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Original Research Article

Immunohistochemical Analysis of P-53 and Ki-67 Expression in Surface Epithelial Ovarian Tumor

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Conflict of interest: Nil

Abstract

Background: Ovarian cancer is the sixth most common female cancer worldwide and third most common gynaecological cancer. Surface epithelial tumors of ovary (SEOT) are commonest among of all ovarian tumors and their malignant forms represents about 90% of malignant ovarian tumors. It is well known for its late presentation at advanced stage. Recently molecular and proliferative markers including p-53 and Ki-67 have emerged as promising adjuvant diagnostic tool for early diagnosis and identification of their biological behavior. Over expression of p-53 and Ki-67 has been claimed to be a marker of aggressiveness in SEOT.

Aim And Objectives: To study and interpret the expression pattern of p-53 and Ki-67 in benign, borderline and malignant SEOT and assessing early aggressiveness and tumour progression of SEOT.

Material & Methods: The study was performed on 100 consecutive resected specimen of SEOT received in pathology department over a period of one year. 10% Formalin fixed, paraffin embedded tissue sections were stained by haematoxylin & Eosin stain for histological analysis and immunohistochemical staining was performed using monoclonal antibodies against p-53 and Ki-67 in all the cases. The expression pattern of p-53 and Ki-67 was studied in terms of proportion score in p-53 and Labelling index (LI) in Ki-67.

Result: In the study of 100 cases of SEOT benign lesions (49%) followed by malignant lesions (37%) and borderline lesions (14%). The expression of p-53 was less in benign (7/49, 14.3%) than borderline (8/14, 57.1%) and malignant tumors (33/37, 89.2%). The Ki-67 expression was more in malignant (35/37, 94.6%) than borderline (13/14, 92.8%) and benign tumors (5/49, 10.2%). A positive correlation were observed in p-53 and Ki-67 expression with tumour grade and FIGO stage. Their co-expression was seen in 46% of cases that was statistically significant (p- value < 0.000012).

Conclusion: p-53 and Ki-67 expression correlate with worse prognosis in SEOTs and are useful adjuvant marker for understanding of biological behavior of these tumors which helps in guiding appropriate management and therapy in these patient.

Introduction

Ovarian cancer is the sixth most common female cancer worldwide [1] and accounts for 80% of all the gynecological malignancies in India.2 Indian Cancer Registry data project ovary as an important site of cancer in women, comprising up to 8.7% of cancers in different parts of the country. [3] It has been recognized as most lethal gynecological malignancy [2]. The symptoms of ovarian cancer are not seen in initial stages and most patients presents in their late stages of carcinoma. Among various risk factors associated with ovarian tumors are older age, positive family history, genetic predisposition plays important role. [4] Around 5 – 10% of ovarian cancers are hereditary. Mutations in BRCA-1 and BRCA-2 tumor suppressor gene possess increased risk for ovarian carcinoma [5]. According to WHO Histological classification, ovarian tumors are categorized into 3 major categories: 1) Surface epithelial tumor, 2) sex cord-stromal tumor, 3) Germ cell tumors [6]. Surface epithelial tumors of the ovary account for approximately 2/3rd of all ovarian neoplasm, & their malignant in about 90% of malignant ovarian cases [7]. According to WHO (2020) surface epithelial tumors of the ovary are categorized on the basis of their morphological features as: 1) Serous tumors {benign (60%), borderline (15%), malignant (25%)} 2) Mucinous tumors {benign (80%), borderline (10%) , malignant (10%)}, 3) Endometrioid tumors, 4) Clear cell tumors, 5) Brenner tumors, 6) Seromucinous tumors 7) Other carcinomas. These subtypes differ significantly in their potential sites of origin, molecular signature, prognosis, and response to treatment [8]. Recently various molecular and proliferative markers including p-53 and Ki-67 have emerged as promising diagnostic tool [9]. P-53 is a tumor suppressor gene situated on chromosome 17 [10]. p-53gene mutation results in loss of function of its protein product thus results in uncontrolled cell proliferation. It is the most common tumor suppressor gene involved with human malignancies [11]. Mutations in TP-53 has been reported in high grade and rarely in low grade serous ovarian cancers [12]. Abnormal p-53 protein is resistant to degradation and has a prolonged half-life which allows its detection by immunohistochemical staining [13]. Similarly Ki-67 is a nuclear non-histone protein and is an excellent immunohistochemical marker to determine proliferating cells of the tumor. Ki-67 is a nuclear antigen located on chromosome 10q25, which is only detected in dividing cells (G1-S-G2-M phase) and not in quiescent cells (G0 phase) [14]. The monoclonal Ki-67 / MIB-1 antibody reacts with the nuclear Ki-67 antigen expressed in proliferating cells [15]. Ki-67 correlates well with tumor type and its clinical stage. So, it can be included with routine histopathological study of ovarian surface epithelial tumors, especially in cases of borderline tumor variety for defining their biological behavior and establishing furthur treatment guidelines [16]. Present study aim to assess the utility of tumor markers p-53 and Ki-67 to aid in early diagnosis, risk assessment, aggressiveness, management, and appropriate therapy in

patients of surface epithelial ovarian tumors.

