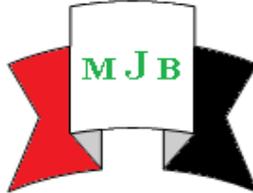


Predictive Power of Serial Serum Beta Human Chorionic Gonadotropin Measurements in the Outcome of Pregnancy

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Abstract

Background: The beta –hCG concentration in a normal intrauterine pregnancy rises in a curvilinear fashion until 41 days of gestation at which time it plateaus at approximately 100 000 IU/L & the mean doubling time for the hormone is from 1.4 to 2.1 days .

Aim of study:To determine whether serial quantitative serum hCG levels obtained 48-72 hours apart are reliable predictors of pregnancy outcome.

Subjects & Methods:A prospective study was done at Babylon hospital for Maternity & Children & private clinic from the period of April 2010 to September 2011 & involved 50 pregnant women in first 6-8 weeks of gestation presented with vaginal bleeding in current pregnancy or abortion in previous pregnancy .Women more than 6-8 weeks of gestation & those with multiple pregnancies were excluded from the study.

The level of B-hCG was estimated by a paired blood samples collected 48-72 hours apart. An automated quantitative measurement of B-hCG in the serum was done by VIDAS instrument using ELFA technique (Enzyme linked Fluorescent ASSAY).

Results:A total of 50 pregnant women in first 6-8 weeks of pregnancy were followed up by serial B-hCG 2-3 days apart.11 women out of 50 (22%) terminate as spontaneous abortion; 39 women out of 50(78%) had term pregnancy.

Mean age was 26.84 years, mean gravida was 3.08, and mean parity was 0.7 .The mean gestational age was 5 weeks +1.3 days.Mean hCG at presentation was 8926.5 miu/ml,& after 2-3 days was 16267.39 miu/ml.

8 women out of 11 (72%) had decline in B-hCG after 2-3 days which is statistically significant (p`0.001).

While none of women with term pregnancies showed decline in B-hCG after 2-3 days.

37 out of 39 women (94%) with term pregnancy had increment more than 60% in B-hCG after 2-3 days (statistically significant p less than 0.001).

Conclusions:

1. Serial quantitative serum hCG 48-72 hours apart provides useful predictors of pregnancy outcome in the first 6-8 weeks of gestation.
2. A single B hCG reading is not predictive regarding the prognosis of pregnancy compared with double readings.

القوة التوقعية للقياسات المتتابعة لهرمون HCG لمعرفة نتيجة الحمل

الخلاصة

دراسة تقديمية اجريت في مستشفى بابل للولادة والأطفال والعيادات الخاصة للفترة من نيسان عام ٢٠١٠ ولغاية أيلول عام ٢٠١١ . تضمنت الدراسة خمسين امرأة حامل في الأسابيع ٦-٨ الأولى من الحمل، واللواتي يعانين من نزف رحمي قليل أو متوسط في الحمل الحالي، أو لديهن اسقاط سابق أو اسقاطات متكررة في السابق.

تم أخذ المعلومات الكاملة عنهن مثل العمر, عدد الولادات أو عدد الأسقاطات السابقة, وتم تحديد فترة الحمل عن طريق معرفة اليوم الأول من آخر دورة شهرية. وتم عزل النساء اللواتي تزيد مدة حملهن عن ٨ أسابيع الأولى من الحمل, وكذلك نواتي الحمل المتعدد من الدراسة. تم سحب ٢-٤ مليلتر من الدم الوريدي, وبعد ترك الدم ليتخثر تم عزل المصل بواسطة عملية الطرد المركزي عند درجة حرارة الغرفة. تم قياس مقدار هرمون B-hCG في الدم بواسطة عينيتين اثنتين تم جمعهن ٤٨-٧٢ ساعة فاصلة بينهما باستخدام طريقة الفايزر والتي تستخدم طريقة الفلوريسين المرتبط مع الأنزيم. تبين من الدراسة ان ١١ امرأة من أصل ٥٠ (22%) قد انتهت بشكل اسقاط ذاتي, وان ٣٩ امرأة (78%) انتهت بشكل حمل كامل. وان ٨ امرأة من أصل ١١ (٧٢%) في مجموعة الأسقاط الذاتي أظهرت انخفاض في مستوى هرمون B-hCG بعد مرور ٢-٣ يوم, والذي يعتبر ذات مغزى احصائي عالي. بينما في مجموعة الحمل الكامل لم يكن هناك أي انخفاض في مستوى هرمون B-hCG بعد مرور ٢-٣ يوم. أستنتج من هذه الدراسة ان قياس كمية هرمون B-hCG في الدم المتعاقب لعينيتين يتم جمعهن ٤٨-٧٢ ساعة فاصلة بينهما يجهز متنبىء مفيد عن نتيجة الحمل في ٦-٨ أسابيع الأولى من الحمل.

