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Role of Her-2 Neu As Prognostic Indicator in Surface Epithelial Tumors of Ovary.

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Abstract
Introduction *: Ovarian cancer is major cause of mortality in women . Now a days so many biomarkers like P53,EGFR, Ki67,CK are used for early detection to predict the prognosis and planning for better treatment.*
Aims Of Study *: To know the role of HER-2/neu expression in surface epithelial tumors of ovary in assessing the prognosis and to know its relation with age of patient, tumor size and grade of tumor.*
Materials & Methods *: Present study conducted for a period of 2 yrs (July 2012 to June 2014) in Government General Hospital, Rangaraya Medical College,Kakinada. During this period we selected total 50 cases of surface epithelial tumors in that we did immunohistochemistry (IHC) with HER 2 neu marker for 32 cases.*
Conclusion : *HER 2 neu expression is not significantly associated with grade of the tumor, type of tumor and age of the patient, but expression somewhat significantly observed in large tumor size (> 10 cm dia).*

I. Introduction

Gynecological malignancy carries a high mortality amongst women of all ages. Ovarian cancer is major cause of mortality in women because of its typical insidious onset and late diagnosis as the ovaries lie in spacious pelvic cavity, they become larger without producing symptoms of pain and pressure. Hence in 70% of patients has spread beyond ovary and in 60% spread beyond the pelvis at the time of diagnosis. Ovarian tumours are heterogeneous group of malignancies that arise from various cell types of ovary like epithelial, sex cord stromal and germ cell[1]. Epithelial ovarian cancer is the leading cause of death from gynecological malignancies[2,3]. During postmenopausal period epithelial tumours are predominant.[4].

There is desperate need for a better understanding of molecular pathogenesis of ovarian tumours, so that new targets and biomarkers that facilitate early detection and treatment can be identified (5). Now a days so many biomarkers like p53, EGFR, Ki 67, cytokeratin are used to predict the prognosis and planning for better treatment.Present study was carried out to know the "Role of HER2/neu expression in surface epithelial tumours of ovary" in assessing the prognosis by correlating with the age of the patient, tumour size and grade of tumour.

II. Materials And Methods

Present study was conducted in the department of Pathology, in cooperation with the department of Gynecology and obstetrics at Rangaraya Medical College (RMC), Kakinada, from July 2012 to June 2014. We selected a total of 50 cases of surface epithelial tumours on application of exclusion and inclusion criteria. In those 32 cases for which immunohistochemistry were done in our institute were included in the study. Immunohistochemistry was used for determining HER2/neu status. Correlation of HER2/neu expression with histologic type of tumour and grade of the tumour was studied. Specimens were fixed in neutral buffered formalin.

Technique Of Analysis Used

1. Preparation of paraffin blocks for histopathological examination was done.
2. Subjected to haematoxylin & eosin staining.
3. Immunohistochemical analysis was performed manually in the department of pathology, RMC, Kakinada.

The scoring of the stained sections was done by two independent observers & the average value taken as the expression of protein.

Interpretation:
Scoring system:

The criterion for positive immunoreaction is dark brown membrane staining. While the intensity of the staining was assessed by counting the percentage of positive cells in 100 malignant cells at objective 40 total magnification. The immunostaining was calculated as the percentage of immunoreactive cells per total number of malignant cells. Each sample was scanned for at least five fields with a high power magnification.

HER2/neu scoring was done according to Sophia K. et al. scoring system at objective 40 and as follows:

- **Score 0** (negative)-no membrane staining observed.
- **Score +1** (negative)-faint partial membrane staining in >10% of cancer cells with rare or absent circumferential staining.
- **Score +2** (equivocal)- weak circumferential membrane staining in >10% of cancer cells but the membrane staining ring is thin.
- **Score +3** (positive)-intense circumferential membrane staining in >10% of cancer cells and the membrane staining ring is thick.

  IHC stained slides were evaluated according to the quick score which takes proportion of cells stained and intensity of staining into consideration. The staining was evaluated on the invasive component only. Well preserved and well stained areas of the sections were assessed, the average value was taken as the protein expression.

HER2/Neu Expression According To CAP Guidelines:

Tumours that show strong circumferential staining (referred to as +3staining)[Graph-3] in > 30% of cells by IHC.Tumours that show moderately strong circumferential membrane staining (referred to as +2 staining) Tumours that show little or no protein expression by IHC (referred to as 0 or +1 staining)[Graph-2]. Because of the uncertain biologic significance of cytoplasmic HER2 staining, only membrane staining was evaluated for this study. Eligibility was restricted to patients with a tumour that demonstrated +2 or+3 over expression by immunohistochemistry. Gene amplification or gene expression by fluorescence in situ hybridization [FISH] was not specifically evaluated in this clinical trial.