Material and Methods

An observational Prospective study was done on 100 resected surface epithelial ovarian tumor specimens of oophrectomy and total abdominal hysterectomy received in the Department of Pathology, B.R.D. Medical College, Gorakhpur for histopathological examination during period from July 2021 to June 2022. All samples of patients of ovary were selected on the basis of inclusion and exclusion criteria. Oophrectomy specimens were fixed in 10% formal saline and subjected To histopathological examination using paraffin embedding technique. Histopathological diagnosis was made and then freshly cut sections were also used for immunostaining. Immunohistochemical staining for p-53 was performed (monoclonal mouse antibody, anti-p-53 manufactured by, Genome Me ready to use) as per standard protocol. Ki-67 immunohistochemical stain was performed (monoclonal rabbit antibody, anti-Ki-67 manufactured by, GenomeMe ready to use) as per standard protocol. Positive and negative controls were run simultaneously with all patient's specimens. Immunohistochemical expression of p-53 & Ki-67 was analyzed in all surface epithelial ovarian tumors and its correlation with histopathological grading was studied.

Immunohistochemical Interpretation: Positive cells were determined by counting 1000 cells in at least 10HPF(X40) for each case. The distribution of p-53 immunoreactivity in SEOT were quantitatively assessed as –ve [<10%cells], 1+ [10-30%cells], 2+ [30-50%cells], 3+ [>50%cells] positive cells. The percentage of immunoreactive tumor cell nuclei was expressed as a Labelling index (Ki-67 LI). Quantitatively assessed as –ve [<1%cells], 1+ [1-10% cells], 2+ [10-20%cells], 3+ [>20%cells]. Positive immunostaining was observed as brown, granular nuclear staining for both immunomarker [17].

Statistical Analysis: Microsoft excel sheet was used to tabulate the collected data, which was additionally analyzed using Statistical package for Social Sciences (SPSS, version 21). Categorical data was represented as frequencies and percentage. Chi- square test used as test of significance for categorical data. P-value <0.05 was considered as statistically significant.

Results

Total 100 specimens of surface epithelial ovarian tumors were received in the Department of Pathology were analysed histopathologically and IHC for p-53 and Ki-67 protein was done. The age of patients ranged from 20 to 80 years. Peak age for benign lesion was seen in third and fourth decade (88.2%) while majority of malignant lesions (83.3%) was seen in seventh decade of life in the patients studied. Most of patients were premenopausal (56%) compared to postmenopausal patients (44%). Majority of patients presented with complaint of abdominal mass (64%), followed by abdominal pain (20%) and ascites (13%) while 3% patients were asymptomatic. Majority of tumors were unilateral (89%). Bilateral tumors were seen in only (11%). On gross appearance, (53%) tumors had smooth surface while (47%) tumor surface had nodularity. Most of the tumors (58%) were cystic, (11%) were solid and (31%) tumors revealed both solid and cystic areas. (49%) tumors were uniloculated and were (51%) multiloculated.

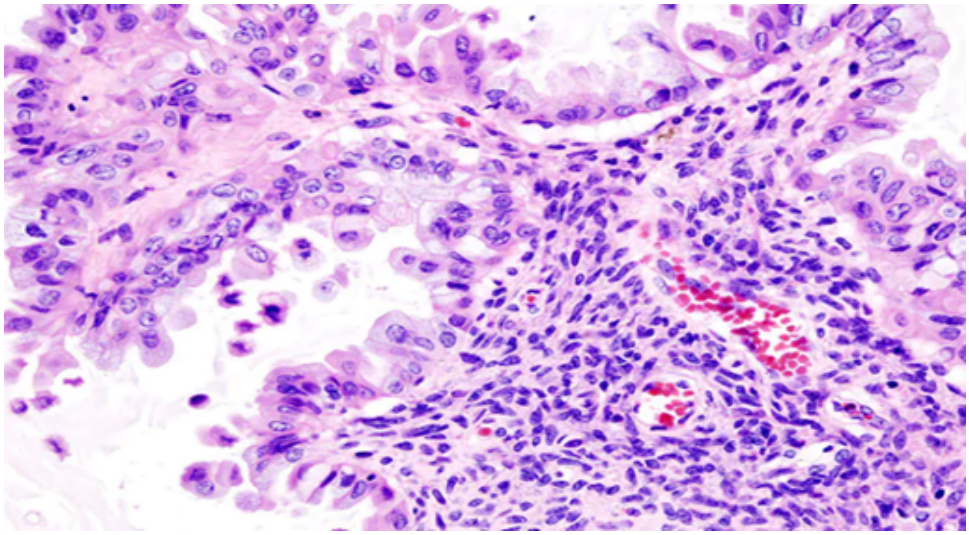

Figure 1: Serous borderline carcinoma showing papillary fronds lined by cells with moderate atypia, without stromal invasion (400x)

