Immunohistochemical Expression of Her-2/Neu Oncogene in Endometrial Carcinoma and its relation with Histopathological Variables

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Abstract :

Objective and background: Her-2/neu oncogene amplification has been implicated in the development and progression of many human cancers like breast cancer, pancreatic cancer and ovarian cancers and it is the target for therapeutic intervention with the help of humanized monoclonal antibody to Her-2/neu (Herceptin). Smaller studies suggest that Her-2/neu oncogene may be involved in the tumerogenesis of endometrial adenocarcinoma and targeting Her-2/neu may be beneficial for a selected group of patient showing Her-2/neu overexpression. The aim of this study was to evaluate Immunohistochemical (IHC) expression of Her-2/neu oncogene in endometrial carcinoma (EC) and its correlation with Histopathological features in order to define its potential prognostic value in Endometrial Carcinomas.

Patients and methods: 42 Cases of Endometrial Carcinoma with stage I-IV were enrolled into this study. Demographic, clinical and pathologic information was obtained and recorded. Her-2/neu expression was evaluated by immunohistochemistry (IHC) on paraffin embedded tissue sections with Her-2/neu antibody. Overexpression was defined as complete, membrane, moderate to strong intensity staining in greater than 10% of cells.

Results: Positive HER-2/neu expression is seen in 54.76% cases of endometrial carcinoma. Her-2/neu overexpression was associated in patients with advanced stage ($p<0.01$), High Grade ($p<0.01$), myometrial invasion ($p<0.05$), cervical invasion ($p<0.01$), and lymph node metastasis ($p<0.01$) but not associated with histological type of the Carcinoma ($p>0.05$).

Conclusion: Our study demonstrated strong HER-2/neu overexpression in considerable proportion of the patients with Endometrial Carcinoma, higher rate HER-2/neu overexpression in patients with high grade, advanced stage, myometrial invasion, cervical invasion and lymph node metastasis, indicating Her-2/neu overexpression is related to most of the prognostic factors of Endometrial carcinoma thus suggesting HER-2/neu IHC can be incorporated into the criteria for determination of tumor aggressiveness as a prognostic marker and the use of anti-Her-2/neu (Herceptin) therapy in Patients harbouring Endometrial carcinoma with proven amplification or strong Her-2/neu overexpression.

Key words: Endometrial Carcinoma, Immunohistochemistry, Her-2/neu oncogene, Prognostic factor.

I. INTRODUCTION

Endometrial carcinoma (EC) is one of the most common malignant tumour of the female genital system and is the third most common gynaecological malignancy in South-Eastern Asia following cervical and ovarian carcinomas.[1] The majority of patients have favourable outcomes as they are diagnosed at an early stage.[2] The overall 5-year survival rate for EC is approximately 80%.[3] The disease is rare before the age of 40 and 80 % of the patients are in postmenopausal age at the time of diagnosis. Majority of the carcinomas occurs sporadic with 5–10% having a hereditary basis.[4] The major risk factors are related to prolonged endometrial stimulation such as unopposed Oestrogen therapy, polycystic ovarian disease and oestrogen-producing tumours, other risk factors include obesity, age, nulliparity, diabetes, hypertension and breast cancer patients treated with tamoxifen therapy.[5]

The endometrium shows tightly controlled hormone-dependent variation during the each menstrual cycle which is disturbed during endometrial hyperplasia and carcinomas leading to series of changes which initiate and promote the progression of endometrial tissue toward malignant phenotype.[3] These changes can be further divided into distinct steps like self-sufficiency in growth signals, insensitivity to growth inhibition, evasion of apoptosis, angiogenesis, invasion and metastasis involving genetic alteration/activation of various oncogenes/other proteins.[4] Oncogenes are usually in present inactive state, and activation stimulates the process of cell division. Only few oncogenes are known to be altered in EC. Amplification and increased expression of oncogene HER-2/neu is observed in 10% - 40% of ECs and correlates with a worse prognosis and more aggressive tumour behaviour.[5]