Introduction

HCG (human chorionic gonadotrophin) is a glycoprotein composed of 244 amino acids. HCG is composed of alpha and beta subunits; the alpha subunit identical to that of luteinizing hormone (LH), follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH); and the beta subunit is unique to HCG. It has a 30- amino acid tailpiece at the cooH terminal .

hCG is hormone produced in pregnancy that is made by developing embryo soon after conception and later by the syncytiotrophoblast.⁽¹⁾

hCG is indispensable for successful progression of pregnancy. Its classical function is to maintain steroid hormones & other growth factors in the corpus luteum.⁽²⁾ In addition ,hCG has been reported to modulate the blastocyst implantation (Srisuparp et al. 2001), uterine vascularization & angiogenesis(Zygmunt et al.,2002; Toth et al. 2001) , uterine quiescent & immunological adaptation during pregnancy.^(3,4,5,6)

Due to its highly negative charge, hCG may repel the immune cells of

the mother & may be a link in the development of peritrophoblastic immune tolerance.⁽⁷⁾

hCG also plays a role in cellular differentiation/ proliferation & may activate apoptosis.⁽⁸⁾

The synthesis of hCG begins shortly after fertilization, the B-subunit of the hormone has been detected in the two-cell stage embryo.⁽⁹⁾

After implantation, hCG is transported into maternal blood stream.

Records to attempts at pregnancy testing have been found as far back as the ancient Greek and ancient Egyptian cultures. The ancient Egyptians watered bags of wheat and barely in the urine of a possibly pregnant woman. Germination indicated pregnancy. The type of grain that sprouted was taken as an indicator of the fetus's sex.

Selmar Aschheim and Bernhard Zondek introduced testing based on the presence of human chorionic gonadotropin (hCG) in 1928.⁽¹⁰⁾

Level of hCG may be measured in the blood or urine; this is done as a pregnancy test.

Many hCG immunoassays are based on the sandwich principle, which uses antibodies to hCG labeled with an enzyme or a conventional or luminescent dye. Pregnancy

urine dipstick tests are based on the lateral flow technique. The urine may be a chromatographic immunoassay with a detection thresholds range from 20 to 100 mIU/ml, depending on the brand of test.⁽¹¹⁾

The serum test, using 2-4ml of venous blood, is typically a chemilnescent or fluorimetric

immunoassay that can detect BhCG levels as low as 5 miu/ml and allows quantification of the BhCG concentration.⁽¹²⁾

The reference levels of hCG are:

Weeks since LMP	miu/ML
3	5-50
4	5-426
5	18-7,340
6	1,080-56,500
7-8	7,650-229,000
9-12	25,700-288,000

Non-pregnant females <5.

