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Analysis of serum human chorionic gonadotrophin levels in normal singleton, multiple and abnormal pregnancies

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Some researchers claim that first trimester β-human chorionic gonadotrophin (βHCG) levels have a constant doubling time; others suggest doubling time increases as pregnancy progresses. This study was designed to settle the debate by analysing a large series of serial serum βHCG determinations from 143 pregnant women whose day of ovulation was precisely determined. Regression analysis was used to evaluate linear and quadratic models for the relationship of HCG with time in normal pregnancies. Doubling times were calculated for three time periods: 10–20 days post-ovulation (period 1); 21–30 days post-ovulation (period 2); >30 days post-ovulation (period 3). Analysis of variance was used to compare the mean doubling time by time period and type of pregnancy (single, multiple, spontaneous abortion and ectopic). The analysis showed that a quadratic model best described the pattern of HCG rise in early normal pregnancy. Furthermore, for normal pregnancies, the mean doubling time increased significantly with advancing gestational age between time periods 1 and 2 and between periods 2 and 3. The mean doubling time was the same for single and multiple pregnancies. The doubling time was prolonged with ectopic pregnancy in period 1; and for aborters reaching ultrasound at 8 weeks, the doubling time was normal in period 1 but prolonged in period 2. Careful observation of the doubling time may aid clinicians in the detection of abnormal pregnancies.

Key words: βHCG/doubling time/ectopics/multiple gestation/spontaneous abortion

Introduction

Monitoring the changing levels of serum β-human chorionic gonadotrophin (βHCG) has become invaluable in early gestations. Numerous authors have shown that an abnormal rate of rise of βHCG can help in detecting abnormal pregnancy, particularly ectopic pregnancy, days before the patient becomes symptomatic (Kosasa *et al.*, 1973; Kadar *et al.*, 1981a; Lenton *et al.*, 1982; Cartwright and DiPietro, 1984; Romero *et al.*, 1986; Kadar and Romero, 1988; Shepherd *et al.*, 1990).

There has been much debate in the literature as to the exact relationship between time and βHCG levels in normal pregnancies. Athough it is well established that βHCG continues to increase during the first few weeks of pregnancy, the exact nature of the increase has come under intense scrutiny. There have been numerous articles analysing the rise of HCG in normal pregnancies (Pittaway *et al.*, 1985a, Kadar, 1986; Kadar and Romero, 1987; Daya, 1987; Fritz and Guo, 1987; Kadar *et al.*, 1990). It is still unclear whether HCG increases in a linear or quadratic fashion during the crucial first 6 weeks of pregnancy (Pittaway *et al.*, 1985b; Kadar *et al.*, 1990). The day of ovulation was not accurately determined in any of these studies but simply estimated from the last menstrual period or the use of basal body temperature charts. Unfortunately, both of these methods can be highly inaccurate.

Few researchers have closely analysed differences in doubling time in abnormal pregnancies, e.g. ectopic pregnancies or those that spontaneously abort. Most authors state that the rise in doubling time in these cases is abnormal, but have not attempted to characterize this difference.

Finally, there have been few reports of the rise of βHCG in early multiple pregnancies (Daya, 1987; Fritz and Guo, 1987). No studies to date have carefully compared normal singleton to multiple pregnancies in terms of characterization of βHCG increases and changes in doubling time during the first few weeks of pregnancy.

In our study, we analysed a large number of normal singleton, abnormal singleton, and normal multiple pregnancies by carefully monitoring both the day of ovulation and the nature of the HCG rise in early pregnancy. We had three objectives: firstly to characterize the βHCG rise in normal singleton pregnancies, either following ovulation inducing drugs or in the absence of follicular medications, secondly to determine whether βHCG changes help distinguish normal from abnormal pregnancies (e.g. ectopic or spontaneous abortions); and thirdly to establish whether the βHCG rises in multiple pregnancies are quantitatively or qualitatively different from those in singleton pregnancies.