Her-2/neu is an oncogene located on the chromosome 17; encoding type 1 tyrosine kinase growth factor receptor.[2] Her-2/neu (c-erbB-2) gene product expression plays an important role in regulating the endometrial growth factor receptor signalling network.[3] When Her2/neu is normally expressed, it leads to

the combination of a few copies of Her-2/neu heterodimers and the Her-2/neu-mediated signalling is weak resulting in a normal cell growth.[7] Her-2/neu Gene amplification results in 50-100 genes per cell leading to the expression of atleast 2,000,000 Her-2/neu proteins per cell in the tissue, instead of the 20,000-50,000 Her-2/neu proteins per cell in physiologic conditions leading to increase tyrosine kinase activity causing increased mitogenic cell signalling and increased cell proliferation and antiapoptotic and neoangiogenic effects.[8]

Her-2/neu oncogene amplification is seen in many human cancers like breast cancer, pancreatic cancer and ovarian cancers and it provides an attractive therapeutic target. A humanized monoclonal antibody to Her-2/neu, Herceptin, have a significant therapeutic effect in patients with Her-2/neu positive breast carcinomas, and its therapeutic efficacy has been investigated in tumour exhibiting strong immunoreactivity for this protein. Targeting Her-2/neu may be beneficial for a selected group of patient with endometrial carcinoma showing Her-2/neu overexpression. Our study aims to evaluate Immunohistochemical expression of Her-2/neu protein in endometrial carcinoma, its pattern of expression and correlation with histological type, and grade of tumour and clinical stage in order to define its potential prognostic value in Endometrial carcinomas.

II. Materials And Methods

Materials: 42 cases of endometrial carcinoma with stage I-IV were enrolled into this study from January 2010 to June 2012 who were diagnosed and treated at MNJ Cancer Hospital and Research Institute, Osmania Medical College, Hyderabad.

The material included in the present study was obtain from hysterectomy specimens which were sent to the Department of Pathology, MNJ Cancer Hospital and Research Institute and diagnosed as Endometrial Carcinoma and Tumor staging was performed according to the International Federation of Gynecology staging revised in 2009 and TNM staging system.Demographic and clinical information like age at time of diagnosis, clinical presentation and parity was obtained and recorded.

Methods:

Formalin-fixed, paraffin embedded tissue blocks of each of 42 cases were obtained and all hematoxylin & eosin-stained sections were reviewed, the quality of the material was checked, and the best sections from each specimen were selected. All microscopic slides were reviewed to confirm the pathological diagnosis and grading was done according to the International Federation of Gynaecology and Obstetrics (FIGO) grading system. Tumour staging was performed according to the FIGO classification and TNM staging system. Patient's medical records and clinic pathologic characteristics were reviewed. 3 μm sections were taken on positively charged slides for IHC with HER-2/Neu

IHC was performed for HER-2/Neu using standard IHC protocol. Thin-sliced sections of 3 microns thickness were taken on polylysine coated slides, de-paraffinized in xylene and dehydrated using alcohol. After rehydration with water, retrieval of antigen was achieved by micro waving the sections in citrate buffer (pH 6.0). After blocking the endogenous peroxidase activity with 0.3% hydrogen peroxide for 5 -10 minutes, the sections were stained for HER-2/neu using polyclonal anti-rabbit cerb-B2 antibody according to instructions of the manufacturer and using reagents included in cerb-B2 detection kit (DAKO). The excess antibody was washed away with TRIS wash buffer (pH, 7.5-7.6). The tissue was incubated with Peroxidase/DAB, Rabbit/Mouse detection system (DAKO) for half an hour. Excess antibody was washed away with TRIS wash buffer again. Peroxidase was demonstrated with 0.5% diamino-benzidine for 5 minutes,and the sections were counterstained with Hematoxylin for 1 minute. Sections were washed under tap water for 2 minutes, Dehydrated and mounted with DPX. Parallel positive control section from a well fixed, preserved breast carcinoma were included and negative controls were obtained by omitting the primary antibody.