Postmenopausal females < 9.5.^(13, 14)

hCG appears in the blood and urine of pregnant women as early as 10 days after conception. Quantitative hCG measurements help determine the exact age of th fetus. It can also diagnose abnormal pregnancies, such as ectopic pregnancies, molar pregnancies, and possible miscarriages.^(15, 16)

hCG levels significantly increase during early pregnancy with levels doubling approximately every 48 hours.⁽¹⁷⁾

Serum level of hCG rise linearly during the first 6 weeks of pregnancy, during this early stage of pregnancy, hCG levels double every 1.3 to 2 days. But if the pregnancy more than six weeks, hCG results may be the best judge of whether the pregnancy is viable. As pregnancy progresses, it is normal for hCG levels to take longer to double.^(18, 19, 20, 21, 22, 23, 24)

hCG is produced by syncytiotrophoblast, and its serum level represents the trophoblast mass.⁽²⁵⁾

hCG levels dynamically increase during early gestation and the levels are significantly greater in viable pregnancies than in ectopic gestation, biochemical pregnancy, or spontaneous abortions.^(26,27,28,29,30,31,32)

Tools used by clinicians to evaluate vaginal bleeding or pain in the first trimester include transvagina;l ultrasound(TVUS) and serial serum B-hCG measurements.⁽³³⁾ Even when combined with risk factors for pregnancy loss (serum levels of estradiol, Inhibin A, and Inhibin; maternal age, smoking, past history of spontaneous miscarriage and vaginal bleeding), TVUS is not accurate at predicting early pregnancy loss.^(34, 35)

A suboptimal rise in B-hCG(<66% after 48 hours has historically been used to indicate possible miscarriage or ectopic pregnancy,⁽³⁶⁾ but studies have found similarly low rates of increase in some viable pregnancies, as well. And B-hCG measurements need to be done on more than one occasion, making this an inconvenient means of predicting miscarriage.^(37, 38, 39)

Subjects and Methods

A prospective study was done at Babylon Hospital for Maternity & Children & at a private clinic from the period of April 2010 to September 2011 & involved 50 pregnant women in their first trimester of pregnancy.

The inclusion criteria was pregnant women in their first 6-8 weeks gestation having mild –

moderate vaginal bleeding or other miscarriage symptoms in the current pregnancy or patient with history of recurrent abortions or abortion in the previous pregnancy.

A full history was obtained regarding their age, parity, gestational age and previous abortions.

Their gestational age was assessed accurately from last menstrual period.

Pregnant women more than 8 weeks gestation & women with multiple pregnancies were excluded from the study.

The blood test, using 2-4 ml of venous blood that can detect B-hCG levels as low as 2mi.u/ml & allows quantification of the B-hCG concentration.

VIDAS hCG is an automated quantitative test for use on the VIDAS instrument, for the quantitative measurement in human serum (lithium heparinate or EDTA) using the ELFA technique (Enzyme linked Fluorescent Assay).

The assay principle combines an enzyme immunoassay sandwich method with a final fluorescent detection. The intensity of the fluorescence is proportional to the concentration of antigen present in the sample.

The entire assay steps are performed automatically by the instrument .The assay

will be completed within approximately 30 minutes.

At the end of the assay, results are automatically calculated by the instrument using calibration curves which are stored by the instrument (4-parameter logistic model), & then printed out & the concentrations are expressed in miu/ml.

Blood was collected by venipuncture, allowed to clot & serum separated by centrifugation at room temperature.

The technique used in the present work is the diagnostic kit prepared by Eurogenetcs.

When hCG levels are doubling as expected within 48-72 hours, it is a good sign that the pregnancy is developing normally.

Results:

A total of 50 women were followed up, from period of April 2010 to September 2011; 39 women (78%) of them progressed to term pregnancy, while 11 women (22%) ended in spontaneous abortion.

The mean age was 26.84 years (range 16-43 years); mean gravid was 3.08 (range 1-7) ; mean parity was 0.7 (range 0-4) .The mean gestational age by reported LMP was 5.06 weeks ±1.3 days (range 3-6weeks +6 days).Mean HCG at presentation was 8926.5 miu/ml ; mean hCG after 2-3 days was 16267.391mi.u/ml.

Table 1-Demographic variables for women with spontaneous abortions compared with viable term pregnancies.