Materials and methods

Data were collected on a group of women who became pregnant between February 1988 and June 1989 all of whom had been closely monitored as to their exact day of ovulation. To be included in the study all patients had to have proper documentation of the luteinizing hormone (LH) surge or had been given βHCG to induce ovulation. Cases in which the day of the LH surge or HCG injection could not be determined were not included in the analysis. Patients had to have had at least two HCG measurements <6000 mIU/ml. The patients in the study were representative of a general infertile population; indications included anovulation, luteal phase defects, clinical factor, endometriosis and tubal factor. LH was measured by double antibody radioimmunoassay (Amersham Corporation, Arlington Heights, IL, USA) and the βHCG levels were determined by fluorometric enzyme immunoassay (American Dade, Miami, FL, USA) using the first or second international standard.

In all, 143 patients had 681 serial serum βHCG determinations made at frequent intervals during their first trimester. An average of 4.1 determinations were made per patient at intervals ranging from 1−7 days. Each patient was monitored by ultrasound and serum LH, progesterone and oestradiol to document the day of ovulation. In addition, all patients were followed at least into their third trimester of pregnancy. The progesterone levels were measured by an immunoassay technique based on enhanced luminescence (Amersham Corporation, Arlington Heights, IL, USA) and oestradiol levels were measured by solid-phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA).

The women were divided into six groups: Group 1 (n = 11), normal singleton pregnancy, not taking ovulation inducing drugs; Group 2 (n = 57), normal singleton pregnancy, with ovulation induction; Group 3 (n = 14), ectopic pregnancy; Group 4 (n = 22), normal twin pregnancy; Group 5 (n = 7), normal triplet pregnancy; Group 6 (n = 32), spontaneous abortion. Spontaneous abortions were further categorized as: (i) spontaneous abortion before ultrasound at 8 weeks gestation; (ii) spontaneous abortion−non-viable ultrasound; (iii) spontaneous abortion−small for dates but heart beat on normal ultrasound; (iv) spontaneous abortion−normal ultrasound but subsequent abortion. The HCG doubling time between pairs of HCG tests was calculated using the formula: doubling time = $(t_2\text{-}t_1)*\ln(2)/\ln(HCG_2/HCG_1)$ (where t_2 = number of days post-ovulation when the second HCG value in the pair was obtained, t_1 = number of days post-ovulation when the first HCG value was obtained, HCG_2 = HCG value obtained at time t_2, HCG_1 = HCG value obtained at time t_1, ln = natural logarithm). Doubling times were calculated for each of three time periods: period 1 = days 10−20 post-ovulation; period 2 = days 21−30 post-ovulation; period 3 = >30 days post-ovulation. If a woman had more than two HCG values in any given time period, then the doubling time for that period was calculated using the initial and final βHCG values obtained in that period. Analysis of variance was used to compare the mean doubling time by time period and type of pregnancy. A 5% level of significance was used.

Further, to investigate the rate of increase of βHCG over time in normal pregnancies, regression analysis was used to evaluate the fit of both a linear and a quadratic model to the data. The models tested considered the natural logarithm of HCG as a function of time, where time was measured as the number of days post-ovulation. The regression analysis was performed for each of the three groups of normal pregnancies (singleton pregnancies−no ovulation induction, singleton pregnancies after ovulation induction and multiple gestations). These were then compared for any differences.

Results

Mean βHCG doubling times in the normal pregnancy groups for the three time periods analysed are presented in Table I. There were no differences in doubling time during any of the three time periods between patients with normal singleton pregnancies with or without ovulation induction. These two groups were therefore combined for further analysis. Furthermore, in the group with normal singleton pregnancies, the doubling time significantly increased with increasing gestational age both between the clinically important time periods 1 (10−20 days) and 2 (21−30 days) (P < 0.05) from 1.40 ± 0.40 to 2.40 ± 3.48 and between time periods 2 and 3 (>30 days) from 2.40 ± 3.48 to 7.18 ± 6.60.

There were no significant differences between the mean doubling times when comparing singleton pregnancies to multiple pregnancies in any of the three time periods. As with the singleton pregnancies, there was a significant increase in doubling time from period 1 to period 2 and from period 2 to period 3.

