for steroids as previously suggested or abnormal function of eaıly trophoblast for hCG secretion ([8]).

Prior data suggest that 35% of EP have ratios sufficiently unique to permit differentiation from IUP and AB. Data of the present study confirm this concept and further suggest that when present, altered ratios characteristic of EP persist throughout the gestation. This persistence suggests that failure to achieve specific landmarks in trophoblastic maturation may characterize EP and that abnormal placentation may be a primary event in EP manifested clinically by altered hCG ratios. These alterations in hCG secretion may characterize one-third of EP and may be of clinical utility in the diagnosis of EP by analysis of a single serum sample, regardless of timing.

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