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UTILITY OF HER2/NEU AND KI67 IMMUNOSTAINING AND THEIR CORRELATION WITH HISTOLOGICAL TYPES, GRADES & STAGING OF SURFACE EPITHELIAL TUMOURS OF OVARY

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Abstract

Background: Ovarian carcinoma is one of the most common gynecologic malignancies in women with highest mortality rate. It was reported that ovarian cancer affected 238,719 women and causes over 150,000 deaths annually as patients were diagnosed in late stages of the disease

Aim: To evaluate the HER2/neu expression and Ki-67 expression in ovarian tumor.

Method: A hospital based prospective study was conducted in eastern India. All the cases of ovarian tumor undergone surgery were included. Results and data were analyzing in the department of pathology of Hi-Tech Medical College Bhubaneswar between2018-2020 for diagnostic accuracy by using stata.

Results: Out of 75 cases consisting 46 cases of Benign mors,9 case of border line and 20 cases of malignant tumors. Mostly elderly age groups were diagnosed as malignant tumors. HER2/neu and Ki67 expression increase with increasing grade.

Conclusion: HER2/ neu over expression in malignant tumors & can be utilized in different types of cancers for targeted therapy and more comprehensive management of patients.

Keywords: ovarian tumor, HER2/neu, Ki-67, targeted therapy.

INTRODUCTION

Cancer is the most common cause of mortality in most parts of the world[1]. Most common gynecologic malignancies in women with highest mortality rate is the ovarian cancer and it was ranked as 3rd after cervical and uterine cancer[2]. World Health Organization (WHO) classifies ovarian tumors according to their most probable cell of origin and histomorphological features[3]. Ovarian cancer has a high death rate because to the tumor's secretive and silent growth, delayed onset of symptoms, and inadequate screening, which leads to an advanced stage diagnosis. Consequently, this malignancy has been named as silent killer [4-6].

Ovarian neoplasm can emerge from germinal epithelium, germ cell, sex cord, ovarian stoma. Surface epithelial tumors are most common followed by germ cell tumor. Epithelial tumors 90%, germ cell tumors 30%, and sex cord stromal tumors 6%[7,8]. During postmenopausal period epithelial tumors predominance. Tumors arising from surface epithelium include serous, mucinous, endometrioid, clear cell carcinoma, Brenner's and transitional cell carcinoma. Clinical presentation is quite variable. 70% of ovarian cancers present with disseminated disease and only 19% of tumors are organ confined at diagnosis. Some of the theories for the pathogenesis of Epithelial Ovarian Carcinoma include: (1) repeated ovulation with trauma[9] (2) increased estrogen concentrations as a result of excess gonadotropin secretion (3) high androgen concentrations and (4) stromal hyperactivity.

The most common presentation is abdominal pain, a lump, or menstrual irregularities[10].

The grading indicates regarding important implications of prognostic and therapeutic. The prognostic factors include the FIGO stage, histological types, tumor grade, and clinic-surgical parameters including residual disease[11].

The HER2/ neu (c-erbB2) proto-oncogene encodes a transmembrane receptor protein which is structurally related to the epidermal growth factor receptor. The HER2 (c-erb-B2) gene, located on chromosome 17q11, encodes the HER2 protein which is normally involved in the signal transduction pathways leading to cell growth and differentiation. This proto-oncogene is mainly expressed in epithelial tissue and activated due to its amplification[12]. Over expression of extracellular domain of Her2/neu is common as ovarian carcinoma progress. Over expression of HER2 initiates intracellular signaling pathways involved in cell proliferation, differentiation, migration and apoptosis. Recent research has demonstrated that this gene's amplification or over expression plays a significant role in the etiology and progression of several aggressive forms of breast cancer. Over time, it has developed into a significant biomarker and therapeutic target for 30% of breast cancer patients. Her-2/neu over expression/amplification has been reported in ovarian cancer and is associated with poor clinical outcome but the exact percentage of HER2/ neu expression in ovarian carcinomas varies widely in the literature between 8% and 66%. So, the aim of our study is to evaluate expression of HER2/neu in surface epithelial ovarian carcinomas.