Mean βHCG doubling times for women with abnormal pregnancies are presented in Table II. Patients with ectopic pregnancies had a prolonged doubling time as early as the first time period compared to patients with normal pregnancies. By the second time period, βHCG levels in women with ectopic pregnancies had begun to decrease as shown by a negative doubling time.

The pattern of βHCG rise in women with spontaneous abortion was found to vary according to the type of abortion (see Table II). Women who aborted before an ultrasound was feasible had decreasing βHCG levels as early as the first time period. Aborters with non-viable ultrasound findings had a prolonged doubling time in period 1 followed by sharply decreasing βHCG levels as shown by a negative doubling time. Late aborters who had viable ultrasound results but were small for dates had the same mean doubling time as normal pregnancies in period 1 but had a significantly prolonged doubling time by period 2.

Regression analysis showed that the best equation to fit the data was a second order quadratic equation. The regression coefficients for each of the normal pregnancy groups (singleton pregnancies without and with ovulation induction, and multiple gestations) are presented in Table III. The regression coefficients were not significantly different (P > 0.05) for all three normal groups. A comparison of the least squares regression equation for normal singleton pregnancies and multiple gestations is seen in Figure 1. A plot of the data points for normal singleton pregnancies together with the polynomial model fitted to the data is presented in Figure 2.

Discussion

Kadar *et al.* (1981b) were the first to show that serial βHCG levels could be useful in distinguishing an ectopic from a normal pregnancy. In these early studies, βHCG rose in a log linear fashion and the doubling time for βHCG was constant at ~2.7 days. Although a number of subsequent studies by these authors

Table I. Mean serum human chorionic gonadotrophin (HCG) doubling times in days in normal singleton and multiple pregnancies[a]

Type of pregnancy	Period 1 (10–20 days post-ovulation)	Period 2[b] (21–30 days post-ovulation)	Period 3[c] (>30 days post-ovulation)
Singleton pregnancy:			
No ovulation induction	1.44 ± 0.44 (6)	2.60 ± 0.64 (6)	5.00 ± 3.90 (3)
Ovulation induction	1.39 ± 0.40 (32)	2.49 ± 2.80 (40)	7.45 ± 6.96 (24)
All	1.40 ± 0.40 (38)	2.40 ± 3.48 (46)	7.18 ± 6.60 (27)
Multiple pregnancy:			
Twins	1.42 ± 0.19 (15)	2.50 ± 0.38 (7)	6.65 ± 4.43 (4)
Triplets	1.30 ± 0.27 (3)	2.28 ± 0.21 (3)	4.86 (1)
All	1.40 ± 0.20 (18)	2.40 ± 0.33 (10)	6.30 ± 3.90 (5)

[a]Data are presented as mean ±SD; sample size in parentheses.
[b]$P < 0.05$ when comparing mean HCG doubling time in period 1 to period 2 in each group.
[c]$P < 0.05$ when comparing mean HCG doubling time in period 2 to period 3 in each group.

Table II. Mean serum human chorionic gonadotrophin (HCG) doubling times in days in abnormal pregnancies: ectopic and spontaneous abortions[a]

Type of pregnancy	Period 1 (10–20 days post-ovulation)	Period 2 (21–30 days post-ovulation)	Period 3 (>30 days post-ovulation)
Ectopic	4.03 ± 4.18 (9)	− 3.73 ± 14.96 (9)	4.21 ± 0.62 (2)
Spontaneous abortion			
Before US[b]	−0.13 ± 3.67 (3)	− 0.64 ± 7.42 (3)	−2.87 ± 23.88 (3)
Non-viable US	2.36 ± 1.39 (6)	−20.91 ± 84.72 (13)	31.69 ± 78.04 (7)
Normal US small for dates	1.57 ± 0.33 (8)	5.44 ± 7.85 (9)	− 1.28 ± 24.41 (9)
Normal US late abortion	1.59 (1)	2.48 ± 1.43 (2)	3.99 (1)

[a]Data are presented as mean ±SD; sample size in parentheses.
[b]US = ultrasound.

