COMPARATIVE STUDY OF DIFFERENTIAL EXPRESSION OF CYCLIN-E IN NORMAL PLACENTA, HYDROPIC ABORTUS AND GESTATIONAL TROPHOBLASTIC DISEASE

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ABSTRACT

BACKGROUND

Gestational Trophoblastic Disease (GTD) encompasses a heterogeneous group of lesions, characterised by proliferation of pregnancy associated trophoblastic tissue. According to WHO, GTD was classified into three subgroups, such as- Neoplasm, non-neoplastic lesions and molar pregnancies. Clinical presentation, prognosis and management of each subgroups varies from one another. So, it is necessary to perfectly identify each subgroup among themselves and from their non-GTD mimickers, which is difficult to achieve based on histopathology alone. So, we employed Immuno-Histochemical (IHC) staining for Cyclin-E, that complements the histological diagnosis and hence in proper management of cases.

MATERIALS AND METHODS

This is a prospective study conducted in S.C.B. Medical College and Hospital in Odisha. Placentas of abortion cases, premature delivery as well as normal delivery were taken for study along clinical serological and radiological finding. The samples were subjected to histopathological examination and IHC analysis using Cycin-E as immunomarker.

RESULTS

This study includes 52 cases, out of which 38 were of GTD, 11 cases were of placenta of different gestational age those had no signs of molar changes or GTD, and 3 cases were of hydropic abortus. All trimester placenta showed a moderate score for cyclin-E, whereas maximum hydropic abortion sample not stained for cyclin-E (66.67%-score 0). 50% of CHM cases had score 3+ and maximum PHM cases had score 2+. Choriocarcinoma cases showed high score and also same for samples with exaggerated placental site.

CONCLUSION

IHC using cyclin-E as an immunomarker, has role as an adjunct to histopathological diagnosis in cases of GTD. Also, it helps in differentiating between the subgroups of GTD. However only one marker is not sufficient for definite diagnosis. So, more immunomarkers should be used in studies including a greater number of cases for a definite conclusion.

KEYWORDS

GTD, IHC, Cyclin-E.

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BACKGROUND

Gestational trophoblastic disease (GTD) encompasses a heterogeneous group of interrelated lesions, characterised by proliferation of pregnancy associated trophoblastic tissue.[1,2] GTD is mainly divided in three groups namely gestational trophoblastic neoplasm (GTN), Non-neoplastic lesions and Molar pregnancies. GTN includes choriocarcinoma (CC), placental site trophoblastic tumor (PSTT), epithelioid trophoblastic tumor (ETT). Molar pregnancy again has three subgroups such as, Complete Hydatidiform Mole (CHM), Partial Hydatidiform Mole (PHM) and Invasive mole (IHM). Non-neoplastic lesion has two entities such as, exaggerated placental site reaction (EPS) and placental site nodules or plaque. The incidence of hydatidiform mole ranges from 23 to 1299 cases per 100,000 pregnancies. The incidence of malignant forms of GTD is much lower constituting only about 10% of the incidence of hydatidiform mole.[3]

The reported incidence of GTD from Asia and South America is significantly higher than the reported incidence of GTD from Europe and North America.[4] per 1000 live births and one per 967 pregnancies.[5] Several potential risk factor have been attributed to development of complete hydatidiform mole and the established risk factors that have been emerged are maternal age and prior molar pregnancy.[6]

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The extremes of maternal age i.e. age less than 15 years and more than 45 years has a significant risk for GTD. Following one molar pregnancy, the risk of molar pregnancy was about 1% & after two molar pregnancy it rises to 23.1%.[7] Several risk factor such as prior miscarriage, blood group, OCP, socioeconomic status, family history have been attributed to GTD.[8,9,10] Despite well described histological criteria the distinction between spontaneous abortion from hydatidiform mole and CHM from PHM remain a problem due to inter observer variability.[11,12] Therefore IHC as an adjunct to histopathology using cyclin-E helps differentiating these entities and provide insight into behaviour of GTD.

Cyclin-E is normally expressed strongly by cytotrophoblast, implantation site and chorionic type intermediate trophoblast and also weakly by trophoblastic column intermediate trophoblast. Syncytiotrophoblast does not express cyclin-E. Progression of cell cycle is regulated by the coordinated interaction of various proteins and cyclin-E is one of them. It forms a complex with Cdk-2 and controls the G_1-S transition by inactivating retinoblastoma protein (pRb), which is a cell cycle inhibitor. Thus cyclin-E promotes cell cycle and cell multiplication. Many studies have proved significant role of cyclin-E in diagnosis of various human cancers, but limited studies have recorded its role in GTD. Therefore, this study was undertaken to analyse role of cyclin-E in GTD along with their clinical parameters.

