Original Article

Beta human chorionic gonadotropin: A prognostic marker of in vitro fertilisation outcome

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Abstract

Background: Pregnancies occurring post assisted reproductive program are at higher risk of adverse obstetrical outcome. Serum β HCG is the first confirmatory test for establishing a pregnancy. Present study has been undertaken to evaluate the predictive value of β HCG level at 16th day post embryo transfer to predict pregnancy outcome in an ART cycle. **Method:** 1722 women with initial β HCG value greater than 50mIU/ml post embryo transfer were enrolled in the study. β HCG test was done 16 days after embryo transfer. The period of study was from 01 Jan 2013 to 31 Dec 2015. **Results:** A significant correlation was found in β HCG values between viable and non-viable pregnancies. More than 90% of multiple pregnancies occurred when β HCG ≥ 550 mIU/ml. Median value of β HCG for Frozen Embryo Transfer (FET) and In Vitro Fertilization (IVF) remains almost similar but was high for oocyte / embryo donor cycle. However, the p value was not statistically significant. β HCG value depends on the outcome of pregnancy; the number of embryos transferred did not affect β HCG value. The majority of pregnancies were associated with the frozen type of transfer. The results were statistically significant ($p < 0.01$) that implies that a better outcome can be predicted with frozen method of embryo transfer. **Conclusion:** β HCG level is a useful marker for forecasting early pregnancy well being and for predicting multiple pregnancies. The number of embryos transferred and age of patient does not alter the β HCG level.

Keywords: β HCG, Viable pregnancy, Non-viable pregnancy, Frozen embryo transfer (FET), In vitro fertilization (IVF).

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INTRODUCTION

It is very important to predict the outcome of pregnancies following an ART procedure as these pregnancies are at increased risk for an adverse outcome compared with women who conceive naturally. IVF pregnancies are also at increased risk of obstetrical interventions, such as induced labor and cesarean delivery. IVF conceptions have higher chances of having multiple gestations. Risks of multiple pregnancies include higher rates of perinatal morbidity and mortality, gestational hypertension, placental abruption, placenta previa and multi-fetal pregnancy reduction. Therefore, it is important to predict pregnancy outcome as early as possible, for refinement of monitoring and treatment for a better outcome. A gestational sac can be seen on ultrasound by 3 weeks post embryo transfer. The period between the first pregnancy test (blood/urine) and a viable pregnancy on the ultrasound is extremely stressful for the couple awaiting the result of IVF. A direct correlation has been established between the anxiety, stress and poor IVF outcome in various studies[1]. There has been ongoing effort to look into endocrine markers or hormones, which can diagnose pregnancy in earlier stage and predict its outcome.[2,3] Human Chorionic Gonadotropin is the first and commonly used marker for diagnosis of pregnancy[3]. HCG is a 237 Amino Acid A glycoprotein and has a molecular mass of 25.7 kDa[4]. It has an alpha subunit identical to thyroid stimulating hormone (TSH), luteinizing hormone (LH) and Follicle-stimulating hormone (FSH), and beta subunit that is unique to hCG.

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The alpha subunit contains 92 amino acids whereas beta subunit is 145 amino acid long. β-HCG represents the functional activity of placental trophoblastic tissue. Its best-known biologic function is the maintenance of the corpus luteum of pregnancy. It has been hypothesized that HCG may act to promote uterine vascular vasodilatation and myometrial smooth muscle relaxation.[3] Low levels of β-HCG are associated with early pregnancy loss or poor outcome. Various markers such as serum progesterone, interleukin-8, estradiol, inhibin, and specific glycoproteins of pregnancy have been thoroughly investigated to distinguish between viable and non-viable pregnancies.[2, 5-9] However, the clinical role of these markers have not been well corborated.[8,9] Therefore, a single, cost effective, and reliable laboratory test, which can predict pregnancy outcome, will be of great importance.

MATERIALS AND METHODS

This was a retrospective cohort study, in which 1722 infertile females who conceived following IVF / ICSI from 01 Jan 2013 to 31 Dec 2015 were included. The study was done at the ART Centre of tertiary care center. The purpose of this study was to determine the predictive value of serum β HCG measurement on day 16 after embryo transfer in IVF / ICSI treatment cycles for pregnancy outcome.