Table III. Coefficients of polynomial regression model for normal pregnancies

Group	Constant	Coefficient of t (t = no. of days post-ovulation)	Coefficient of t^2 (t = no. of days post-ovulation)	Standard error of estimate
Singleton no ovulation induction	−4.772	0.791	−0.010	0.883
Singleton ovulation induction	−2.778	0.677	−0.008	0.603
Multiple	−3.435	0.739	−0.009	0.778

and others have confirmed a constant doubling time for normal pregnancy in the range of 1.4–3.5 days (Kosasa *et al.*, 1973; Kadar *et al.*, 1981a, 1990; Cartwright and DiPietro, 1984; Romero *et al.*, 1986; Kadar, 1986; Kadar and Romero, 1987, 1988; Shepherd *et al.*, 1990) other researchers have shown that the doubling time actually increases as the pregnancy progresses (Pittaway *et al.*, 1985a,b; Fritz and Guo, 1987). Pittaway *et al.* (1985b) showed that the HCG doubling time increases and the exponential rate of increase of serum HCG significantly decreases with gestational age. They concluded that the rate of increase could be better described by a quadratic rather than a linear equation. Based on this assumption they proposed that estimates of normal rises of βHCG should be established for short sampling ranges and short intervals of gestation. Fritz and Guo (1987), in a study of 16 women supported this conclusion. Conflicting evidence showing a constant doubling time in the first 6 weeks of gestation has been obtained by other workers (Kadar and Romero, 1988; Kadar *et al.*, 1990), leading to a questioning of the Pittaway *et al.* (1985b) analysis. It should be noted that these researchers measured βHCG levels in relation to the last menstrual period or rise in basal body temperature charts, both of which are inaccurate methods of determining the day of ovulation.

Our data, based on a highly accurate determination of the day of ovulation, support the thesis of Pittaway *et al.* (1985b) that the doubling time increases throughout early pregnancy. From the time periods corresponding to 3 5/7–4 6/7 weeks gestation age and from 4 6/7–6 2/7 weeks gestational age, the doubling time increased from 1.40 ± 0.40 days to 2.40 ± 3.48 days ($P < 0.05$). The doubling time increased to 7.18 ± 6.60 in the period after 6 2/7 weeks. Any deviation from these markers must alert the clinician to the strong possibility of an abnormal pregnancy, i.e. ectopic pregnancy or spontaneous abortion.

Interestingly, we found no difference in doubling time in patients having ovulation induction versus those without. The concern about a vanishing second or third fetus in patients on