Results: Positive-Brown stain of the cytoplasmic membrane.

Estimation of HER-2/Neu expression was evaluated under light microscope and uniform criteria were used according to Hanaa O Badr Eldin et al [3] and Grushko TA et al.[10]

The intensity of HER-2/Neu staining were scored from

Negative (0) : with no staining is observed.

+ 1: incomplete membranous staining or complete membranous staining in less than 10% of the tumour cells.

+ 2: moderate intensity; complete membranous staining in more than 10% of the tumour cells.

+ 3: strong staining intensity, complete membrane staining is observed in more than 10% of the tumour cells.

In our study, specimens with 2+or 3+ staining were considered positive for HER-2/Neu antibody overexpression.The X^2 test and Fisher exact test were used to evaluate the relation between histopathological

parameters and immunohistochemical markers. The level of statistical significance was established at $P < 0.05$.

III. Results:

Clinicopathological findings: Among 42 patients with endometrial adenocarcinoma, 48% patients were between 46 – 55 years age group,7 cases (16.6%) were between 56- 60 years, 5 cases (11.9%) between 61 – 65 years age group, 4 cases (9.52%) of endometrial carcinoma were aged between 66 -70 years and 2 cases (4.76%) were above 70 years. Results are depicted in Table - 1

Table 1: Age wise distribution

AGE (years)	Endometrial carcinoma (%)
35- 40	01(2.38)
41-45	03(7.14)
46-50	10(23.80)
51-55	10(23.80)
56-60	07(16.66)
61-65	05(11.90)
66-70	04(9.52)
71-75	02(4.76)
Total	42cases

Clinical presentation: 83.33% (35) of patients presented with postmenopausal bleeding and 16.6% (07) of patients with menorrhagia. Regarding parity in patients with Endometrial carcinoma, 47.61% (20) of patients were of Para 2, 23.80 %(10) of patients were of Para 1, 19.04%(08) patients were of Para 3 and 4.76% (02) were of Para 4.Results are depicted in Table - 2

Table 2: Parity wise distribution

Parity	**Endometrial carcinoma**
Para 0	02 (4.76%)
Para 1	10 (23.80%)
Para 2	20 (47.61%)
Para 3	08 (19.04%)
Para 4	02 (4.76%)

Type and Grade wise distribution of Endometrial carcinoma: Among 42 cases of endometrial carcinoma, 38 cases (90.47%) are of endometrioid adenocarcinoma(type 1) and 4 cases (9.52%) are of serous adenocarcinoma(type 2). 17 cases (40.47%) were Grade I, 10 cases (23.80%) were Grade II and 15 cases (35.71%) were Grade III. Results are depicted in Table – 3

Table 3: Grade wise distribution of endometrial adenocarcinoma

Grade	**No. of patients**	**Percentage %**
Grade I	17	40.47%
Grade II	10	23.80%
Grade III	15	35.71%

Endometrial carcinoma staging:

The present study showed 17 cases of Stage I (Tumour confined to the corpus uteri only) of which 4 cases (9.52%) were Stage Ia, 10 cases (23.8%) of Stage Ib and 3 cases (7.14%) of Stage Ic. Followed by 9 cases of Stage II (Tumour invades cervix) of which 4 cases (9.52%) showed Endocervical glandular involvement only (Stage IIa) and 5 cases (11.9%) showed Cervical stromal invasion (Stage IIb).

