Human chorionic gonadotropin and free beta subunit secretion in three events of pregnancy

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Summary: Serum hCG and free beta hCG subunit were measured in intrauterine pregnancies (n = 21), ectopic pregnancies (n = 20) and spontaneuos abortions (n = 19) matched for gestational age. Significantly higher concentrations of both dimer and free beta subunit hCG were detected in normal pregnancies (86,336 IU/L) and 21.02 IU/L respectively) compared to abortions (10,460 IU/L and 3.73 IU/L) and ectopic pregnancies (3,900 IU/L and 3.73 IU/L) ($p < .05$). When the ratio of free beta hCG/intact hCG (%) was studied, however, EP had significantly higher ratios (0.09 ± 0.09) than IUP ($p < .05$). Assessment of the relative distributions of these ratios revealed that 100% of IUP and AB and 65% of EP had ratios < 0.10. Most notably, 35% of EP were uniquely characterized by ratios > 0.10. This ratio was sufficiently higher in 35% of EP to define a profile completely unique to EP. These data suggest that an increased free beta to dimer hCG ratio of > 0.10 may assist in the differentiation of ectopic from intrauterine pregnancies of spontaneous abortions and provide insight into a possible trophoblastic mechanics in these clinical events.

Key words: ectopic pregnancy; hCG subunit secretion.

INTRODUCTION

Recent advances in assays for serum human chorionic gonadotropin (hCG) have provided improved methods in diagnosing complications in pregnancy. To diagnose an ectopic pregnancy (EP) or spontaneous abortion (AB) from a normal intrauterine pregnancy (IUP), prolonged doubling time and lower absolute values for hCG are well established indices. These profiles however are not sufficiently specific to differentiate EP from AB [1, 2]. HCG is a glycoprotein comprising two dissimilar subunits, alpha and beta joined noncovalently. During pregnancy dimer hCG production may be accompanied by free subunit secretion. A number of studies have evaluated whether the measurement of free hCG subunits can complement hCG assays. These studies have shown increased free alpha hCG production in ectopic pregnancy, gestational neoplasia, and spontaneous abortions [3, 4, 5]. However, no

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strict comparisons of subunit secretion in these events has been made and the exact role of the subunit testing remains unclear. In the present study, we evaluated whether free beta hCG measured alongside hCG may aid in the diagnosis of EP and distinguish this clinical event from spontaneous abortions and normal intrauterine pregnancies.

MATERIALS AND METHODS

Patient population:

An analysis of serum hCG and beta subunit concentrations was made on a single serum specimen from 21 patients with IUP, 19 patients with AB and 20 patients with EP. Patients with EP were matched to patients with IUP and AB on the basis of gestational age to obviate any potential influence of this parameter on hCG secretion. All patients had a history of regular menses prior to conception, a reliable last menstrual period (LMP) by history and were similar in gestational age when sampled. All patients were selected from a population presenting for clinical evaluation with the following diagnoses:

1. Intrauterine Pregnancy: Gestational age was confirmed by at least one pelvic ultrasound in the first trimester and clinical examinations that were consistent with estimated gestational age by LMP and ultrasound. All pregnancies continued normally into the second trimester.

2. Ectopic Pregnancy: All patients were managed by either salpingectomy, salpingotomy or salpingostomy. Histologic diagnosis confirmed the presence of chorionic villi on all excised specimens.

3. Spontaneous Abortions: All patients presented for evaluation with clinically diagnosed spontaneous (threatened, inevitable) abortions. Patients were managed by dilatation and curettage and histologic diagnosis (chorionic villi) was confirmed on all specimens.

Serum aliquots were stored at −70 degrees C before being assayed in random batches. All assays for dimer hCG and beta subunit were performed under a numerical code without information descriptive of clinical diagnosis of the patient.