Aims and Objectives

To find the expression of Cyclin-E in normal placenta, hydropic abortous and GTD and to compare the differential expression of the immunomarker in above mentioned cases.

MATERIALS AND METHODS

The present study is a prospective study carried in S.C.B. Medical College and Hospital in Odisha for a period of two years i.e. from January 2016 to December 2017, in collaboration with Department of Obstetrics & Gynaecology. This study has been approved by Institutional Ethical Committee.

Clinical and radiological parameter including age, parity, presenting symptoms, gestational age at presentation, were analysed in each cases.

Inclusion Criteria

Cases histologically diagnosed to have GTD, hydropic abortion and normal placenta.

Exclusion Criteria

Non-gestational choriocarcinoma.

Scoring System

For the immunomarker cyclin-E, we considered homogeneous nuclear staining as positive. Occasional cytoplasmic staining was considered non-specific and not included in evaluation. We used a quantitative scoring system based on the number of trophoblasts having positive nuclear staining among 100 trophoblastic cells.

Score 0 ----negative;
Score 1----1-10% trophoblast stained positive;
Score 2----11-25%;
Score-3----26-50%;
Score 4---- >50%

The ultimate score attributed to the lesion was the highest score found in several regions analysed. The respective H&E slides were examined by two pathologists independently without knowing the previous H&E diagnosis or IHC pattern. Pearson Chi-square test was applied to calculate p value and result was considered statistically significant if p value was less than 0.05.

RESULTS

This study includes 52 cases, out of which 38 were of GTD, 11 cases are of placenta of different gestational age those had no signs of molar changes or GTD, and 3 cases were of hydropic abortus. Among 38 cases of GTD, 16 cases were of CHM (42.1%), which was the most common type of GTD we found in our study period. 26.31% were of PHM cases, only one cases (2.6%) was of IHM, 15.79% of cases of choriocarcinoma, 13.15% of EPS and rest CHM cases were the subgroups of GTD in our study population. (Table-1)

In the present study 20 out of 38 cases of GTD (52.63%) belongs to age group 21-30 years followed by 9(23.68%) were in age group 31-40 yrs. (Table-2) 71.05% of the patients were multipara. Rest of cases were paucipara. (Table-2)

Most of the patients of GTD presented in their 1st trimester of pregnancy (78.95%) and only 21.05% of cases were presented in 2nd trimester.

In all trimester of normal placenta, Cyclin-E differential expression was nearly similar i.e. a score 2+. (Figure-1) In case of hydropic abortus only 33.33% case showed weak expression i.e. score of 1+. (Figure-2) Rest hydropic abortion sample were negative for IHC with cyclin-E. CHM cases showed stronger IHC score for cyclin E (50% -3+ & 37.5% - 4+) (Figure-3) in comparison to PHM (80% - 2+) (Figure-4). Maximum number cases of choriocarcinoma (Figure-6) & EPS (Figure-5) showed strong positivity for cyclin E with score-4+.

DISCUSSION

GTD are a spectrum of abnormal gestation and neoplasm arising from villous or extra villous trophoblast associated with pregnancy. Trophoblastic cells have been divided into three type such as, cytotrophoblast, syncytiotrophoblast and intermediate trophoblast (IT) and it is essential to distinguish them because, in different type of GTD the constituting cells are different and show different immunophenotyping.[13,14]

In the present study, cyclin-E, which has role in cell cycle, used as a immunomarker to establish its role in differentiating between various subgroups of GTD and closely resembling non-trophoblastic lesions.

Most of the patients in the study were in between 21-30 years, 23.68% patient were in 31-40 years. 18.4% cases

belonged to age group <20 years. Least number of cases i.e. only 5.26% were >40 years. Mean age of presentation was 26.22 ± 6.98 years with youngest being 18 years and oldest 43 years. This result was comparable to result obtained by Pariyar J et al (2013), who reported 51.2% cases belonging to 21-30 years age group. (Table-2)

As regards to parity, highest incidence was recorded in para 2, in contrast to Ben Temime Riadh et al in 2009 reported highest incidence in paucipara.[15] but Parivar J et al (2013)[16] reported higher incidence among primi gravida.