16 cases were of Stage III (Local and/or regional spread of tumour) of which 13 cases(30.95%) showed Metastases to pelvic and / or para-aotic lymph nodes(Stage IIIc),whereas in 2 cases(4.76%) Tumour invasion into the B/L ovaries was seen and only in single case (2.38%) Vaginal involvement was seen. Results are depicted in Table – 4

Table 4: Stage wise distribution of endometrial carcinoma

Endometrial carcinoma staging	No. of cases (42)	Percentage (%)
Stage Ia	04	9.52%
Stage Ib	10	23.80%
Stage Ic	03	7.14%
Stage IIa	04	9.52%
Stage IIb	05	11.90%
Stage IIIa	02	4.76%
Stage IIIb	01	2.38%
Stage IIIc	13	30.95%

Immunohistochemical analysis of HER-2/neu expression:

Immunohistochemistry findings were demonstrated in Table 5 and figures (6-12). 15/42 cases (35.71%) of the cases showed strong complete membrane staining in more than 10% of the tumour cells (3+). 08/42 (19%) cases showed moderate complete membranous staining in more than 10% of the tumour cells (2+). 16/42 (38.09%) cases showed weak incomplete membranous staining or complete membranous staining in less than 10% of the tumour cells(1+) and only 03(7.14%) cases showed no membrane staining for HER-2/neu expression. The results are depicted in Table no. 5

Table 5: HER-2 Immunoexpression in Endometrial carcinoma

Diagnosis	HER-2/neu expression assessment			
Endometrial carcinoma	Absent (%)	1+ (%)	2+ (%)	3+ (%)
	03 (7.14%)	16 (38.09%)	08 (19.04%)	15 (35.71%)

Correlation of HER-2/neu expression with histological type:

In the present study, of 42 case of endometrial carcinoma 38 were Endometrioid Adenocarcinoma (type 1) and 4 cases were Serous Adenocarcinoma (type 2).

28.94% (11 of 38) of cases of Endometrioid Adenocarcinoma showed 3+ HER-2/neu expression while 100% (4 of 4) of cases of Serous Adenocarcinoma showed 3+ HER-2/neu expression. The results are depicted in Table no. 6

Table 6: Correlation of HER-2/neu expression with histological type

Histopathological type	No. of cases	POSITIVE HER-2/neu expression (3+ & 2+)	Percentage (%)
Endometrioid Adenocarcinoma	38	23	60.52%
Serous Adenocarcinoma	04	00	100%

Correlation between histopathological variables and Her-2/neu overexpression by IHC in Endometrial carcinoma:

The relations between Her-2/neu expression and various clinic-pathologic variables of EC are listed in Table No 7.

Patients with stag I-II disease (limited to the uterus) were compared to those with stage III-IV (extrauterine tumor spread) regarding Her-2/neu expression. Statistically significant relation was reported between Her-2/neu expression and FIGO stage where 07/25 (28%) of patients in stage I-II demonstrated Her-2/neu expression, while 16/17 (94.12%)of patients with stage III-IV expressed Her-2/neu ($p < 0.01$). According to grade of differentiation, Her-2/neu overexpression was reported in 08/27 (29.63%) cases with Low to intermediate (Endometrioid Grade-1 and Grade-2) and 15/15(100%) cases with High(Endometrioid Grade-3 and serous Adenocarcinom) the difference was significant ($P < 0.01$). No statistically significant relation between Her-2/neu overexpression and the histological type of the patients could be detected.

Patients with lymph node metastases demonstrated significantly higher rates of Her-2/neu expression as compared to patients without lymph node metastases (92.31% vs 37.93%, $p<0.01$). Significant correlation was also reported in myometrial invasion, Her-2/neu overexpression was reported in 09/23 (39.13%) with <50% mymetrial invasion and in 14/19 cases with ≥50% myometrial invasion, the difference was significant ($P < 0.05$). Significant correlation ($P<0.01$) was also reported in cervical invasion, with 17/22 (77.27%) cases with cervical invasion showing Her-2/neu overexpression and 6/20 (30%) case without cervical invasion showing Her-2/neu overexpression.

