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Original Research Article

Study of clinical significance and histopathological correlation of serum β- hCG level

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ABSTRACT

Background: β-hCG is a marker useful in diagnosis of gestational trophoblastic disease (GTD), ectopic gestation (EG), spontaneous abortion (SA) and malignant germ cell tumors (MGCT) and it is helpful to clinician as an excellent tumor marker. It is useful to monitor treatment whether tumor is responding to treatment or the disease is progressing.
Methods: β-hCG is a marker useful in diagnosis of gestational trophoblastic disease (GTD), ectopic gestation (EG), spontaneous abortion (SA) and malignant germ cell tumors (MGCT) and it is helpful to clinician as an excellent tumor marker. It is useful to monitor treatment whether tumor is responding to treatment or the disease is progressing.
Results: p value is highly significant in Gestational Trophoblastic Diseases, EG and SA, as p value is < 0.005 in all these three categories. But in case of MGCT it is 0.452 which is not significant because study group was very small and one case who developed recurrence affected the value significantly. These findings suggest that β-hCG has definitive prognostic role (p value<0.005) in GTD, EG and SA.
Conclusions: ELISA is rapid, sensitive, reliable and cost effective test for measurement of β-hCG. Pre-and post-therapeutic β-hCG serum levels seem to be useful in the therapy monitoring of trophoblastic gynaecological conditions i.e. GTD, EG and SA.

Keywords: ELISA, Gestational trophoblastic diseases, Neoplasia, Spontaneous abortion

INTRODUCTION

The tumor markers include cell surface antigens, cytoplasmic proteins, enzymes and hormones which indicate the presence of tumor. Many tumor markers are also used as immunohistochemical stains.[1] A tumor marker is measured in serum or other body fluid in the clinical laboratory, often utilizing immunoassays. β-hCG is a marker secreted from syncytiotrophoblastic cells of placenta and appear in patient's serum.[2]

The aim of the study is to study the use of serum beta-HCG level as a diagnostic and prognostic tool and to see serum beta-hCG level with histopathological correlation and as a marker to see the response of treatment in germ cell tumors, gestational trophoblastic neoplasia and ectopic gestation.[3]

METHODS

Total 100 cases were studied. These cases were patients admitted to various departments. These included patients with suspected GTD, ectopic pregnancy, spontaneous abortion, malignant mass in ovary, testes, benign germ cell tumor, various adenocarcinoma of uterus, lung, pancreas, colon, stomach and squamous cell carcinoma(SCC) of lung. Pre-operative value of β-hCG was measured in every case for diagnostic purpose. Pretreatment serial β-hCG value was measured in every case twice at the interval of two days to measure the

doubling time of β-hCG for diagnostic purpose in ectopic gestation, spontaneous abortion and normal intrauterine gestation. Pre-and post-operative values of β-hCG were measured for prognostic purpose as well as to detect recurrence or metastasis.

Procedure

Blood is collected by venipuncture. 2mL Sample was collected in sterile plain vacutte and allowed to clot and then serum was separated by centrifugation at room temperature. β-hCG is measured by ELISA method by Enzyme immunoassay for the quantitative determination of beta-hCG in human serum.

Principle of the Assay

The beta-hCG assay is based on the simultaneous capture of hCG by a monoclonal antibody immobilized on the microplate and directed against the β-hCG fraction, and another monoclonal antibody conjugated with peroxidase horseradish (HRP) and directed against the α-HCG fraction. After the incubation, the bound/free separation is performed by a simple solid-phase washing. The enzyme HRP in the bound-fraction reacts with the substrate (H2O2) and the TMB substrate and develops a blue color that changes into yellow when the stop solution (H2SO4) is added. The color intensity is proportional to the β-hCG concentration in the samples. The β-hCG concentration in the sample is calculated based on a calibration curve.