Our study had shown that, 78.95% patients of GTD were in first trimesters of pregnancy at the time of diagnosis, which was similar to the study done by Nirmala CK et al(2013)[17] and Simms- Stewart D et al (2013)[18] reported the median gestational age by date as 12 wks.

In the present work Cyclin E was uniformly expressed at moderate levels (2+) in different stages of normal placental development, where basal cytotrophoblasts nuclei surrounding the villi have taken the stain; this result was similar to study done by Olvera M et al. (2001)[19]

Out of all cases of CHM 50% showed Cyclin E IHC score 3+ and 37.5% CHM had a score 4+, with the rest 12.5% had 2+ score which was significantly different from PHM cases which had a score 2+ in 80% of cases. Choriocarcinoma, had a Cyclin E score 4+ in (83.3%) cases (Table -3). In our study Cyclin E over expression was observed in GTD in comparison to normal placenta and hydropic placenta with a p value of 0.001, which is statistically significant, similar work of Kim and colleagues. (2000).[20] But Olvera M et al. (2001)[19] found that partial moles did not exhibit the same level of cyclin E overexpression found in complete moles and choriocarcinoma, same as our study (Table-3).

EPS cases showed a high cyclin-E score, i.e. 80% cases had score 4+ and 20% had 3+, which is comparable to the results obtained for CC cases. But EPS is a non-neoplastic lesion whereas CC is a malignant lesion with completely different biological behaviour. So only cyclin-E can't help to differentiating these two entities

CONCLUSION

IHC using cyclin-E as an immunomarker, has role as an adjunct to histopathological diagnosis in cases of GTD. Higher levels of cyclin-E expression in complete moles provides useful adjunct to separate it from partial mole. A previous study states that cyclin-E over accumulation might play a role in malignant transformation of trophoblast.[20] Our data supports this interpretation.

However other markers such as $p27^{kip1}$, Ki-67, Cdk-2, E2F-1 can also be used to supplement the findings and results.

Type of Cases		**No. of Cases**	**Percentage of GTD**
Normal Placenta		11	
Hydropic Abortus		3	
GTD (n=38)	CHM	16	42.10
	PHM	10	26.31
	IHM	1	2.6
	Choriocarcinoma	6	15.79
	EPS	5	38.15
	Total	**38**	**100**
Total no. of Cases		**52**	

Table 1. Distributions of Cases of this Study (Total Cases 52, GTD= 38)

		CHM	**PHM**	**IHM**	**CC**	**EPS**	**Total**	**Percentage**
Age in Years	<20	2	2	0	2	1	7	18.4
	21-30	10	5	1	3	1	20	52.63
	31-40	3	3	0	1	2	9	23.68
	41-50	1	0	0	0	1	2	5.26
Parity	P0	5	3	0	2	1	11	28.95
	P1-P4	11	7	1	4	4	27	71.05
	P5	0	0	0	0	0	0	0
Gestational Age at Presentation	1st Trimester	14	8	1	4	3	30	78.95
	2nd Trimester	2	2	0	2	2	8	21.05
	3rd Trimester	0	0	0	0	0	0	0
		16	10	1	6	5		

Table 2. Clinical Data of GTD Cases (n =38)

Type of Cases		0	1+	2+	3+	4+
Normal Placenta	Early	0	5 (83.33%)	1	0	0
	Mid	0	3 (100%)	0	0	0
	Late	0	2 (100%)	0	0	0
Hydropic abortus		2	1	0	0	0
CHM		0	0	2 (12.5%)	8 (50%)	6 (37.5%)
PHM		0	1	8 (80%)	1	0
IHM		0	0	0	1	0
Choriocarcinoma		0	0	0	1	5 (83.3%)
EPS		0	0	0	1	4 (80%)

Table 3. Expression of Cyclin–E in Gestational Trophoblastic Disease, Normal Placenta, and Hydropic Abortus

0- no staining; 1+, 1-10% positive cells; 2+, 11-25% positive cells; 3+, 26-50% positive cells; 4+, >50% positive cells.