Ki-67 protein is a cellular marker for proliferation. It is an excellent marker to determine the growth fraction of given cell population. The proportion of tumor cells expressing Ki-67 (known as the Ki-67 labeling index) is frequently associated with the progression of cancer [13].

Proliferative activity and steroid hormone receptor status along with clinical and morphological characteristics of serous ovarian carcinoma possess prognostic significance and may be used for evaluation of the disease course. The need of the hour is for a better understanding of the pathogenesis of ovarian tumors immuno histochemical markers facilitates early diagnosis, prognostic assessments and targeted therapy. Many biomarkers like p53, EGFR, Cytokeratin, ER PR etc. are used to predict the prognosis and planning for better treatment.

Present study was for estimating the prognosis by linking the patient's age, tumor size, and tumor grade.

OBJECTIVES

1. Assessment of expression of HER2/neu and ki67 in different surface epithelial tumors of ovary.
2. Correlation of expression of these antibodies with histological type, grading and staging of different tumors.

MATERIALS AND METHODS

The retrospective study of '**Utility of HER2/NEU and Ki67 Immunostaining and Their Correlation with Histological Types, Grades & Staging of Surface Epithelial Tumours of Ovary'** was carried out over a period of 2 years from 2018 to 2020 in the Department of Pathology in collaboration with the Department of Gynecology of Hi-Tech Medical College and Hospital, Bhubaneswar.

Ethics Approval

The study was approved by the Institutional Ethics Committee.

Inclusion Criteria

Patients included in our study were: All the diagnosed cases of surface epithelial tumor of ovary undergone surgery.

Exclusion Criteria

l. Non neoplastic lesions, Non surface epithelial tumors and metastatic lesions were excluded.
2. Those who did not give consent to IHC
3. Inadequate tissue samples
4. Improperly preserved tissues

Consent

Patients were explained about the study and a written consent was taken using standard questionnaire format.

Data were collected following parameters (like Age, Menstrual status,. Mode of presentation e.g. Abdominal mass and pain, ascites, pleural effusion, GI and urinary Symptoms, Investigation- X-ray, USG of chest, USG of abdomen, Cytological examination of ascitic and pleural fluid) and results of each case after doing histopathological and immunohistochemical study in the Pathology Hi-Tech Medical College and Hospital, Bhubaneswar in collaboration with Obstetrics and Gynecology department. The embedded tissue in paraffin blocks were sectioned at 5micron thickness by using Rotary microtome machine for H&E staining and Section at 3 micron thickness was mounted on the poly –L-lysine coated slides for immunohistochemistry for HER2/neu and Ki-67. The immunostaining was carried out by using avidin biotin peroxidase method. The antibodies and chemicals were obtained from Rabbit Anti-c-erbB2 Monoclonal Antibody to HER2 surface antigen (clone SP3) was used for IHC evaluation of HER2/neu and anti Ki-67 monoclonal antibody were used as primary antibodies incubated for 60 minutes. For secondary antibody slides were tagged with HRP (horseradish peroxidase) was added and incubated for 30 minutes at room temperature. Addition of chromogen: DAB solution was added on slides and kept for 5 - 10 minutes. • DAB solution - 1 ml of DAB buffer + 2 drops of DAB chromogen + 10µl of hydrogen peroxidase and counterstained with Mayer's hematoxylin. Granular brown staining of membrane of epithelial cells showed positive for Her2/neu. Intranulear brown staining of tumor cells were considered as positive for Ki-67

Guidelines for Interpretation of HER2/neu (according to **Sophia K.et.al.** scoring system at 40x) (2016) [14].

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.

Guidelines for Interpretation of Ki-67 LABELLING INDEX (taken from YUSRA ABDUL KHALIQ QUASIM et al, 2017) [15]

The nuclear staining for Ki-67 was graded by counting Ki -67 labeling index (Li) as a percent of positively stained tumour nuclei in 1000 tumour cells in hot spot area of tumour[14].