III. Results

Total 32 cases of ovarian epithelial tumours were subjected to HER2/neu immune stain. 18 out of 32 showed HER2/neu positivity, remaining 14 were negative. In present study, among 18 (56.2%) HER2/neu positive cases,13cases (72.2%)were less than 50 years age and 5 cases (27.8%) were above 50 years. while among 14 (43.5%) negative cases, 11 cases (78.6%) were present in age less than 50 years and 3 cases (21.4%) were above 50 years. From the above data we can conclude that age had no relation with expression of HER2/neu. Among 18 cases (56.2%) which were HER-2/neu positive, 4 cases (22.2%) had size less than 10 cm and 14 cases (77.8%) had size more than 10 cm . Among 14 cases (43.8%) which were HER2/neu negative, 7 cases (50%) cases had size less than 10 cm and 7 cases (50%) had size more than 10 cm, indicating that HER2/ neu positivity was more in tumours greater than 10cm.

Histopathological analysis included 32 cases of ovarian tumours of which 15 cases (46.8%) were serous and 17 cases (53.2%) were mucinous tumours[graph 1]. Assessment of histopathological grade of differentiation in total 7 malignant ovarian cases revealed that 3 cases (42.86%) are well differentiated, 2 cases (28.57%) are moderately differentiated, and 2 cases (28.57%) are poorly differentiated. Regarding intensity of HER2/neu immunostaining, 33.4% of grade I serous tumours(Fig5&6) revealed score +1 (negative), 66.6% revealed score+3. Grade II (Fig 3&4) revealed score +1(negative) in 50% of cases and score +3 (positive) in other 50%. Grade III revealed 50% score +2, and 50% score +3 with significant difference only for grade III. Among 13 benign tumours HER2/neu positivity was seen in 7cases with intense positivity (+3) in 2 cases, 5 cases are showing equivocal positive staining (+2)[Table-1]. 3 cases of mucinous cystadenomas(Fig.1&2) are showing absent (0) staining and 3 cases are showing score +1 staining. In borderline tumours both the cases are showing 1+ positivity (negative staining) only. 2 (100%) cases of mucinous cystadenocarcinomas are showing intense (+3) positivity.[Table-2] Both cases are grade-I carcinomas. Pertaining to intensity of immunostain out of total 9 malignant tumours +3 positivity was seen in 80% of grade-I tumours whereas only 50% of grade II and grade III tumours. It clearly indicates that the intensity of immunostain is not significantly increasing with the grade of the tumour.[Graph-4] **.**

IV. Discussion

HER2/neu oncogene which belongs to epidermal growth factor receptor family has been implicated in malignant transformation and may have a driving force in the carcinogenesis of several human cancers including ovarian cancer.[6,7,8,9]. Several studies have examined the prognostic significance of HER2/neu expression in epithelial ovarian cancer. The role of HER2/neu immunohistochemistry in ovarian cancers is not yet clear with contradicting results and conflicting data. Thus, the prognostic influence of HER2/neu is still a matter of debate

since the percentage of HER2/neu positive patients varies considerably, among different individual studies dealing with different samples.[10,11]

In present study we evaluated the expression of HER2/neu in ovarian lesions, its relationship with the type of tumour and correlation with the clinicopathological factors like age of patient, size of tumour and histological grading to asses whether HER2/neu like in breast cancer can be considered as an important prognostic indicator or not. Increased expression of HER2/neu oncogene has been reported to occur in ovarian tumors and possibly which may correlate with its biologic behavior and prognosis. Hence the present study is designed to correlate the HER2 /neu over-expression with the grade of differentiation of ovarian surface epithelial carcinoma and to assess whether such relation could be used as a prognostic factor for the early diagnosis of the malignancy.[9,10,11,12]

Role of HER2/neu in ovarian cancers is a matter of debate. Various studies have reported that between 5% and 30% of ovarian tumors overexpress HER2/neu (Hellstrom I *et al* 2001).[6,7,9,13]

In Sapna et al study, among 24.3% HER2/neu positive cases,66.7% were <50 years age and 33.3% were above 50 years while among 75.7% negative cases, 67.9% were present in age less than 50 years and 32.1% in more than 50 years.