Variable	Spontaneous abortion		Term pregnancy		P-value
	No.	Percentage %	No.	Percentage %	
1-Age(years)					
<20	5	45	2	5	0.001
20-24	Zero	Zero	11	28	0.001
25-29	3	27	15	38	0.001
30-34	2	18	4	10	>0.05
35	1	9	7	17	>0.05
2-Parity					
0	7	63	25	64	>0.05
1	Zero	Zero	7	17	>0.05
2	3	27	4	10	>0.05
3	1	9	1	2	>0.05
4	Zero	Zero	2	5	>0.05

3-Gestational age at presentation(weeks)					
3-4	7	63	14	35	>0.05
5-6	3	27	23	58	>0.05
7-8	1	9	2	5	>0.05
4-Prior spontaneous abortion					
0	1	9	8	20	>0.05
1	1	9	16	41	<0.05
2	3	27	10	25	>0.05
3	5	45	2	5	<0.001
4	Zero	Zero	3	7	>0.05
5	1	9	zero	Zero	>0.05

P- Value<0.05 was statistically significant.

Table 1 showed that overall women with spontaneous abortions were younger than women with term pregnancies. Women under 20 years were more likely to terminate as spontaneous abortion than other age groups. No statistically significant association was

found between spontaneous abortion or term pregnancy and parity, and gestational age at presentation. History of spontaneous abortions was associated with increased risk of spontaneous abortion.

Table2-Comparison of clinical factors at presentation in women with spontaneous abortion compared with term pregnancy.

Variable	Spontaneous abortion No. 11		Term pregnancy No. 39		OR	P-value
	No.	percentage%	No.	percentage%		
1-Abdominal pain	8	72	23	58	1.85	<0.406
2-Vaginal bleeding(mild-moderate)	9	81	17	43	5.82	<0.025

OR=odd ratio

Table2 showed there was a statistically significant association between vaginal bleeding (mild-moderate) and risk of spontaneous abortion (p value less than

0.025). Women with initial HCG less than 500 I.U/ml had increased risk of spontaneous abortion.

Table3- Comparison of HCG level in women with spontaneous abortion compared with term pregnancy.

Variable	Spontaneous abortion		Term pregnancy		p-value
	No.	Percentage	No.	Percentage	

1-Initial hCG(miu/ml) at presentation					
0-500	4	36	Zero	Zero	<0.001
501-2000	3	27	2	5	<0.05
2001-4000	2	18	6	15	>0.05
4001-6000	Zero	Zero	15	38	<0.05
>6000	2	18	16	41	>0.05
2-hCG(miu/ml)after 2-3 days					
0-500	5	45	Zero	Zero	<0.001
501-2000	3	27	1	2	<0.01
2001-4000	Zero	Zero	Zero	Zero	>0.05
4001-6000	Zero	Zero	4	10	>0.05
>6000	3	27	34	87	<0.001

P-value < 0.001(extremely significant).

P-value <0.01 (highly significant)

Table 3 showed women in spontaneous abortion group had lower initial hCG at presentation than women in term group; 4 women out of 11 (%36) had initial hCG level less than 500 miu/ml, compared to zero in

term group. Also they had lower HCG level after 2-3 days; 5 out of 11 women (%45) had hCG less than 500 miu/ml compared to zero in term group.

Table 4- Changes in hCG level within 2-3 days in women with spontaneous abortion and term pregnancy.

Increment in hCG	Spontaneous abortion		Term pregnancy		p-value
	No. .	Percentage%	No.	Percentage%	
<50%	Zero	Zero	2	5	>0.05
50-70%	1	9	7	19	<0.001
71-90%	2	18	30	76	<0.001
Decrement in HCG					
50-70%	4	36	Zero	zero	<0.001
71-90%	2	18	Zero	zero	<0.01
>90%	2	18	Zero	Zero	<0.01

Odd ratio (OR) was 1.667.