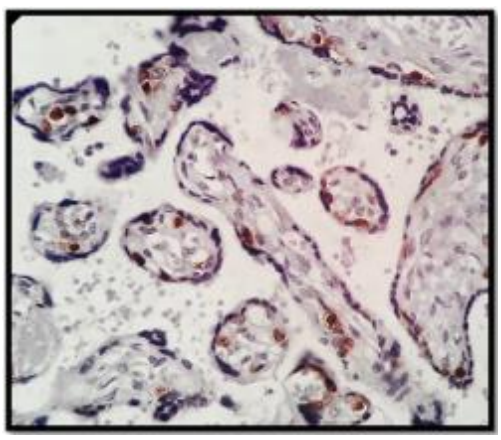

Figure 1. Picture Showing IHC for Cyclin-E, Score 2+ in Normal Placenta (400X)

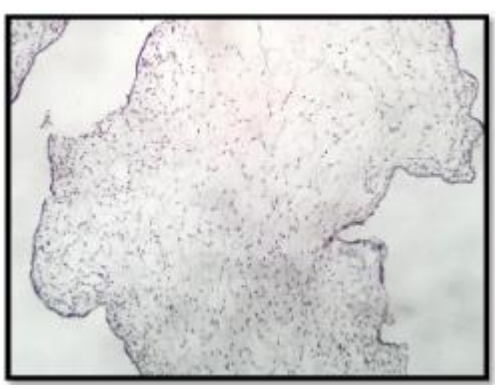

Figure 2. Picture Showing IHC for Cyclin-E, Score 1+ in Hydropic Abortus (100X)

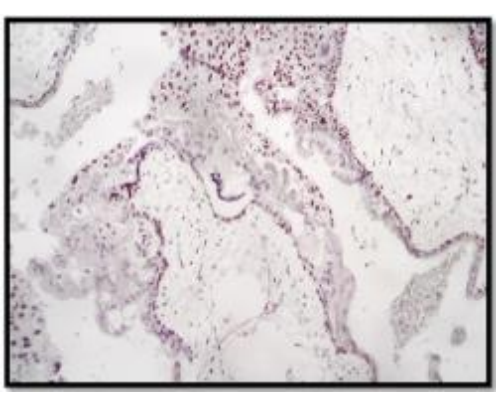

Figure 3. Picture Showing IHC for Cyclin-E, Score 3+ in CHM (100X)

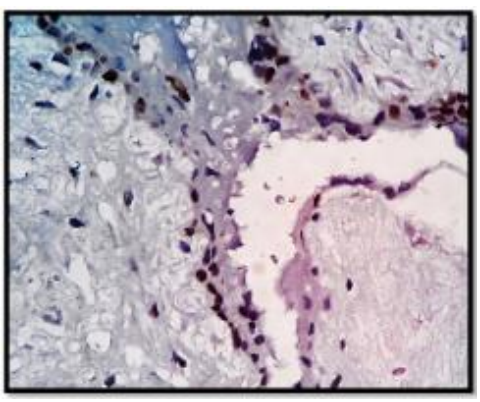

Figure 4. Picture Showing IHC for Cyclin-E, Score 2+ in PHM (400X)

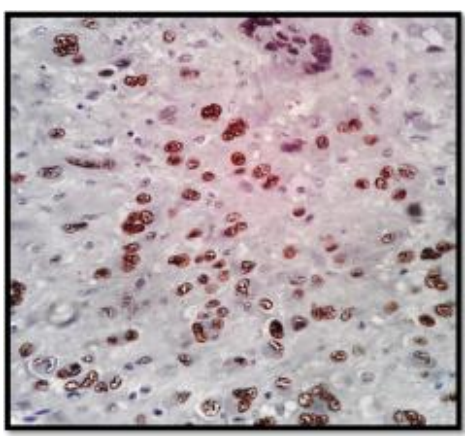

Figure 5. Picture Showing IHC for Cyclin-E, Score 4+ in EPS (400X)

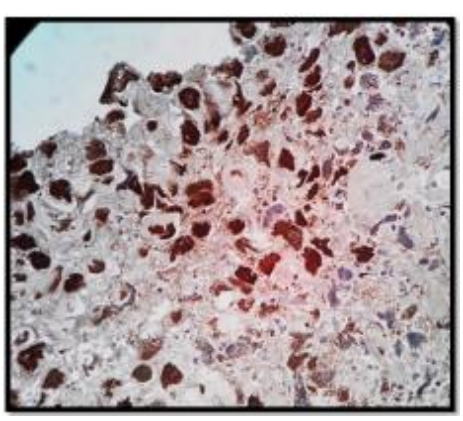

Figure 6. Picture Showing IHC for Cyclin-E, Score 4+ in Choriocarcinoma (400X)

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