≤ 10% - NEGATIVE
10% - 20% - +
30% - 50% - ++
≥ 50% - +++

Table no. 1: Immunohistochemical slides were scored for Ki-67 as follows (Giurgea et al.2012).

Number of cells with nuclear positivity	**Score**
0	0
1-10 %	1
10-50 %	2
50-100 %	3

Statistical analysis
It was constructed to be met with the correlation of histopathology, clinical and immunohistological data was changed into a master chat, then chi square was used for statistical analysis. All were done in the form of percentage and represented as table & figures.

RESULTS
This study included 75 patients which were proven neoplastic lesions on histopathology study, out of these 46 cases were benign, 9 cases were borderline and 20 cases were under malignant.

Table no. 2: Age Distribution of patients studied

SL NO	**AGE GROUPS**	**BENIGN**	**BORDERLINE**	**MALIGNANT**	**TOTAL**
1	11-20	0	0	0	0
2	21-30	11	0	0	11
3	31-40	6	4	0	10
4	41-50	17	5	6	28
5	51-60	8	0	7	15
6	61-70	4	0	4	8
7	71-80	0	0	3	3
Total		46	9	20	75

In the present study age ranged from 22 to 77 years and mean age was 46.16years. Majority cases about (28 cases -37.33%) belonged to 41-50 years. Majority of cases 46(61.3%) belonged to Benign type.

Table no. 3: Distribution of surface epithelial tumor on Histopathology.

SL NO	HISTOTYPE	NUMBER	PERCENTAGE
1	SEROUS TUMOUR	45	60
2	MUCINOUS TUMOUR	28	37.34
3	BRENNER TUMOUR	1	1.33
4	CLEARCELL TUMOUR	1	1.33
Total		75	100

In our study majority of cases were serous tumours 45(60%).

Figure 1: Distribution of surface epithelial tumor and laterality of involvement.