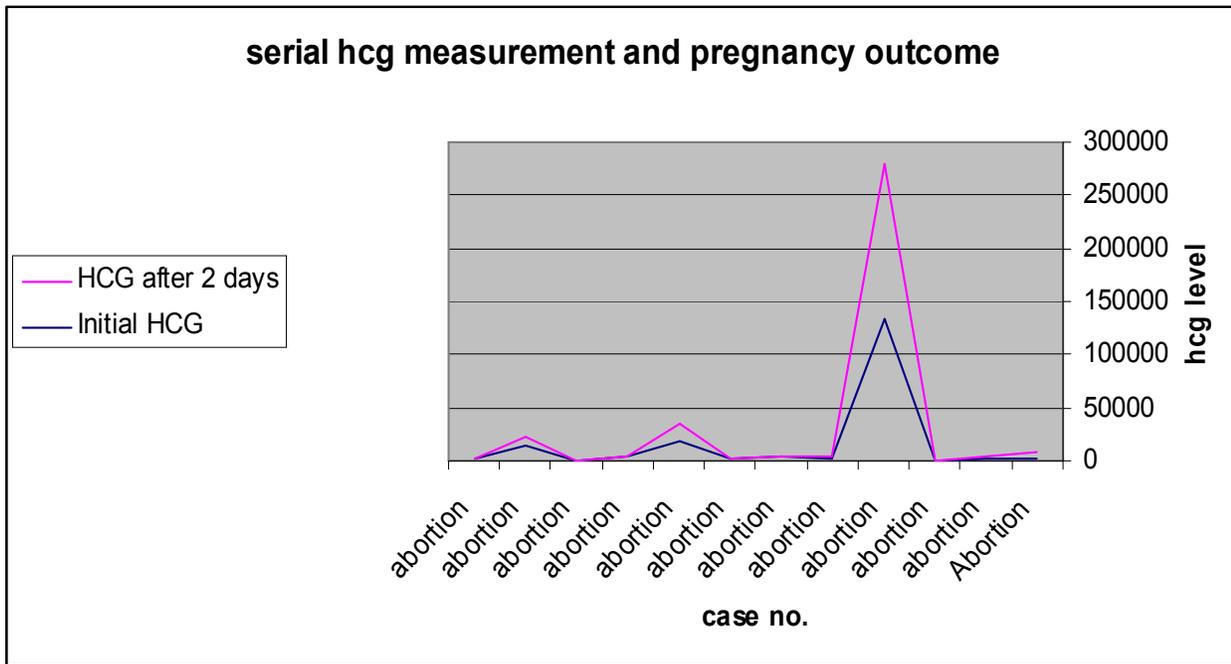
Two women out of 39 (5%) in term group had increment in hCG less than 50% after 2-3 days .The remaining 37 women (94%) the increment was more than 60% ; which is highly significant(p-value less than 0.001).

Eight out of 11 patients with spontaneous abortions(72%)had decline in hCG level after 2-3 days ;and only three women (27%) had