Immunoassays:

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 Hybritech, 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 μg 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. Beta hCG assays, hCG, LH, FSH and TSH cross-reacted 0.6%, 0.3% and 0.5% respectively. In the dimer hCG assay, FSH, LH and TSH cross-reacted 1.3%, 1.1% and 0.01% respectively, but beta hCG did not significantly cross-react in the hCG assay.

Data analysis:

Comparisons of dimer hCG, free beta subunit hCG and exact gestational ages for each group, were carried out using an analysis of variance. Significance was tested at the 0.05 level using a Bonferroni T test.

RESULTS

Serum concentrations of dimer hCG in IUP were significantly higher than in EP or AB ($p<.05$); however, no significant differences in concentrations of dimer hCG between EP and AB were noted. The range of dimer hCG for these clinical entities varied widely as would be expected (Table 1). The mean gestational age of the three groups was not significantly different at the time of serum sampling, being 7.4, 7.3 and 7.8 weeks for IUP, EP and AB respectively.

Concentrations of the free beta subunit were significantly higher in IUP than in EP or AB ($p<.05$) (Table 1). Though the mean value of beta hCG for EP was 2.07 IU/L and the range 0.1 to 15.1 IU/L, it was unusual to find elevated levels of beta hCG. In 75% (15/20) of patients, beta hCG concentrations were less than 1.5 IU/L and only two patients had concentrations greater than 2.0 IU/L. Similar but not identical circumstances

Table 1. – *Concentration or hCG and beta hCG subunit in intrauterine pregnancies (IUP), ectopic pregnancies (EP) and spontaneous/missed abortions (AB) at six to eight weeks gestation.*

	Intact hCG * IU/L	Beta hCG * IU/L
IUP	86,336±94,498 (6,400 to 287,000)	21.01±29.95 (1.4 to 136.6)
EP	3,901±6,459 (285 to 26,450)	2.07±3.61 (0.5 to 15.1)
AB	10,462±13,778 (180 to 46,600)	3.73±6.89 (0.03 to 28.9)

* Mean±SD; range in parenthesis.

prevailed for AB patients in which a broader and somewhat higher range of beta hCG was noted; however 50% (9/19) of patients had concentrations less than 1.5 IU/L. These lower concentrations of free beta subunit for EP and AB are in contrast to higher concentrations for IUP in which group only one patient had a value less than 2.0 IU/L.

When the ratio of free beta hCG/intact hCG (%) was studied, EP had significantly higher ratios (0.09±0.09) than IUP or AB (0.04±0.03 and 0.03±0.02 respectively) ($p<.05$). Analysis of the relative distributions of these ratios revealed that 100% of IUP or AB and 65% of EP had ratios less than 0.10 (Figure 1).

Most notably, 35% of EP were uniquely characterized by ratios greater than 0.10. This finding of a beta hCG/intact hCG (%) ratio greater than 0.10 was noted only in EP and not associated with either IUP or AB. Thus, a ratio greater than 0.10 was helpful in distinguishing an EP from IUP or AB in 35% of cases.

This finding also provides insight into the trophoblastic events that may be involved in each event of pregnancy and suggests a hypothesis that an arrest of trophoblastic maturation and differentiation may characterize EP.

DISCUSSION

Data of the present study are in agreement with prior investigations suggesting that abnormalities in hCG production do exist in both EP and AB ([4, 7]). Decreased production of intact hCG in these events suggest an alteration in the secretory abili-