25 uL sample diluents (as blank), standards samples or controls dispensed into the assigned wells. Immediately, 100 uL of biotinylated capture antibody (blue colour) dispensed into the assigned wells. It was incubated for 120 minutes at room temperature. The incubation mixture was removed and rinse the wells five times with washing buffer (300 uL/well//each rinse). 100 uL enzyme conjugate dispensed into each well except the blank well. It was incubated for 60 minutes at room temperature. The incubation mixture was removed and washed five times with the washing buffer (300 uL/well/each rinse). The 100 uL of TMB solution dispensed into each well including the blank well. It was incubated for 30 minutes at room temperature. The reaction was stopped by adding 50 uL of stop solution to each well. Zero a microreader on the blank and the absorbance of each well was measured at 450nm**.**

RESULTS

This prospective study included 100 cases. These cases were from different wards like surgery, gynaecology, radiotherapy, etc. All the cases were studied in detail including proper history, clinical diagnosis and supportive investigations like USG finding, CT scan. Pre-Treatment value of β-hCG was measured in all cases. Histopathological confirmation for each case was done before stamping the diagnosis and all cases (except NIUG) were categorised into four main groups, i.e. GTD (Gestational trophoblastic disease), EG(ectopic gestation), NIUG (normal intrauterine gestation.) and MGCT (Malignant germ cell tumor). Clinical stage and histopathological grade were noted. Post-operative value of β-hCG were measured in 45 cases. Serial measurement of β-hCG were done in all cases of EG, SA and NIUG for determining doubling time and pattern of fall and rise in β-hCG level. Patient with raised post-operative β-hCG value were further investigated and confirmed as having recurrence or persistence by histopathological diagnosis. All the cases were above 9 years of age with most of cases being between the age of 21-40 years.[5]

For pre-treatment diagnosis, cut off value of β-hCG was >5mIU/mL. Sensitivity of β-hCG as a diagnostic measure was calculated for each β-hCG producing conditions separately and was 96%, 62.5%, 100%, and 100% for GTD, MGCT, EG and SA respectively.[6] Other rare β-hCG producing tumors and non β-hCG producing tumors were taken in other two group to determine significance of β-hCG in various adenocarcinoma and as a negative control respectively. For post-operative follow up interval taken was 45 days after surgery and after 3 cycles of radio/chemo therapy. Significance of post-operative follow up was measured using paired 't' test. P value<0.005 was considered significant and p value was <0.005 in GTD, EG and NIUG which was highly significant.

While in case of MGCT p value was 0.452 which is not significant because of small study group. β-hCG value in H. mole was from minimum 6850 mIU/mL to maximum 210500 mIU/mL. 40.9% cases shown above 1 lakh mIU/mL of β-hCG. One case of choriocarcinoma had β-hCG value 256700 mIU/mL. PSN did not expressed elevated level of β-hCG. As far as GTD is concerned, incidence was 1:120 with comparison to total deliveries was commonest in para-2 and most common symptom was bleeding per vagina (100%) followed by amenorrhoea, usually height of uterus was larger for period of gestation in complete mole and smaller for partial mole, H. mole most frequently graded into grade-I.

The rise in β- hCG for women with EG and SA was slower than the mean increase reported for a NIUG. Fall is more commonly associated with SA while rise (abnormal) is with EG. Geographical mean of β- hCG of NIUG was substantially high than mean of EG an SA. Mean increase in β- hCG was 52%, 40% and 108% in EG, SA and NIUG respectively.

DISCUSSION

Total-100 cases were studied. Preoperative value of β-hCG was measured in every case for diagnostic purpose. Pretreatment serial β-hCG value was measured in every case twice at the interval of two days to measure the doubling time of β-hCG for diagnostic purpose in ectopic

gestation, spontaneous abortion and normal intrauterine gestation. Pre-and post-operative values of β-hCG were measured for prognostic purpose as well as to detect recurrence or metastasis.[7] Pre-operative β-hCG value measured of every patient before surgery or before starting chemo/radiotherapy. Post-operative values of 5 cases (out of 8 cases) of malignant germ cell tumor were measured after one and half month post operatively and after 3 cycles of radiotherapy. Postoperative β-hCG value was measured in 14 cases of ectopic pregnancy, 6 cases of spontaneous abortion and 20 cases of GTD to detect the prognosis of treatment. β-hCG non-related malignancy, cases which was not traced or died during treatment were not included in post-operative follow up. One patient died during operation due to disseminated disease and cardiac complications, was also not included in post-operative follow up.