Table 7: Correlation between histopathological variables and Her-2/neu overexpression by IHC in Endometrial carcinoma

Variables	Types	No. Of Cases	Positive Her-2/Neu Overexpression (3+ & 2+)	Negative Her-2/Neu Expression (1+, 0)	X^2	P Value
Stage	Early (stage IA through IIB)	25	07 28.00%	18 72.00%	17.86	<0.01
	Advanced (stage IIIA to IIIc)	17	16 94.12%	01 5.88%		
Grade	Low to intermediate (endometrioid G1, G2)	27	08 29.63%	19 70.37%	19.28	<0.01
	High (endometrioid G3, serous)	15	15 100.00%	00 0.00%		
Histological type	Endometrioid	38	19 50.00%	19 50.00%	3.652	0.11
	NonEndometrioid	04	04 100.00%	00 0.00%		
Lymph-node status	Negative	29	11 37.93%	18 62.07%	10.71	<0.01
	Positive	13	12 92.31%	01 7.69%		
Myometrial invasion	<50% invasion	23	09 39.13%	14 60.87%	5.015	<0.05
	> 50% invasion	19	14 73.68%	05 26.32%		
Cervical invasion	No invasion	20	6 30.00%	14 70.00%	9.450	<0.01
	Cervical invasion	22	17 77.27%	5 22.73%		

Discussion

Overexpression of the HER2/neu proto-oncogene, either by gene amplification or through transcriptional deregulation is seen in approximately 25-30% of breast and ovarian carcinoma and confers worse biological behavior and poor prognosis.[24] Smaller studies suggest that Her-2/neu overexpression may be involved in the tumorigenesis of endometrial carcinoma. Prognostic and therapeutic implication of Her-2/neu overexpression and amplification in endometrial carcinoma continue to evolve over the past few years. In the literature, the reported rates of Her-2/neu overexpression and amplification in endometrial carcinoma range from 17% to 80%.[3,13,16]

In the study done by Grushko et al,[10] HER-2 gene amplification was detected in 44%. There was a significant association between the grade and HER-2 amplification among non-serous tumours. Similarly Hanaa O Badr Eldin et al[3] also demonstrated a higher rate of Her-2/neu protein overexpression in endometrial carcinoma (42.1%) Other studies which reported higher Her-2/neu expression are Cherchi *et al.*[17], 2001 (55.2%), Villella et al.[18] 2006, *(*71%), and Santin et al.[19] 2002(80%). Kohlberger et al.[20] found 21% HER-2/neu oncoprotein expression and according to their study, clinical stage, histological stage, grade and depth of invasion did not correlate with HER-2 expression.

In our study, analysis of HER-2/neu overexpression in endometrial carcinoma showed 23/ 42 (54.76%) of cases expressing a moderate to strong intensity complete membrane staining in more than 10% of the tumour cells (3+ & 2+) . This is comparable with Hanaa O Badr Eldin et al[3] study which demonstrated 42.1% (8/19) HER-2/neu overexpression. In Ivana Markova et al[5] study HER2/Neu overexpression (3+) was seen in 28.4% cases of endometrial carcinoma, whereas in the study done by Bahman Saffari et al [22] 52% endometrial cancers were characterized as showing moderate or high HER-2/neu immunostaining.

The wide range of HER2/Neu overexpression in the previous studies may be due to the different detection methods used, Protein targets may differ among the IHC methods used Or difficulty with the definition of Her-2/neu overexpression. The criteria for Her-2/neu positivity require complete moderate to strong intensity membranous staining in greater than 10% of the tumor cells. In some of the previous studies this criteria was not clearly defined. Some studies did not distinguishing complete from partial staining. Partial or incomplete membranous staining may be responsible for higher rates of Her-2/neu positivity.

In the our study, we demonstrated a significant relationship between Her-2/neu overexp-ression and FIGO stage of the tumor, lymph node metastases, myometrial invasion and cervical invasion indicating that Her-2/neu is related to most of the prognostic factors of Endometrial Carcinoma. HER-2/neu was strongly positive in 16/17 (94.12%)of patients with stage III-IV endometrioid adenocarcinoma whereas 07/25 (28%) of patients in stage I-II demonstrated Her-2/neu expression. 100.00% of Grade 3 tumours showed HER-2/neu overexpression while only 29.63% of Grade 1 & 2 showed HER-2/neu overexpression.