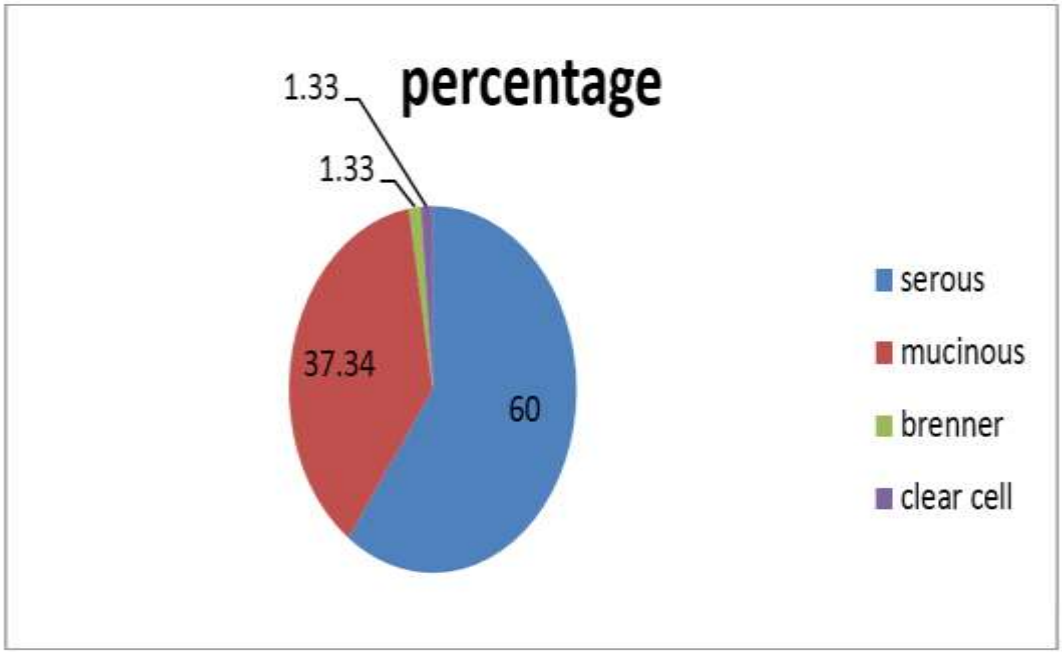


Table no. 4: Distribution of Epithelial ovarian tumors based on gross morphology

FEATURES	GROSS			Unilateral	Bilateral	TOTAL
LESIONS	Solid	Cystic	Both			
BENIGN	0	42(91.3%)	4(8.7%)	43	3	46
BORDERLINE	0	7(77.78%)	2(22.2%)	09	0	9
MALIGNANT	5(25%)	1(5%)	14(70%)	11	9	20

In our study, Most of the Benign tumors i.e. 42 (91.3%) were cystic on cut surface and most of the Benign tumors were unilateral 43 (93.48%) in presentation. 11 out of 20 (55%) of Malignant tumors were bilateral.

Table no. 5: Distribution of malignant tumors according to FIGO staging system.

	STAGE I	STAGE II	STAGE III	STAGE IV	TOTAL
NO OF CASES	9	8	2	1	20
PERCENTAGE	45%	40%	10%	5%	100

In our study, there were 9 malignant tumors in stage I (45%), 8 tumors in stage II (40%), 2 tumors in stage III (10%) and 1 in stage IV (5%).

Figure 2: Distribution of malignant tumors according to FIGO staging system

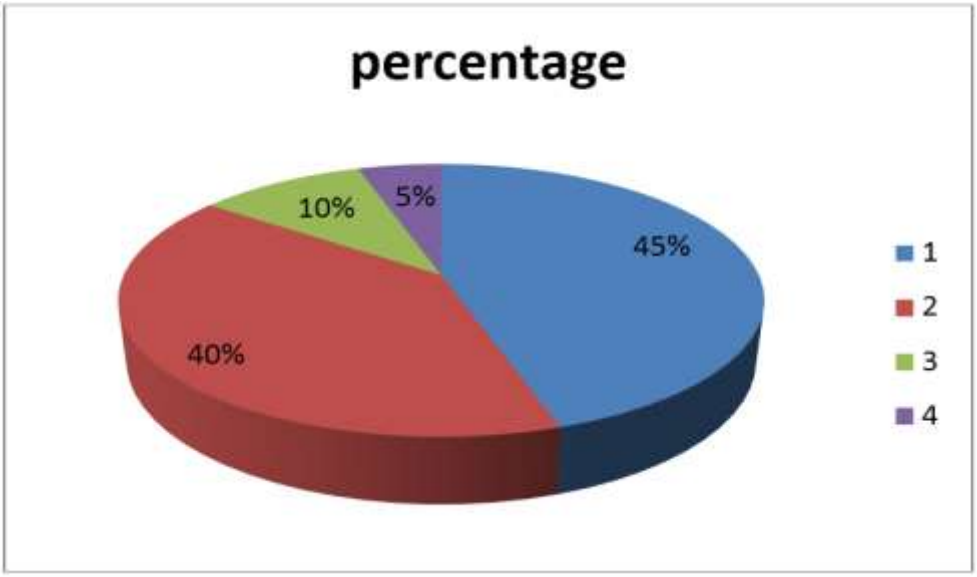
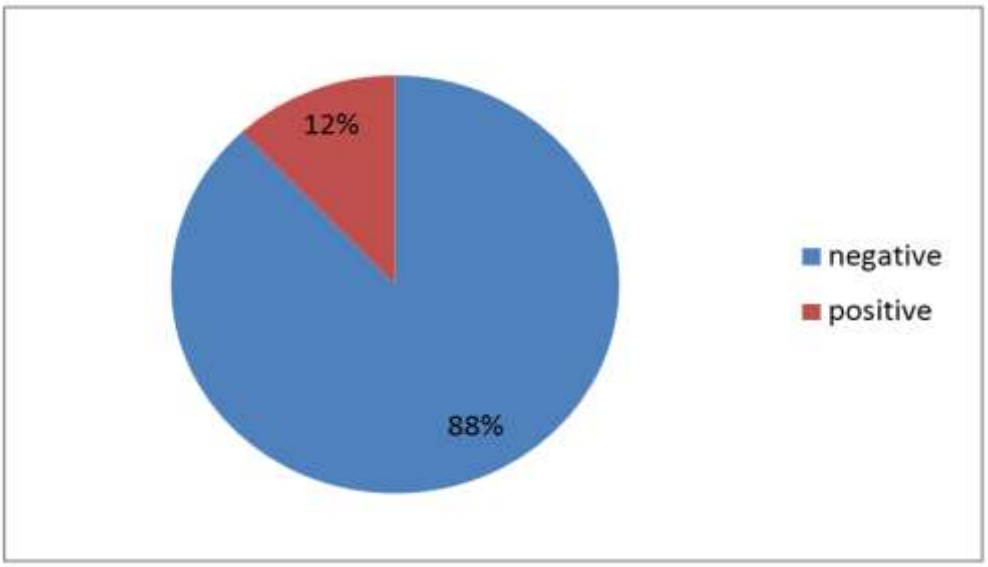