In present study, among 18 (56.2%)HER2/ neu positive cases ,13 cases (72.2%) were less than 50 years age and 5 cases(27.8%) were above 50 years, while among 14(43.5%) negative cases ,11 cases (78.6%) were present in less than 50 years age and 3 cases (21.4%) in more than 50 years but the difference was not statistically significant. Hence we can conclude that age had no relation with expression of HER2/neu.[Table-4]

In present study, among 18 cases (56.2%) HER2/neu positive 4 cases (22.2%) had size less than 10cm and 14 cases (77.8%) had size greater than 10 cm. Among 14 cases (43.5%) HER-2/neu negative cases, 7 cases (50%) had size less than 10cm and 7 cases 50% had size greater than 10cm. In present study tumours with size greater than 10cm showed significant association with HER2/neu positivity.[Table-3]

In Sapna et al study, among 24.3% tumors which were HER2/neu positive, 50% had size less than 10 cm and 50% had size more than 10 cm. Among 75.7% cases which were HER2/neu negative, 39.3% cases had size less than 10 cm and 60.7% had size more than 10 cm.

Similar results were shown by Sueblinvong T *et al* 2007 who found no correlation between HER2/neu and clinicopathologically analyzed factors for 74 cases of surface malignant ovarian tumours.

In contrary, in present study HER2/ neu expression was more in tumours greater than 10cm size indicating that HER2/ neu expression was associated with size of the tumour.

In present study total 56.2% ovarian tumours showed HER2/neu positivity. In addition to malignant tumors, we also observed its expression in both benign/borderline tumours.

Many other studies revealed various degrees of detection rate for HER2/neu immunostaining such as Berchuch et al 1990 (32%), Salmon et al 1989 (26%), Bookman et al 2003 (11%), Dimova et al 2006 (11%), Nielsen JS et al 2004. (35%), and Malamou-Mitsi V 2007 (18%).

The positivity of HER2/neu overexpression has a very wide range of variation in various studies i.e, 2% to 100%. In many studies the intensity of staining does not correlating with the grade of the tumour. Even 2 benign cases are also showed intense (+3) HER2/neu positivity.

After revised all the studies mentioned above, finally concluded that HER2/neu expression is not correlating with clinicopathological prognostic indicators like age of the patient and grade of the tumour except for the size of tumour. It still needs further evaluation as a prognostic factor in ovarian neoplasms.

V. Conclusion

The HER2/neu immunoexpression in the analysis of surface epithelial tumors of ovary, showed positivity in 18 cases (56.25%) out of 32. In these 18 positive cases 10 cases are benign tumours, one is borderline tumour and 7 cases are cystadenocarcinomas. In present study, 57% cases of serous carcinomas showed intense HER2/neu positivity (+3), whereas not even a single case was noted as +3 HER2/ neu positivity in both benign and borderline categories. 50% of benign and borderline serous tumours were HER2/neu negative and remaining 50% were shown equivocal positivity(+2).Most of the mucinous cystadenomas were showing equivocal staining but 2 cases are with intense positivity (+3 score). Even though two mucinous cystadenomas were showing intense positivity, the percentage of HER2/neu positivity was increasing from benign to malignant tumours i.e., from 15% to 100%. In present study no significant correlation was observed between HER2/ neu positivity and clinicopathological factors like age of the patient and grade of the tumour. But HER2/ neu expression was significantly observed in tumours greater than 10cm Even though HER2/neu positivity was significantly associated with carcinomas (77.7%), most of the benign lesions (52.6%) are also showing HER2/neu positivity indicating that positivity was not significantly correlating with the type of the tumour. In present study intense positivity was more in well differentiated (grade I) tumours (80%)when compared to moderately & poorly differentiated tumours (grade II &III) indicating that its expression was not significantly associated with poorly differentiated tumours. Though grade of a tumour is one of the important

prognostic indicator, HER2/neu positivity is not significantly associated with it. Hence we suggest that HER2/neu deserves further evaluation as a prognostic marker in epithelial ovarian cancers. Additional studies are warranted to ascertain the value of HER2 as a predictor of ovarian tumors that are resistant or responsive to specific chemotherapeutic agents or high-dose chemotherapy.

VI. Summary

In the present study the independent prognostic impact of HER2 overexpression in ovarian epithelial tumours is assessed. As HER2 / neu expression is predominantly seen in surface epithelial tumours we studied 32 cases of these tumours and found that 18 were HER2/neu positive and 14 were HER2/neu negative.