Regarding lymph node involvement, 92.31% of cases with lymph nodal metastasis showed strong HER-2/neu overexpression and only 37.93% of cases without lymph node metastasis showed HER-2/neu overexpression. 73.68% of cases with >1/2 myometrial invasion and 77.27% (12/22) of cases with cervical invasion showed strong HER-2/neu overexpression.

This finding is comparable with Hanaa O Badr Eldin et al[3] study, where 66.7% of Grade 3 and 30% of Grade 2 showed HER-2/neu overexpression, 85% of advanced stage (stage III and IV) showed overexpression.71% with lymph node metastasis and 70 % with >1/2 myometrial invasion showed strong HER-2/neu overexpression. Similarly, Cherechi et al.[23] (2001) also demonstrated a positive correlation between Her-2/neu expressions and grading, myometrial invasion and FIGO stage which indicate a more malignant tumour phenotype.

The findings in our study support the notion that Her-2/neu overexpression may be a major prognostic factor and Her-2/neu Immunohistochemistry could be incorporated as a prognostic variable in patients with endometrial carcinoma.

There have been numerous studies in breast cancer patients showing that first-line treatment with trastuzmab, a humanized anti-Her-2/neu antibody, in combination with chemotherapy increases response rates, time to progression, and overall survival in the subset of women whose breast tumours demonstrated Her-2 /neu overexpression. In analogy to breast cancer, patients harbouring disease with proven amplification

or strong Her-2/neu overexpression, endometrial carcinoma patients with overexpression of Her-2/neu may potentially benefit from Herceptin therapy.

CONCLUSION

The Present study demonstrated strong HER-2/neu overexpression is seen in 54.76% cases of endometrial carcinoma. The present study also demonstrated higher rate HER-2/neu overexpression in patients with high grade, advanced stage, myometrial invasion, cervical invasion and lymph node metastasis, indicating that Her-2/neu overexpression is related to most of the prognostic factors of Endometrial carcinoma. Patients harbouring Endometrial carcinoma with proven amplification or strong Her-2/neu overexpression may potentially benefit from Herceptin therapy

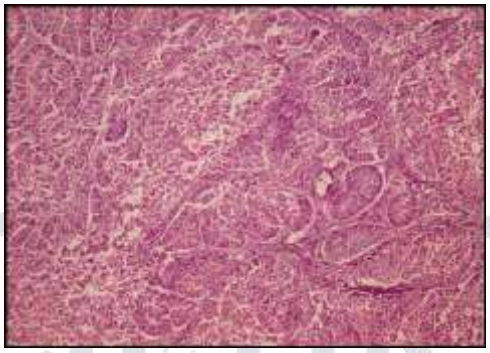

Figure 1: Section showed Well differentiated Endometrioid Endometrial carcinoma.Glands show back – back arrangement with complex folds and stroma is scanty. (H & E Scanner)

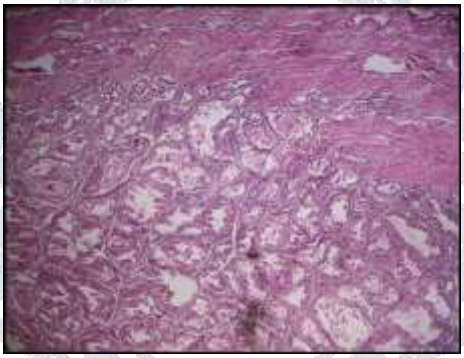

Figure2: Endometrial carcinoma with myometrial invasion. Endometrial carcinoma showing >1/2 myometrial invasion (H & E Scanner)