Table no. 6: Distribution of ovarian tumors according to HER2/neu expression

Her 2 neu	Benign	Borderline	Malignant	Number of cases	percentages
Negative	46	9	11	66	88%
Positive	0	0	9	9	12%
Total	46	9	20	75	100%

Figure 3: HER2/neu status in ovarian tumour



In our study, 12% of cases were Her 2 neu positive

Table no. 7: Immunohistochemistry: Expression profile of HER2/neu in Malignant tumors.

SL. NO	HISTOTYPE	NEGATIVCE	POSITIVE	TOTAL
1	HIGH GRADE SEROUS CARCINOMA	0	9	9
2	LOW GRADE SEROUS CARCINOMA	5	0	5
3	MUCINOUS CARCINOMA	4	0	4
4	CLEAR CELL CARCINOMA	1	0	1
5	MALIGNANT BRENNER TUMOUR	1	0	1

In present study, HER2/neu expression is higher in high grade serous carcinoma when compared to low grade tumour, mucinous, clear cell and brenner tumour.

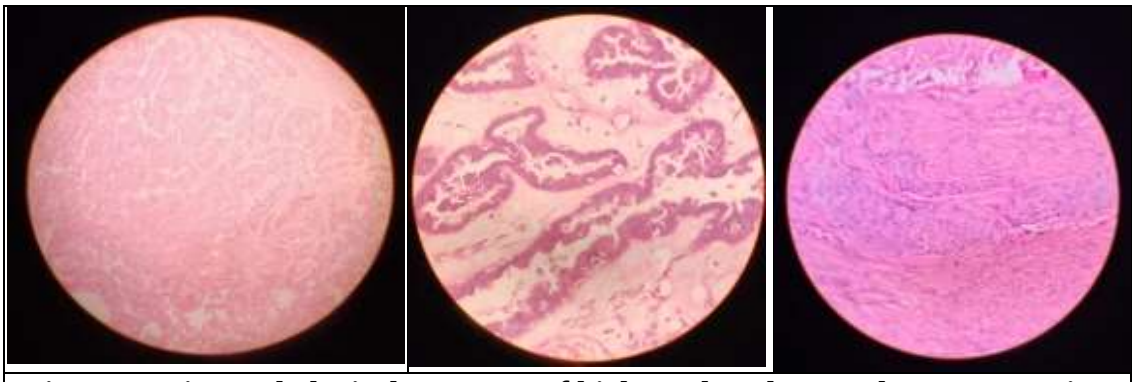


Figure 4: Histopathological spectrum of high grade to low grade serous ovarian tumor in upper panel.