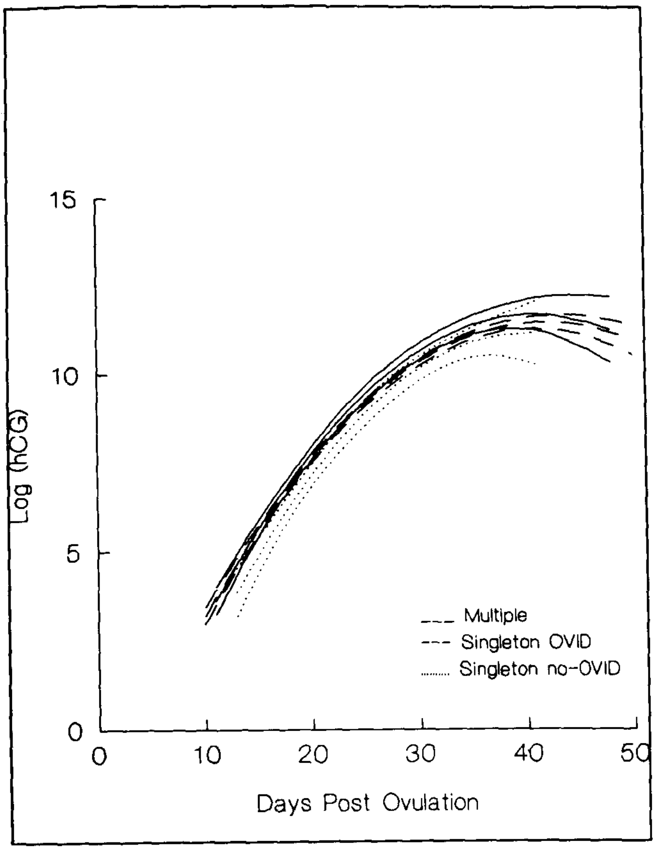

Fig. 1. Comparison of the polynomial regression model of the logarithm of serum β-human chorionic gonadotrophin (HCG) concentrations in normal pregnancies as a function of the number of days post-ovulation for singleton pregnancies – no ovulation induction (OVID) (dotted lines); singleton pregnancies – OVID (dashed lines), and multiple gestations (solid lines). 95% confidence limits are also plotted for each group.

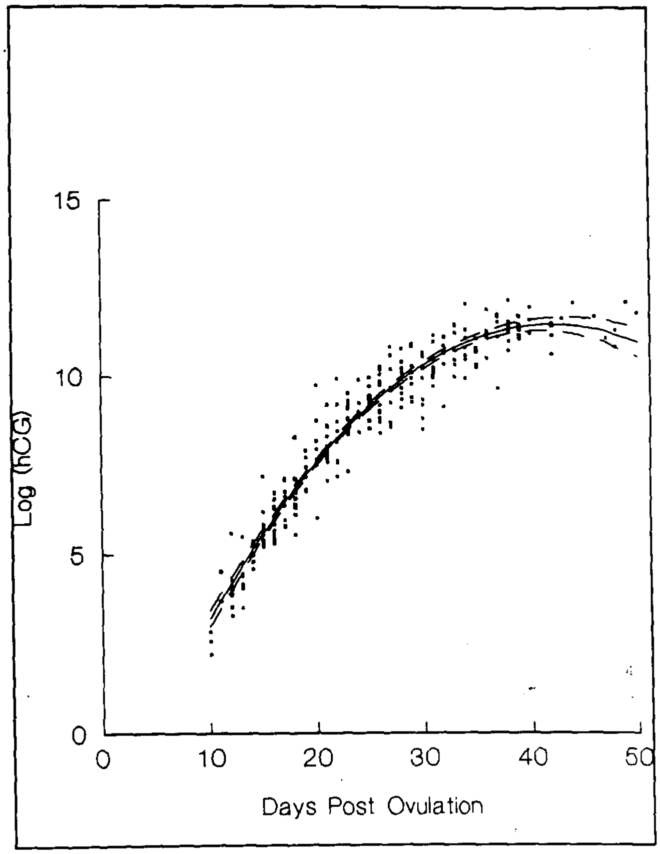

Fig. 2. Plot of the polynomial regression model for normal singleton pregnancies. The natural logarithm of the serum β-human chorionic gonadotrophin (HCG) concentrations was plotted as a function of time, where time was measured as the number of days post-ovulation.

ovulation induction affecting the βHCG rise does not seem to be clinically relevant when analysing βHCG changes in early pregnancy.

The results of our regression analysis also supported the finding of Pittaway *et al.* (1985b) that the rise in HCG levels in singleton pregnancies is better described by a quadratic equation.

Although clinicians sometimes suspect multiple pregnancies when they find unusually elevated early βHCG levels, a recent study by Kelly *et al.* (1990) showed no quantitative or qualitative differences in βHCG rise when comparing multiple and singleton pregnancies. Our study supports this finding, as we found no significant difference in doubling time or absolute HCG values between the singleton, twin or triplet pregnancies. Although the clinician will continue to guess that a pregnancy is multiple when he or she finds an early elevated βHCG value, our data suggest that there is no discernible quantitative or qualitative difference in βHCG values in the first few weeks of a pregnancy.

Patients with ectopic pregnancies had prolonged doubling times as early as the first time period analysed. Early spontaneous aborters had decreasing βHCG levels even in the first period. Late aborters had a normal doubling time in the first time period, but prolonged doubling times beginning in the second time period. Careful analysis of the βHCG rise in early pregnancies may help the clinician identify and differentiate between ectopic pregnancies and patients who will eventually have spontaneous abortions.

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