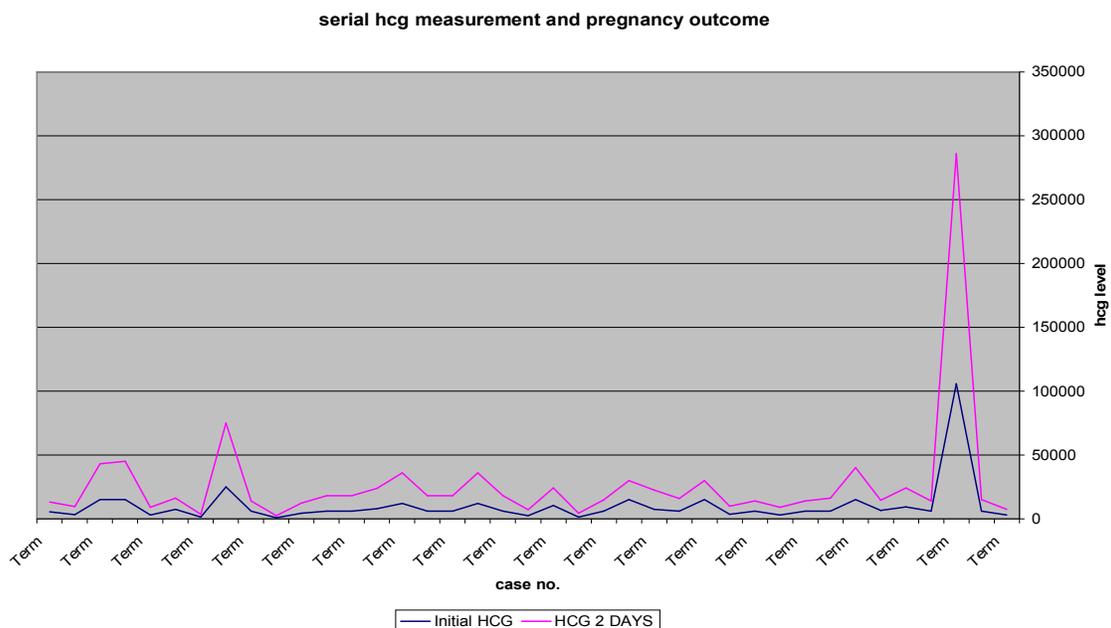
rising in hCG level which is statically significant (p value less than 0.001).

The decrement was between 50-70% in 4women (36%); between71-90% in 2 women (18%); and more than 90% in 2 women (18%). While there was no hCG decrement in term group.