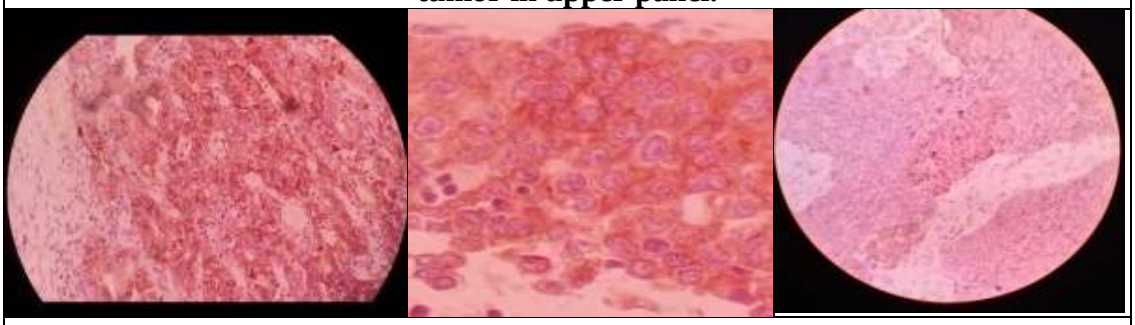


Figure 5: HER2/neu expression over those ovarin tumor (score 3,2,1)

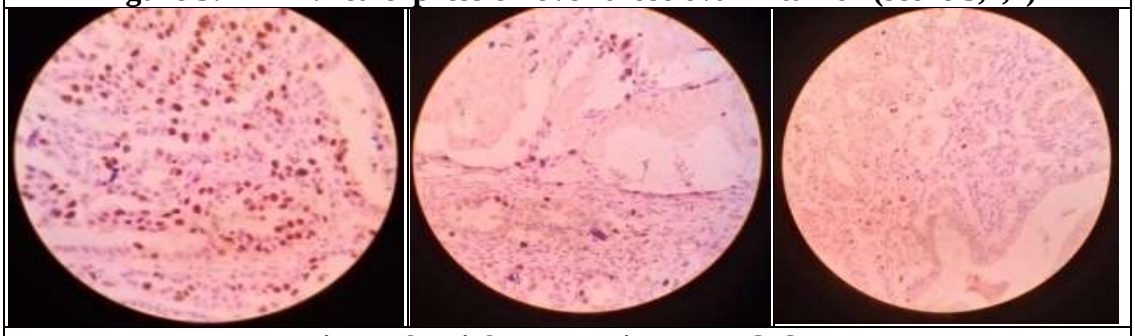


Figure 6: Ki-67 expression score 3, 2, 1

Only+3 staining is included as HER2/neu positive. The expression of HER2/neu in Borderline tumors showed negative expression of HER2/neu. It is observed that all the benign tumors showed negative expression of HER2/neu.

Table no. 8. HER2/neu status with age of the patient

AGE(yrs) n=75	**HER2/neu negative**	**HER2/neu positive**	**P value**
≤ 50	45(68.18%)	3(33.33%)	
≥ 50	21(31.82%)	6(66.67%)	0.16
Total	66(88%)	9(12%)	

Table no. 9: HER2/ neu correlation with tumour grade

TUMOUR GRADE (n-14)	Number of patients	Percentage	HER2/neu negative	HER2/neu positive	P value
High	10	31.30%	0	9	.0001
Low	05	15.60%	5	0	
Total	17	53.10%	5	9	

Table no. 10: HER2/neu with stage of the tumour

STAGE(n=20)	HER2/neu negative	HER2/neu positive	P value
I	9	0	
II	2	6	0.34
III	0	2	
IV	0	1	
Total	11	9	

Table no. 11: Correlation of histological grade with HER2/neu score

Histological Grade	HER2/neu				P value
	0	1	2	3	
Grade 1	2	2	5	1	0.030
Percentage	20.00%	20.00%	50.00%	10.00%	
Grade 2	0	1	2	2	
Percentage	0.00%	20.00%	40.00%	40.00%	
Grade 3	0	0	7	10	
Percentage	0.00%	0.00%	41.20%	58.80%	

Table no. 12: Correlation of histological grade with Ki-67 score

Histological Grade	Ki -67			P value
	0	1	2	3	
Grade 1	2	1	3	4	0.006
Percentage	20.00%	10.00%	30.00%	40.00%	
Grade 2	0	1	2	2	
Percentage	0.00%	20.00%	40.00%	40.00%	
Grade 3	0	0	3	14	
Percentage	0.00%	0.00%	17.60%	82.40%	

DISCUSSION

This retrospective study included 75 cases of surface epithelial ovarian tumor. We used two immunomarkers HER2/neu & Ki -67 for our study for correlation with histological grade.