- 9 serous tumours were HER2/neu positive of which 3 were benign, 1 was borderline and 5 were malignant.
- 9 mucinous tumours were HER2/neu positive of which 7 were benign and 2 were malignant.
- Intense positivity (score+3) was seen in 4 serous cystadenocarcinomas and 2 cases of mucinous cystadenocarcinomas and also seen in 2 mucinous cystadenomas.

1 case of grade I and another grade II malignant serous tumours were HER2/neu negative.

Though stage and grade of a tumor are the most important prognostic indicators, HER2/neu positivity was not significantly correlated with grade of the tumour or other prognostic markers like age of the patient. Hence we suggest that HER2/neu deserves further evaluation as a prognostic marker in epithelial ovarian cancers.

GRAPH-1: Her2/Neu Status In Surface Epithelial Tumours

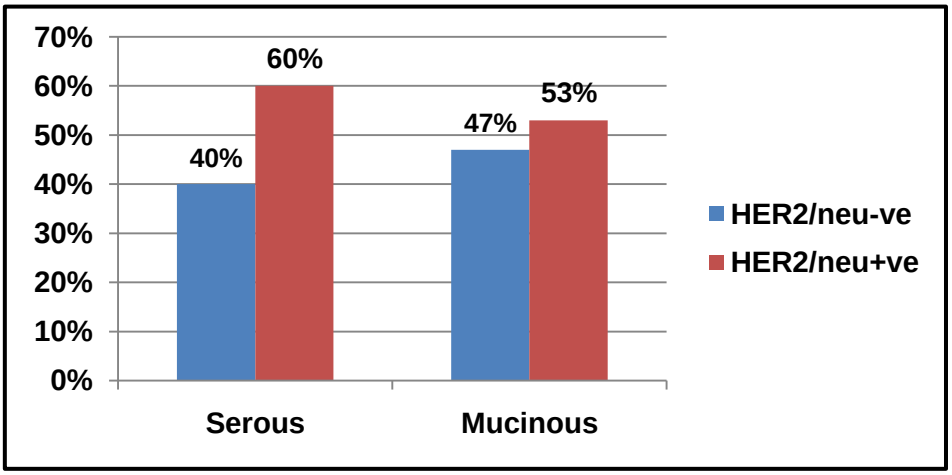


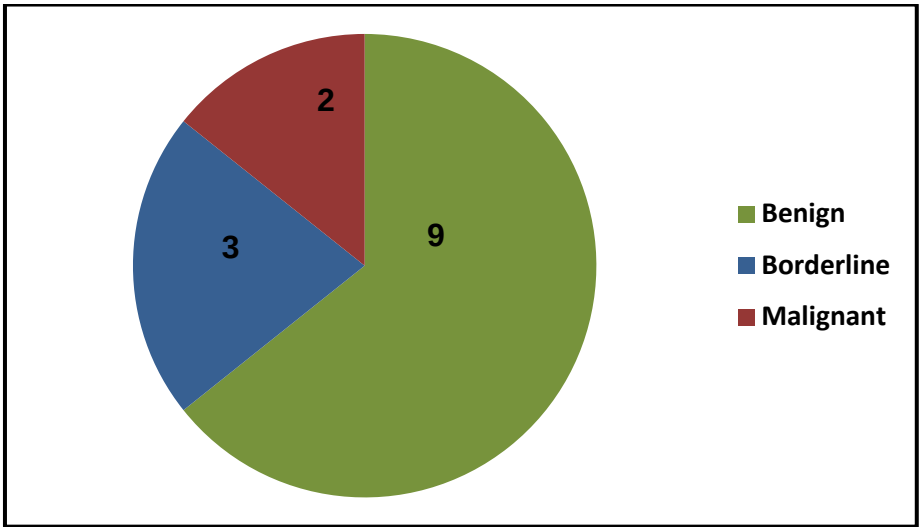
TABLE-1: Her2/Neu Expression In Serous Tumours

Type of tumour	**Scoring of HER2/neu positivity**			
	0	**1+**	**2+**	**3+**
Benign	1	2	3	-
Borderline	1	-	1	-
Malignant		2	1	4