Table 1: B - HCG Level (in mIU/ml) (pre-treatment).

Diagnosis	<05	>05	Total
Gestational trophoblast Diseases	01	24	25
Malignant germ cell tumor	03	05	08
Ectopic gestation	00	30	30
Spontaneous abortion	00	08	08
Normal intrauterine gestation	00	10	10
Mature teratoma	03	00	03
Struma ovarii	02	00	02
Endometrial adenocarcinoma	01	01	02
Lung adenocarcinoma	01	00	01
Gastric adenocarcinoma	02	00	02
Pancreatic adenocarcinoma	01	00	01
Colonic adenocarcinoma	02	00	02
Serous cystadenocarcinoma	01	00	01
Mucinous cystadenocarcinoma	01	00	01
Squamous cell carcinoma	01	00	01
Others	03	00	03

Table 2: HCG level (post treatment Follow-up).

Condition	Decreased	Increased	Total
GTD	20	00	20
Ectopic pregnancy	14	00	14
Spontaneous abortion	06	00	06
Malignant germ cell tumor	04	01	05

As previously mentioned, due lack of patient's interest after treatment and getting benefits from treatment, many patients does not visit back for post treatment check-up, due to some migratory patients and due to death or some other reasons, it was not possible to evaluate the effect of treatment in every patient by measuring post-treatment β-hCG value. 8 Out of 87 cases of β-hCG producing conditions, only 45 cases were included in post-Treatment measurement of β-hCG value for prognostic purpose. 10 cases of NIUG were also not included in post-treatment measurement of β-hCG value. All case (20 cases) of GTD which were tested post-treatment for β-hCG value showed significant decrease in level except two cases of complete H. mole who expressed fall but it was not significant and β-hCG value was higher than 20,000 after 1.5 month of post treatment(evacuation) so as per criteria these cases were included in persistent GTD.[9]

Table 3: P Value.

	β-HCG Level	Mean	N	P* Value
Ectopic gestation	Pre-op.	4035	14	<0.0001
	Post- op.	8.236	14	
Spontaneous abortion	Pre -op.	3179	06	0.006
	Post- op.	8.183	06	
GTD	Pre -op.	97808	20	< 0.0001
	Post- op.	2847	20	
MGCT	Pre- op.	33687	05	0.452
	Post- op.	15713	05	

Both cases of persistent GTD were having β-hCG value more than 1.5 lac mIU/ml initially and after Treatment it was 31200 mIU/ml and 25365 mIU/ml at first measurement. As all case of GTD were in stage- I, so rest of cases responded well to conventional modalities of treatment, like suction and evacuation for HM, and hysterectomy for others with prophylactic chemotherapy. Two cases of persistent GTD were evaluated further for β-hCG level for twice at the interval of two weeks but no significant decline were observed and lastly these two cases were treated successfully with Methotrexate (chemotherapy).

Similarly, all cases of ectopic pregnancy (14) and spontaneous abortion (06) which were included in post-treatment monitoring of β-hCG value shown reduced serum level. Amongst 8 cases of MGCT, 1 patient died in follow-up, and all 5 cases who shown elevated β-hCG were monitored post-treatment for prognostic purpose and effectiveness of therapy or surgery. 4 case showed decreased value of β-hCG and 1 cases showed increased serum β-hCG value because patient developed wide spread dissemination with recurrence.