In our study in which HER-2/neu expression was assessed in 63 cases. In this study HER2/neu status was determined by immunohistochemistry and all IHC 3+ tumors are accepted as HER2/neu positive cases.

Maximum numbers of cases (26 cases, 41.33%) were found in the age group 50-70 years followed by age group 40-50 years (17cases, 27%). Of all ovarian neoplasms approximately two thirds were surface epithelial tumors and their malignant forms represent 90% of all ovarian cancers[16]. In our study, surface epithelial tumors which are consistent with the studies done by Zaman et al[17]. They did not find any correlation with age in the Satyanarayana P et al (2016)[18] Age is described as an independent prognostic factor in ovarian tumours[15]. The incidence of ovarian cancer rises exponentially with age[16]. About 1 in 10 tumors in patients less than 45years is malignant, which was increases to one in 3 in older women. Borderline tumors were found in women in their 25- 30's. The mean age of diagnosis in our study was 48.55 years. The age varied from 14-76 years. The age distribution was comparable with studies done by Zaman et al., Pilli et al., Jha et al., Kayastha et al., and Mankar et al. [17,19,20].

The clues to their nature depend on the laterality of ovarian cancers[21]. Bilaterality is a common feature of metastatic tumors and an important diagnostic clue. But one has to be cautious while diagnosing them, because typical serous or undifferentiated carcinomas can also be bilateral. In our study, majority of the cases (93.6%) were unilateral. Kanthikar et al. also reported higher incidence of unilateral tumors in their study[21] but malignant tumors are bilateral.

Histopathological analysis of the 75 cases of ovarian surface epithelial tumors 46 were benign (61%), 9 borderline (12%) and 20 were malignant (27%). All the patients' with malignant tumors

were above 50 years of age. In general, we found that most of the Patients with ovarian tumors were above 50years of age (n=41.3%). Serous tumors formed the majority with 18 cases (28.57%), followed by 16 cases of mucinous tumors(25.39%), clear cell & endometrioid tumors both of which compromises 9 cases (14.28%), followed by Seromucinous 8 cases(12.79%), Brenner 2 cases(3.19%) & Undifferentiated tumors 1 case (1.5%).

Carcinoma arises in the ovary and is confined to the ovary that is not sure. It usually disseminating into pelvis, abdominal cavity and distant sites forms the basis of International Federation of Gynecology and Obstetrics (FIGO) staging system. Progression of ovarian cancers is poorly understood[22].So to study about precursor of ovarian cancers is difficult as ovaries are not readily accessible for screening, and identification of assumed precursor lesion is based on the microscopic examination of ovary. This means that the natural history of the lesion is not visible[13,22].

Table no. 17: Distribution of different histopathological types of ovarian neoplasms reported in some contemporary studies from Asian countries

SL.NO	Author (Year)	NUMBER	SEROUS	MUCINOUS	OTHERS
1	Jha and Karki (2008)	161	77 (47.8%)	50 (31.1%)	34 (21.1%)
2	Mondal et al. (2011)	702	447(63.68%)	158 (22.51%)	97 (13.8%)
3	Abdullah et al. (2012)	84	28 (33.3%)	13 (15.4%)	43 (51.2%)
4	Jindal (2014)	53	23 (43.4%)	3 (5.7%)	27 (50.9%)
5	Vaidya et al. (2014)	363	158(43.5%)	92 (25.3%)	113 (31.1%)
6	Makwana et al.(2014)	135	60 (44.4%)	18 (13.3%)	57 (42.2%)
7	OUR STUDY	75	45(60%)	28(37.34%)	2 (2.66%)

Table no. 18: Correlation of Her2/neu status on Ovarian Cancer in various studies.