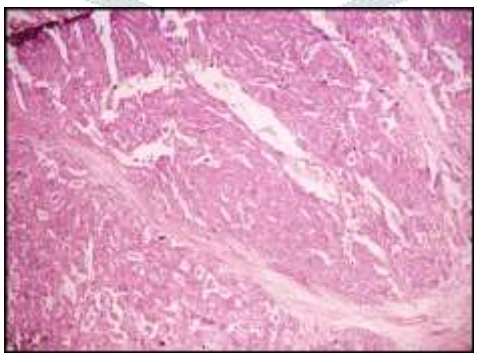

Figure 3: Poorly differentiated endometrial carcinoma shows >50% of solid growth with marked nuclear enlargement and pleomorphism. (H & E X10)

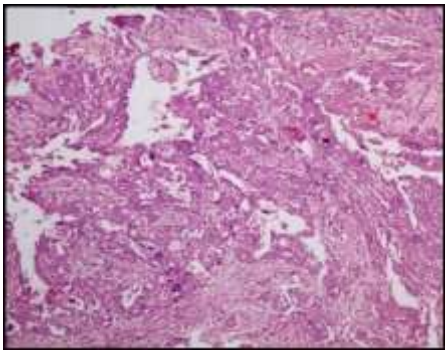

Figure 4: Serous Adenocarcinoma showing papillary architecture with broad fibrovascular core and pleomorphic nuclei with prominent nucleoli (H & E X10)

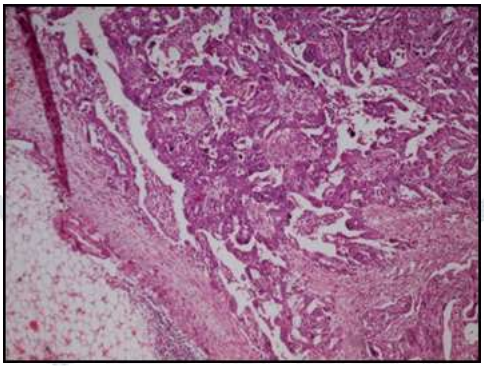

Figure 5: Serous Adenocarcinoma with Omental metastasis (H & E X10)

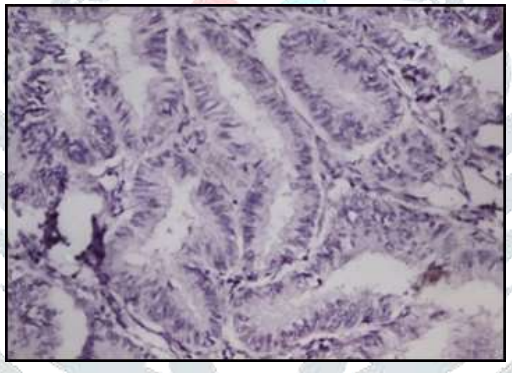

Figure 6: HER-2/neu immunoexpression in well differentiated Endometrioid Endometrial carcinoma (X40) Glands shows absent HER-2/neu immunoexpression.

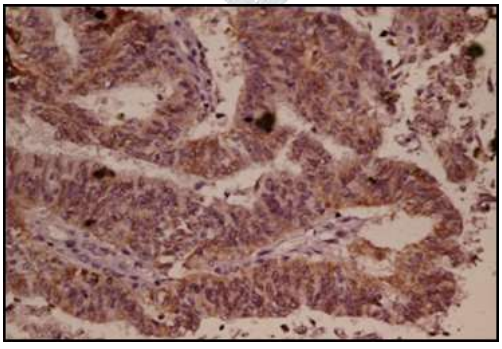

Figure 7: HER-2/neu immunoexpression in well differentiated Endometrioid Endometrial carcinoma (X40) Glands shows +1 HER-2/neu immunoexpression.

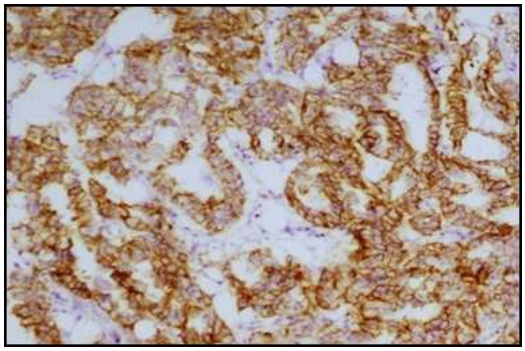

Figure 8: HER-2/neu immunoexpression in well differentiated Endometrioid Endometrial carcinoma (X40) Glands shows +3 HER-2/neu immunoexpression.