Procedure

The protocol used for controlled ovarian stimulation varied on the basis of physician preference, patient and clinical scenario. As part of the study center's routine protocol, embryo transfer was done on D-2 i.e. at 4 - 6 cell stage and serum quantitative β HCG concentrations were taken on day 16 after embryo transfer. Initial ultrasound was done 18 days post embryo transfer to verify intrauterine pregnancy and numbers of gestational sacs followed by a repeat scan after one week. Sonographic follow up continued till 12 weeks of gestation to see the ongoing pregnancy and to rule out missed abortion. Additional β HCG concentrations were drawn according to physician choice to follow a falling concentration or non-visualization of sac on ultrasound. An ongoing pregnancy for this study was defined as one, which progressed to at least 12 weeks gestation with the presence of fetal cardiac activity on ultrasound. Pregnancies defined as not ongoing were ectopic pregnancies and those that had falling β HCG concentrations, which ultimately became negative, an emptygestational sac on ultrasound (anembryonic) or a fetal pole with no cardiacactivity visualized (missed abortion). Multiple gestations were defined by having more than one embryo with cardiac activity. The variables that were gathered from the patients were: patient age, procedure done (IVF, donor cycle, frozen embryo transfer), β HCG level on day 16 post embryo transfer, number of embryos transferred and outcome of pregnancy. Serum β HCG level was measured with a chemiluminescent microparticle immunoassay for total β HCG. The measuring range for β HCG was 0.0-15,000 mIU/ml.

Data analysis

The descriptive statistics was done by using mean or median with standard deviation (SD) or inter quartile range (IQR) for quantitative variables and categorical variables were presented in frequencies along with respective percentages. The best cut-off for β HCG was computed by sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and Receiver Operative Characteristic (ROC) [10]. The other statistical comparison for quantitative values for two groups was done by Mann-Whitney 'U' test and that for three groups was done by Krushka-Wallies "H" test. The categorical variables were compared by Chi-square test. All statistical analyses were performed using. SPSS software (Version 22, SPSS Inc, Chicago, IL, USA). The p value less than 0.05 were considered as statistically significant.

RESULTS

The Mean ± SD age of patients was 28.95 ± 5.92 years. The Mean ± SD duration of infertility was 5.5 ± 5.02 years. 1283 (74.5%) cases were of primary infertility. Male factor infertility constituted 582 (33.8%) cases. With respect to pregnancy outcome, 1428 (82.9%) pregnancies were viable while 294 (17.1%) cases were non viable.

Table 1: Frequency of infertility type, factor, number of fetuses, and pregnancy outcome

Sr No	Variables	Frequency	Percentage
Infertility Type			
(a)	Primary	1283	74.5
(b)	Secondary	439	25.5
Infertility Factor			
(a)	Male	582	33.8
(b)	Female	463	26.9
(c)	Male/female	177	10.3
(d)	Unexplained	500	29.0

Number of foetuses			
(a)	Single	964	67.5
(b)	Twins	357	25.0
(c)	Triplets	107	7.5
Pregnancy outcome			
(a)	Viable	1428	82.9
(b)	Non viable	294	17.1

There was a significant correlation between pregnancy outcome and β HCG concentration. The best cut-off for β HCG was found at 550 mIU/ml (AUC: 83.5%, 95% CI: 80.4%-86.5%, p value < 0.001) with 84.5% of overall classification with respect to viable pregnancy as outcome. The sensitivity and specificity at this cut point was observed as 89% and 69.1% respectively. Using the ROC curve, the ability of serum β HCG concentration on day 16 after embryo transfer was relatively high in differentiating viable pregnancy versus pregnancy failure, indicating it as a good diagnostic modality.

Table 2: Comparison of serum β-HCG and age with respect to viable and non-viable pregnancies

Variables	Outcome		p value
	Viable	Non-viable	
β-HCG			
Mean ± SD	2023.2 ± 1022.6	543.5 ± 1022.6	*< 0.0001*
Median (IQR)	1000 (547.5 -2231.1)	198.9 (93.9 -510.4)	
Age (years)			
Mean ± SD	29.4 ± 4.1	29.7 ± 4.1	0.348

(SD = Standard Deviation, IQR = Inter Quartile Range)

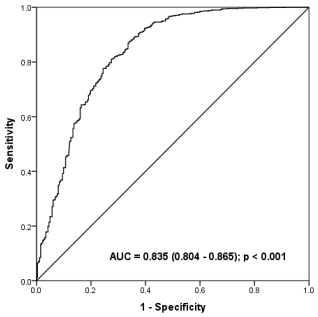

Figure 1: ROC plot for β-HCG with viable pregnancy as a reference outcome.