PREVIOUS STUDIES	HER2/neu positivity percentage
Salmon et al 1989	26%
Bookman et al 2003	11 %
Dimova et al 2006	11%
Tuefferd M et al2007	12.8%
Steffensen KD et al 2008	14.1%
McAlpine et al 2009	18.2%
Sylvia et al 2012	21%
Sapna et al 2014	24.3%
Ajani et al 2016	37%
Ndukwe et al 2018	18%
Azeem et al 2018	8.5%
Our study	27.9%

Our study comprised of maximum number of cases of serous histotype (60%). This was similar to other studies conducted by Mondal et al. (2011)[23]. High grade tumors constituted maximum number of cases in the present study, comprising of 19 cases (57.57%). Our findings were similar to study conducted by Nielsen et al, in which 45% tumors were poorly differentiated followed by 30% well differentiated tumors and 25% moderately differentiated tumors with a significant p value of<0.001.

Maximum number of cases did not express HER2/neu. The study conducted by Salmon et al 1989 showed that HER2/neu was expressed in 26 % epithelial ovarian carcinoma. 65% ovarian carcinomas did not express HER2/neu which is similar to the observation in our study. Another study conducted by Goel et al revealed that 25.67% malignant ovarian tumors and 5.40% borderline tumors did not express HER2/neu. Mucinous tumors were observed to show strong HER2/neu

expression. In comparison to mucinous tumor, serous tumor showed more number of negative HER2/neu expression.

Table no. 19: Correlation of Ki -67 status on Ovarian Cancer in various studies.

PREVIOUS STUDIES	**Ki 67 positivity percentage**
Nesrin Gursan et al	52.70%(Grade III)
Sylvia et al	41.3%(Grade III)
Asha Mahadevan et al	65.34%(Grade III)
Luminita Nkoleta Giurgea et al	61.53%(Grade III)
Rekha et al	75%(Grade III)
Our study	32.80%(Grade III)

HER2/neu an epidermal growth factor receptor family member, overexpressed in 20-30% of ovarian cancer[7]. Berchuck et al., first recognised that, HER2/neu overexpression is related to poor survival of patients with late stages of malignant SEOTs which states that patients with HER2/neu overexpression had worse prognosis than patients with normal HER2/neu expression. In addition, patientswith high HER2/neu expression did not show complete response to therapy[9].

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Identifying early-stage malignant lesions and identifying prognostic biomarkers are the top concerns of recent research. Structural similarity of EGFR and HER2/neu growth factors, have led to the hypothesis that over expression of both tyrosine kinase proteins are involved in signal transduction for the corresponding growth factors and activation of pathways that ultimately lead to ovarian cancer[21]. Ovarian cancer carries a high mortality rate because of its typical insidious onset and late diagnosis, they become larger without producing symptoms of pain and pressure in abdominal cavity[24].By the time of diagnosis, 70% of tumors have spread far away from the ovary and 60 % of the tumors spread far away from the pelvis.
Even with the current established therapies of radical surgical tumor debulking and platinum plus paclitaxel-based chemotherapy, the 5-years survival rate for patients with ovarian cancer is still only about 40%.

CONCLUSION

The present study found Ki 67 expression is seen in 32.80 % cases considering 3+ as positive which consists of high grade tumors. The expression of HER2/neu changed from high to negative as the tumor grade increased from borderline to high grade. We observed a significant association between the nodal metastasis and upregulation of HER2/neu expression. Her2 /neu scoring was found to be significantly associated with grade of tumor grade III being more associated with HER2/neu positivity. HER2 over expression in present scenario was mostly utilized in different types of cancers for targeted therapy; it can be used as a prognostic marker for surface epithelial ovarian tumors that can help the clinician for targeted therapy using TRASTUZUMAB therapy with HER2/neu positivity. A larger sample size and a long term follow up of the patient can further add to the role of Her2/neu in the diagnosis and treatment of patients with surface epithelial ovarian tumors.

Conflict of Interest: Nil

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