This decrease in β-hCG value directly correlated with tumor removal or decrease in tumor size. Patients who had undergone surgery with total removal of tumor had β-hCG value almost near normal after one and half month. In patients with unresectable mass taking radiotherapy had decrease in β-hCG value which was correlated with decrease in size of tumor.

Two patients who developed recurrence had raised β-hCG value than their preoperative measurement. β-hCG value was raised earlier than radiographic findings and

even in asymptomatic patients. These cases were confirmed in histopathology for recurrence.

β-hCG is useful serum marker in various conditions associated with trophoblastic proliferation e.g. GTD, MGCT, ectopic gestation, spontaneous abortion and normal intrauterine gestation.[10] We used 5mIU/mL as cut off (reference) value of β-hCG which was correlated with various studies. Almost all cases (24 cases out of 25), that is 96% of GTD show markedly elevated level of β-hCG, so here sensitivity is 96% for GD.[11]

Table 4: Serial B -HCG value (at interval of 2 days).

Condition	Increased	Mean increased (mIU/mL)	Decreased	Mean decrease (mIU/mL)
Ectopic gestation	25	260	05	324
Spontaneous abortion	03	226	05	380
Normal intrauterine gestation	10	12560	00	00
Total	38		10	

Table 5: Overall sensitivity.

Diagnosis	NO.	<05mIU/ml	>05mIU /ml	Sensitivity (%)
GTD	25	01	24	96
Malignant germ cell tumor	08	03	05	62.5
Ectopic gestation	30	00	30	100
Spontaneous abortion	08	00	08	100
Normal intrauterine gestation	10	00	10	100
Benign germ cell tumor	05	05	00	00
Endometrial adenocarcinoma	02	01	01	50
Lung adenocarcinoma	01	01	00	00
Gastric adenocarcinoma	02	02	00	00
Pancreatic adenocarcinoma	01	01	00	00
Colonic adenocarcinoma	02	02	00	00
Serous cystadenocarcinoma	01	01	00	00
Mucinous cystadenocarcinoma	01	01	00	00
Squamous cell carcinoma	01	01	00	00
Others	03	03	00	00
Total	100	21	79	00

Similarly, all cases of EG (ectopic gestation), SA (spontaneous abortion) and NIUG (normal intrauterine gestation) shown significantly increased value of serum β-hCG, thus sensitivity for all these conditions are 100%. As far as MGCT are concerned, out of total 8 reported cases, 5 cases shown increased level of β-hCG and all these cases were having choriocarcinomatous, seminomatous or embryonal components.[12]

Benign germ cell tumors did not show any increase in β-hCG level above the reference level. Among 10 cases of various adenocarcinoma, only one case of poorly differentiated endometrial carcinoma shown mild rise in β-hCG level which was just 140 mIU/mL, could be due to trophoblastic differentiation, but on sectioning, we did not found any trophoblastic differentiation. Rest of all other cases, including SCC of lung were negative for β-hCG rise above the reference level.

Sensitivity

It detects true positivity. Sensitivity = (true positive/ true positive + false negative) x 100

Sensitivity for GTD = (24/ 24+1) X 100 = 96 %

Sensitivity for MGCT = (5/ 5+3) X 100 = 62.5 %

As all the cases of EG, SA, and NIUG presented with increased level of β-hCG, sensitivity for these conditions in this study is 100 %.

CONCLUSION

- Serum β-hCG is a important diagnostic measure for gestational trophoblastic disease (GTD), ectopic gestation (EG), spontaneous abortion (SA) as well as

upto few extent also for malignant germ cell tumor (MGCT).[13-16]

- ELISA is rapid, sensitive, reliable and cost effective test for measurement of β-hCG. All the cases of Ectopic Gestation, Spontaneous Abortion, and Normal Intrauterine Gestation presented with increased level of β-hCG, sensitivity for these conditions in this study is 100 %.
- Persistence or elevation after treatment indicates residual disease, recurrence and/or dissemination.

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