The median value of β HCG among the viable group was 1000 mIU/ml and among the non-viable group was 200 mIU/ml. The difference between the groups was statistically significant (p < 0.0001). However, the average maternal age was almost equal between viable and non-viable pregnancy group signifying that maternal age does not affect β HCG levels. The β HCG was categorized according to the cut-off value and cross-tabulated with type of pregnancy. The chance of multiple pregnancies was estimated by odds ratio. More than 90% of multiple pregnancies had β HCG levels ≥ 550 mIU/ml. There were 7.1 times more chances of multiple pregnancies if the β HCG level was more than 550 mIU/ml.

Table 3: Comparison of β-HCGcategory with pregnancy type

Variable	Pregnancy type		Odds ratio (95% CI.)	p value
	Single	Multiple		
β-HCGcategory				
≤ 550	327 (34.3)	32 (6.9)	1 (ref)	
≥ 550	625 (65.7)	444 (93.2)	7.1 (4.8 - 10.7)	**< 0.001**
Total	**952 (100)**	**476 (100)**		

Ref = reference

The Krushka-Wallies 'H' test was used to compare the statistical differences between the types of protocol used. The median value of β-HCG for viable pregnancies following frozen embryo transfer and IVF/ICSI remained almost similar but was high for oocyte / embryo donor cycle. However, the p value was not statistically significant.

Table 4: Effect of type of protocol on β-HCGvalues

Protocol type	Median β-HCG value among viable singleton pregnancy	Krushka-Wallies p value
FET	763.1 (418.9 - 1211.1)	
IVF	731.3 (436.4 - 1222.2)	0.053
OD / ED	873.0 (492.4 - 1777.6)	

The median concentration of β-HCG for one embryo transferred was 548mIU/ml while that for two embryos transferred was 873mIU/ml, resulting into a singleton pregnancy **(Table 5)**. This signifies that β-HCG value is determined by number of embryos implanted and not number of embryos transferred.

Table 5: Median β-HCGconcentrations in singleton live pregnancies following transfer of one versus two embryos.

Variables	Embryos transferred in singleton live pregnancies		P value
	One	Two	
β-HCG			
Mean ± SD	756.5 ± 595.9	1540.1 ± 4288.6	*0.005*
Median (IQR)	548 (352.5- 839.6)	873.4 (508.4 - 1748.4)	

DISCUSSION

Pregnancies following ART procedures are at high risk of adverse obstetrical outcome, increased operative interventions and multifetal gestation. The intervening period between serological confirmation of pregnancy and sonological confirmation of location and viability of pregnancy is extremely stressful. Hence, there are ongoing efforts to establish some marker or test, which can forecast the pregnancy outcome at a very early stage to relieve the anxiety of the couple and also the treating clinician. Researchers have done studies on various biochemical markers such as serum progesterone, interleukin- 8, estradiol, inhibin, and specific glycoproteins of pregnancy to distinguish between viable and non-viable pregnancies. There are published data on serum β-HCG as a predictor of pregnancy outcome from the western countries[11, 12] and smaller sample size (71 to 139 cases) in Indian studies.[13, 14] This is a retrospective study which has analyzed data of 1722 patients with IVF conception over a period of two years. In the present study, we correlated serum β HCG levels to number of embryos transferred, procedure carried out (FET, IVF / ICSI, donor cycle) and age of patient to predict the pregnancy outcome in patients undergoing IVF. We measured serum β HCG levels at 16 days post embryo transfer. The study found that the higher values of initial serum β HCG levels were associated with good pregnancy outcome and these pregnancies were likely to continue beyond 12 weeks. The sensitivity of serum β HCG levels to predict ongoing pregnancy was 89.0% and specificity was 69.1% when the initial value was taken as 550 mIU/ml. More than 90% of multiple pregnancies occurred in cases with β HCG levels ≥550 mIU/ml. There were 7.1 times more chances of multiple pregnancies if the β HCG level was more than 550 mIU/ml. The median value of β HCG for the viable pregnancy was 1000 mIU/ml and in the non-viable pregnancies was 200 mIU/ml. The difference between the groups was statistically significant ($p < 0.0001$). The average age of patients was equal between the viable and non-viable pregnancy group. The median value of β HCG for frozen embryo transfer and IVF/ICSI was almost similar but was high for oocyte / embryo donor cycle. However, the p value was not statistically significant. The majority of viable (86.3%) and non-viable (92%) pregnancies were associated with the frozen type of transfer. The results were statistically significant ($p < 0.01$) that complies with the fact that frozen method of transfer has a better outcome.

CONCLUSION

β HCG values have a significant predictability for viable and non-viable pregnancies with statistical significance. The study also deduces that age of patient and number of embryo transferred does not influence the β HCG value. B HCG values ≥ 550 are indicative of multiple pregnancy. The merit of the study is that it was conducted at a single IVF center with a large sample size.

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