Graph.1

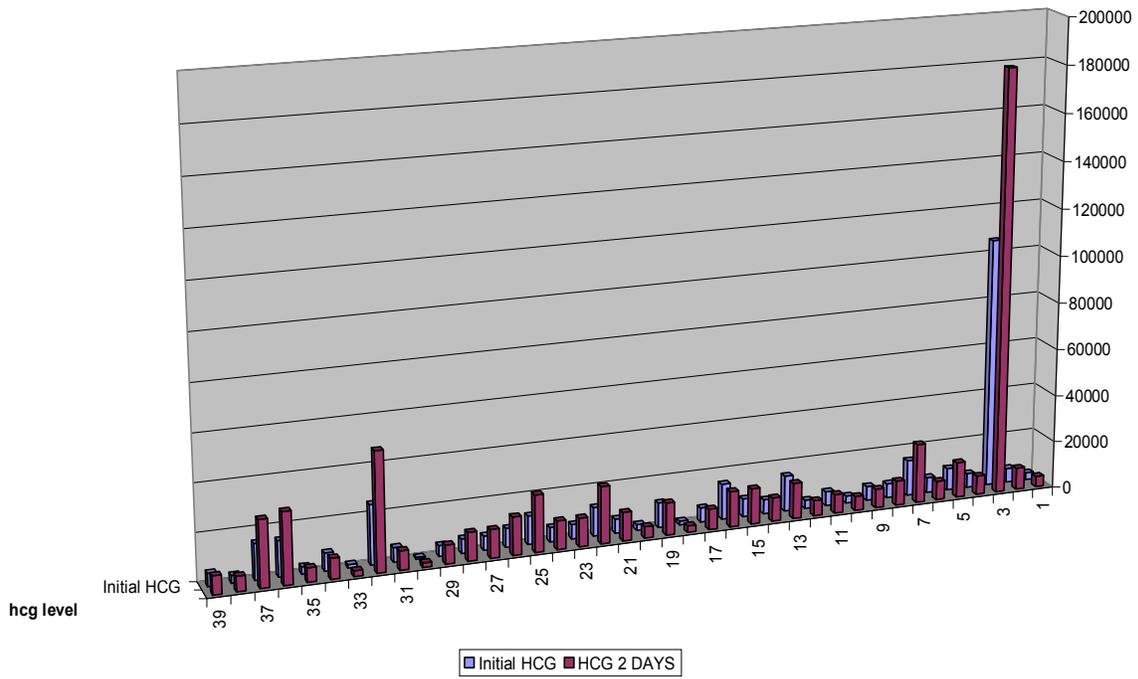


Graph.2



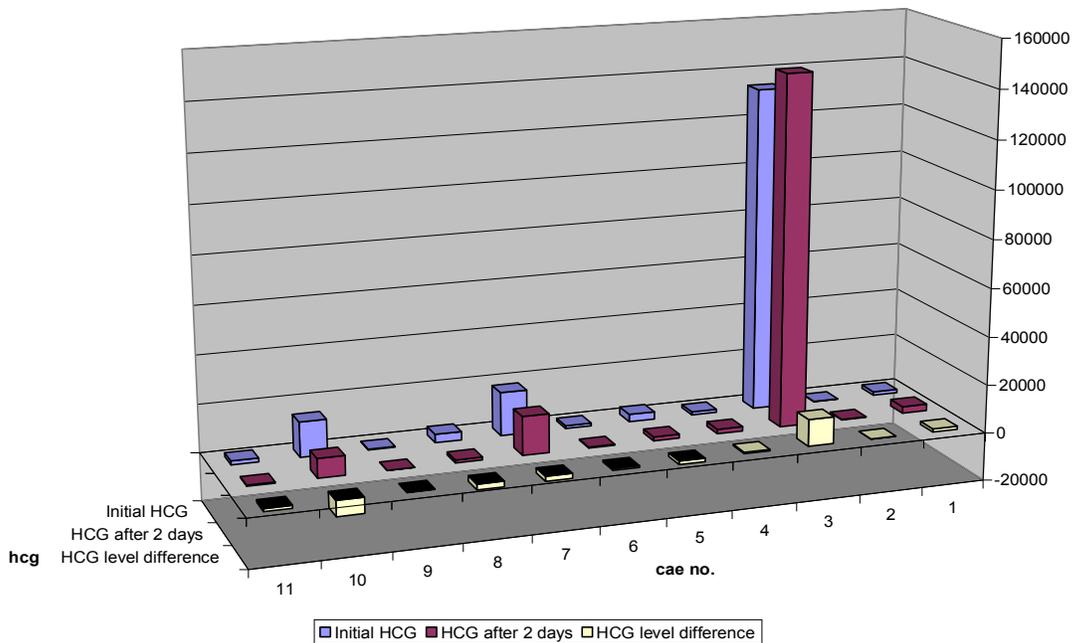
Graph.3

serial hcg measurement in successful pregnancy



Graph.4

hcg difference in failing pregnancy



Discussion

hCG is a glycoprotein produced by the trophoblast of the developing placenta .It is detectable in maternal serum within a few days of implantation. There is wide variability in hCG levels at any given point during pregnancy & there is an increasing doubling time as pregnancy progresses.⁽⁴⁰⁻⁴⁶⁾

A slower rate of rise or a drop in hCG levels during the first 8 to 10 weeks of pregnancy represents death of trophoblastic tissue & can indicate ectopic or non viable intrauterine pregnancy.⁽⁴⁷⁾

Quantitative determinations of hCG are used to predict complications especially in early pregnancy, e.g. pregnancy loss & ectopic pregnancy.

About 20-30% of all pregnancies end in early pregnancy loss (EPL) which often takes place before the pregnancy is clinically recognized.⁽⁴⁸⁾

EPL , which also is called" biochemical pregnancy "is now well recognized (Walker et al. ,1988).This condition is associated with lower than expected hCG levels , & assay of hCG in serum is used to identify it especially in connection with ART.⁽⁴⁹⁾

Studies in viable intrauterine pregnancies have demonstrated that in 85% of these gestations the Beta –hCG concentration raises by at least 66% every 48 hours during the first 40 days of pregnancy; in only 15% of viable pregnancies the rate of rise is less than threshold.

The previous data that normal pregnancies increase by 66% every 48 hours was based on studies of 29 & 36 patients.^(22, 23)

More recent data from 287 patients showed the slowest or minimal rise for a normal viable intrauterine pregnancy was 24% at 1 day & 53% at 2 days (Barnhart).