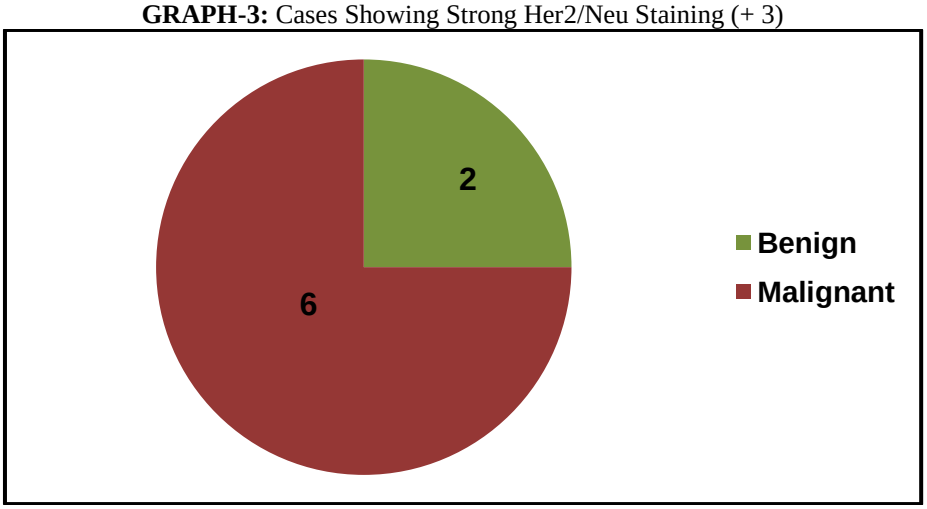
TABLE-2: Her2/Neu Expression In Mucinous Tumours

Type of tumour	**Scoring of HER2/neu positivity**			
	0	+1	+2	+3
Benign	3	3	5	2
Borderline	-	2	-	-
Malignant	-	-	-	2

GRAPH-2: Cases Showing Her2/Neu Negativity (0, + 1**)**



GRAPH-3: Cases Showing Strong Her2/Neu Staining (+ 3)



GRAPH-4: Her2/Neu Positivity According To Grade

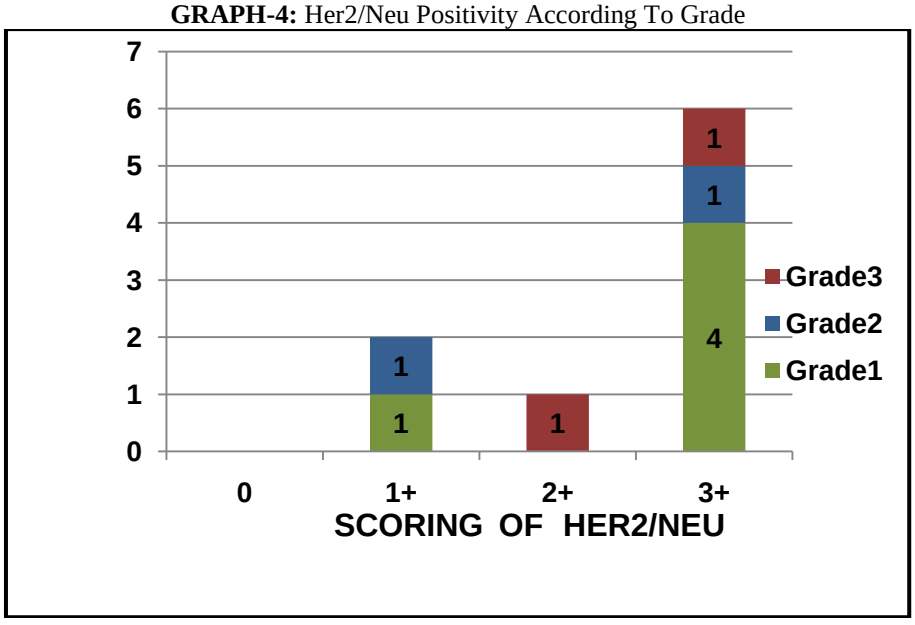


TABLE-3: Her2/ Neu Status With Size Of Tumour

HER2/neu status	**< 10 centimeters**	**>10 centimeters**
HER2 /neu positive (18 cases)	4 cases (22.2%)	14 cases (77.8%)
HER2/neu negative (14 cases)	7 cases (50%)	7 cases (50%)

TABLE-4: Her2/Neu Status With Age

HER2/ neu status	**Age < 50yrs**	**Age>50 yrs**
HER2/neu positive (18 cases)	13 cases (72.2%)	5 cases (27.8%)
HER2/ neu negative (14 cases)	11 cases (78.6%)	3 cases (21.4%)