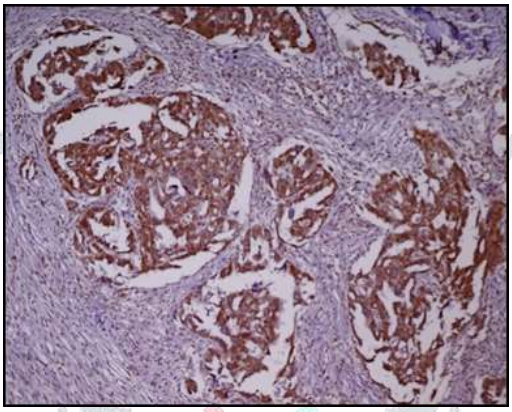

Figure 9: HER-2/neu immunoexpression in poorly differentiated adenocarcinoma (Scanner).Glands shows +3 HER-2/neu immunoexpression

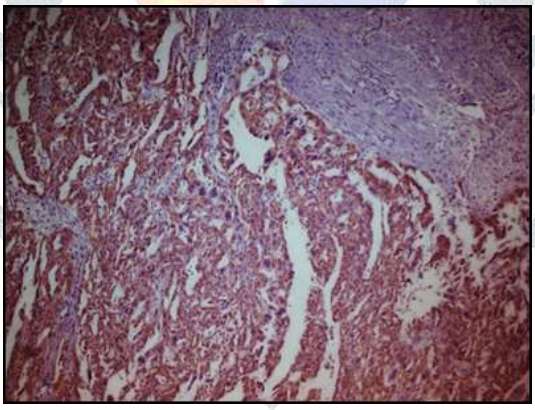

Figure 10: HER-2/neu immunoexpression in poorly differentiated adenocarcinoma (X10) Glands shows +3 HER-2/neu immunoexpression

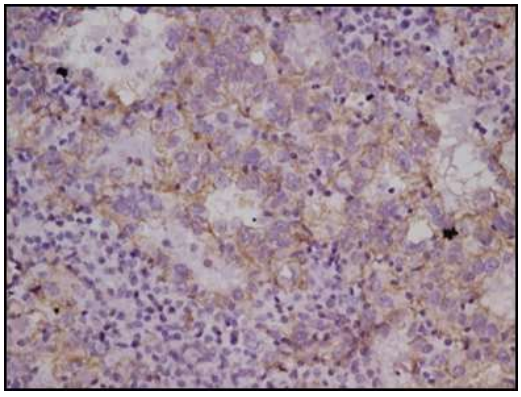

Figure 11: HER-2/neu immunoexpression in poorly differentiated adenocarcinoma (X40) Glands shows +1 HER-2/neu immunoexpression.

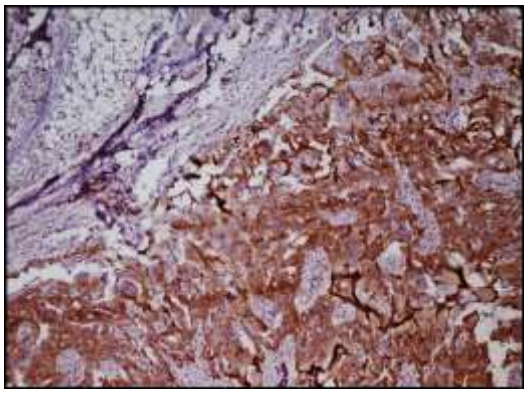

Figure 12: HER-2/neu immunoexpression in serous adenocarcinoma (X10). Glands shows +3 HER-2/neu immunoexpression

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