This is in agreement with results of our study in which the minimal rise was 58%.

The Barnhart et al. data re-define the slowest rise in serial hCG values for a potentially viable gestation & will aid in distinguishing a viable early pregnancy from a miscarriage or ectopic pregnancy .The minimal rise in serial hCG values for women with a viable intrauterine pregnancy is "slower "than previously reported , suggesting that

intervention to diagnosis & treat an abnormal gestation should be more conservative .The use of the more conservative data on hCG rise will hopefully lead to less need for invasive procedures & /or unnecessary use of methotrexate.^(50,51)

However, in clinical practice, a more conservative cutoff of 35% has been suggested to minimize the potential risk of terminating a wanted pregnancy.⁽⁵²⁾

Serum hCG curves for expected HCG declines in spontaneous miscarriages have also been described.⁽⁵³⁾

In a study done by Barnhart et al. in 2004 found, the rate of decline in hCG varies with the initial hCG level. In patients with presenting hCG level more than 2000 miu/ml experienced a mean reduction of 74% in 2 days (in our study decline was 74% in 2 days); whereas those with initial hCG level less than 2000 miu/ml had 71% mean decline in 2 days (this is in agreement with results of our study the decline was 70%).^(50, 51)

In addition, the change in serum HCG level over 48 hours has also been referred to as the hCG ratio (hCG 48 h: hCG 0 h).

An hCG ratio of less than 0.87 (or an hCG decrease more than 13%) has been demonstrated to have a sensitivity of a 92.7% and a specificity of 96.7% for the prediction of a failing pregnancy.⁽⁵⁴⁾

Braunstentn et al. examined the prospective value of serial hCG determinations, and correctly predicted 88.9% of the abortions, and all of the ectopic pregnancies from normal material.⁽⁵⁵⁾

Tong et al. conclude that low hCG levels in very early pregnancy are associated with an increased risk of miscarriage, adding; the mechanisms underlying late first –trimester & second –trimester miscarriages may have begun as early as the first week of implantation.⁽⁵⁶⁾

The use of both serum beta hCG levels & ultra sonograms helps to eliminate the uncertainty.

Between 6-9 weeks, the use of beta hCG can be helpful with an inconclusive ultra sonogram & can predict abortion in 95% of cases.^(57, 58, 59)

Jouppil et al. reported, however, that the beta –hCG level & gestation sac diameter were

usually normal between 6&9 weeks of gestation, even in pregnancies that later aborted.⁽⁶⁰⁾

Nyberg et al. concluded that when ultrasound findings were uncertain, a disproportionately low beta-hCG level is supportive evidence for an abnormal pregnancy, & serial beta-hCG levels complement ultrasound predictions (^{61, 62, 63, 64})

In our study we found the age is an independent risk factor for abortion. Women between 20-24 years had the lowest risk of spontaneous abortion, while those under 20 years had the highest risk.

These findings are consistent with reports demonstrating a higher risk of spontaneous abortion in teenage pregnancies.⁽⁶⁵⁾

Women with vaginal bleeding (mild-moderate) also had increased risk of spontaneous abortion. According to other studies, 92% of abortions are accompanied by vaginal bleeding (in our study 81% of women who abort had vaginal bleeding).⁽⁶⁶⁾

Several studies have found that previous abortion is a strong risk factor for spontaneous abortion. However; the risk seems to be negligible with a history of only one prior abortion. Although studies have reported a risk 2.3 to 8.1 times higher after two or three or more abortions.^(67, 68, 69)

In our study women with three abortions were more likely to have spontaneous abortions than others.

Recommendations

1. In high risk pregnancies, including those in danger of spontaneous abortion, or women with recurrent abortions; the success of pregnancy can be predicted by performing sequential BhCG measurements.

2. A larger study in the future involving larger number of patients & assessed by other hormones such as progesterone, & Estriol, to assess the prognosis of their pregnancies.

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