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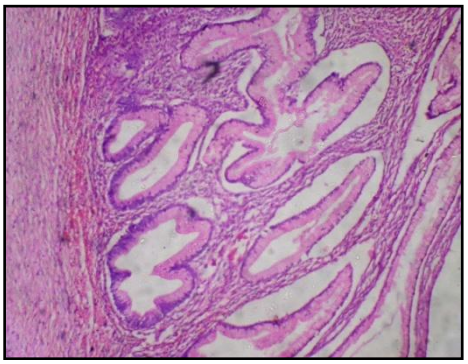

Figure 1: Mucinous cystadenoma H&E Stain- 10X

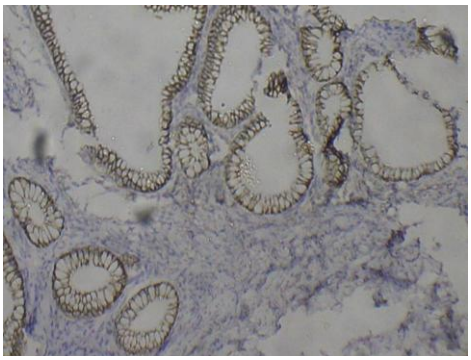

Figure 2: Mucinous cystadenoma – HER2/neuimmunostainpositive (score +3)-10X

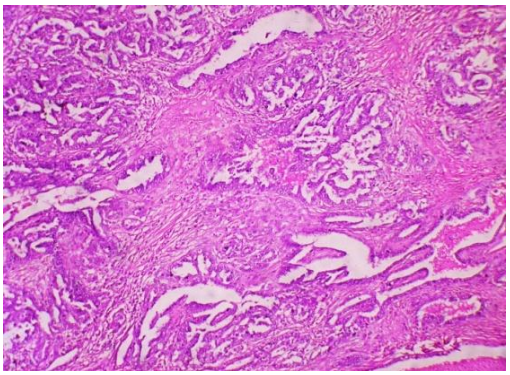

Figure 3: Papillary serous cystadenocarcinoma Grade-2 H&E Stain- 10X

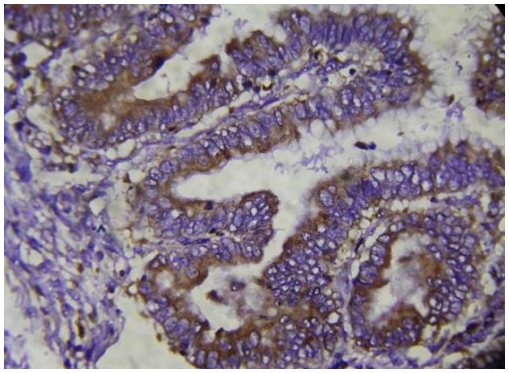

Figure4: Papillary serous cystadenocarcinoma HER2/neuimmunostain positive (score+3) - 40X

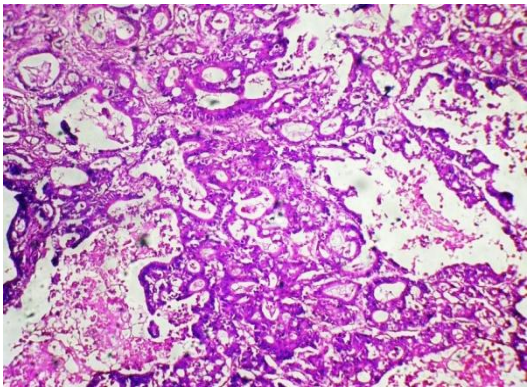

Figure 5: Papillary serous cystadenocarcinoma Grade-1 H&E Stain- 10X

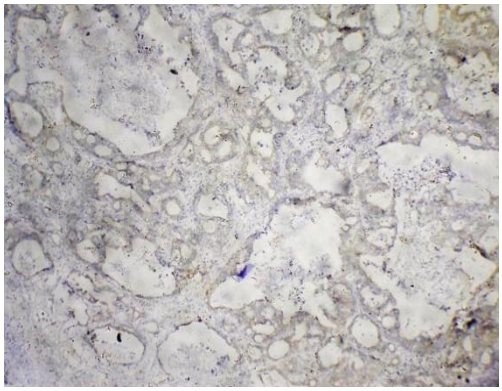

Figure6: Papillary serous cystadenocarcinoma HER2/neuimmunostain negative (score+1) - 10X