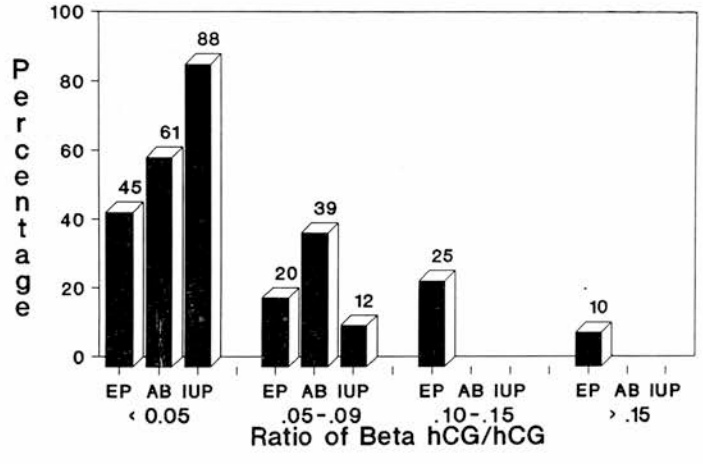

Fig. 1. — Percentage of patients and the ratio of beta hCG/hCG in three events of pregnancy (IUP - Intrauterine Pregnancy, EP - Ectopic Pregnancy and AB - Spontaneous Abortion).

ty of the trophoblast. The overall decreased secretion of free beta hCG subunit in both EP and AB observed in the present study further substantiate an impaired secretory potential. Though inspection of the absolute secretion of intact hCG and its beta subunit suggested decreased production in both EP and AB, a comparison of the relative secretion of each (i.e., ratio of beta hCG/hCG) however revealed characteristic patterns for IUP, EP and AB.

Uncomplicated pregnancies were consistently characterized by ratios <0.10 and in fact 88% of IUP manifested ratios <0.05 (Figure 1). Such a pattern has been previously described during the first trimester when the beta hCG ratio decreased steadily from 4% to $<1\%$ from conception to 10 weeks gestation ([8]). Similar ratios were observed for AB in which all ratios were <0.10; however, only 61% of AB had ratios <0.05. In contrast, EP were characterized by ratios ranging from 0.015 to 0.176. Thirty-five percent of EP were characterized by ratios >0.10, a finding noted only in EP. These ratios may suggest an increased production of beta hCG or decreased production of intact hCG providing a ratio in these cases unique to EP.

A possible explanation and hypothesis for these findings may be found in the histology of trophoblastic tissue in normal and abnormal pregnancies. In situ hybridization probes have localized mRNA for alpha hCG to cytotrophoblasts, while mRNA for beta hCG was localized to intermediate cells and syncytium ([9]).

Thus in trophoblastic villi, as cytotrophoblasts differentiate into intermediate cells, there is a switch to increased beta hCG production. In pathological villi frequently found in EP, there may be impaired and arrested syncytium formation which is reflected in reduced dimer hCG levels and accumulation of intermediate cells secreting free beta hCG. This would be reflected in higher beta hCG/hCG ratio. In contrast, villi in IUP and AB show proliferating syncytium with rising hCG levels and declining intermediate cells which is reflected in low and falling beta hCG/hCG ratios. Data of the present study suggest that these histologic changes may prevail in clinically detectable, abnormal events of pregnancy and may be responsible for the alterations of beta hCG/hCG ratios in EP and AB observed. In 35% of EP, these alterations were of sufficient magnitude to result in a profile characteristic and indicative of EP.

Altered beta hCG secretion suggests that subtle alterations in hCG production and secretion may occur in EP and AB. Data of the present study suggest that alterations in the specific, sequential production of hCG may be associated with abnormal events of pregnancy providing an attractive hypothesis explaining possible trophoblastic mechanics in there clinical events. To distinguish EP from AB or IUP, well established methods include transvaginal ultrasuond, serial hCG and progesterone profiles. These procedures are effective but can be time consuming and have limited application in an emergency. The use of monoclonal antibodies in rapid " stat " procedures for hCG can generate semi-quantitative results within minutes. These methods may serve as an initial screening test on a single serum sample.

The finding of raised beta hCG/hCG in 35% of EP patients indicate that these ratios may enhance hCG results, and be used to distinguish EP from spontaneous abortions and continuing pregnancies especially where sophisticated ultrasound facilities are not readily available. These changes in dimer and free beta subunit hCG secretion also provide insight into possible trophoblastic events in EP which await histologic confirmation.

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