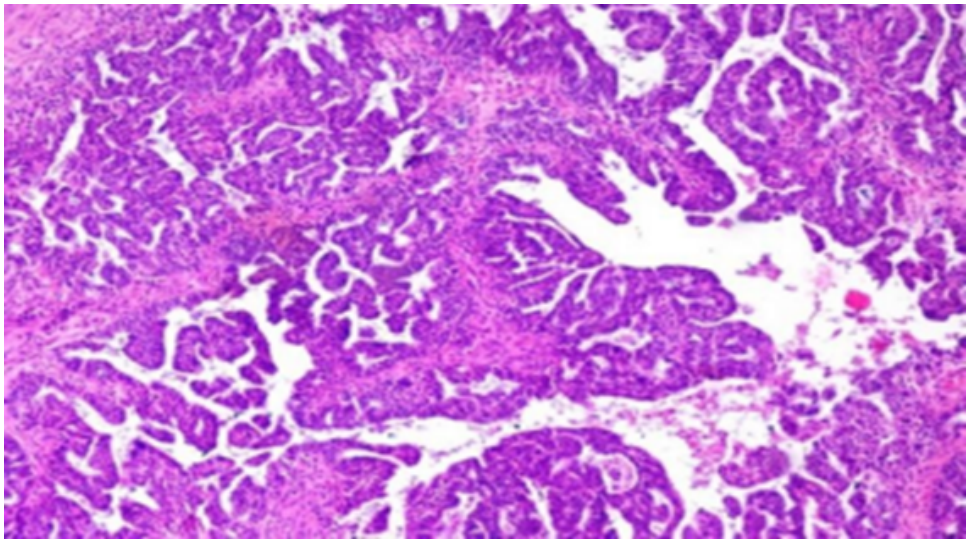

Figure 2: Serous adenocarcinoma showing papillary fronds lined by cells with marked atypia, with stromal invasion (400x)

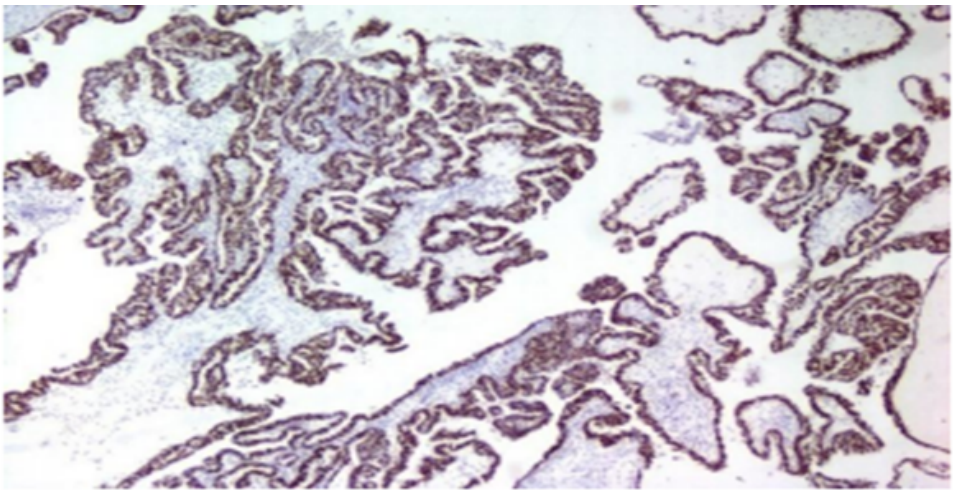

Figure 3: Section of serous adenocarcinoma showing strong p53 expression, nuclear positivity (400x)

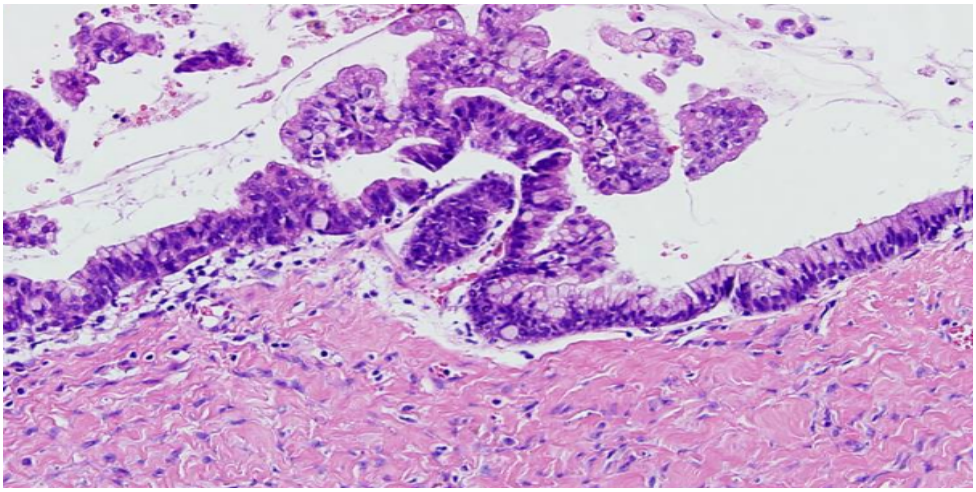

Figure 4: Mucinous borderline carcinoma showing goblet cells and stratification in epithelium (400x)

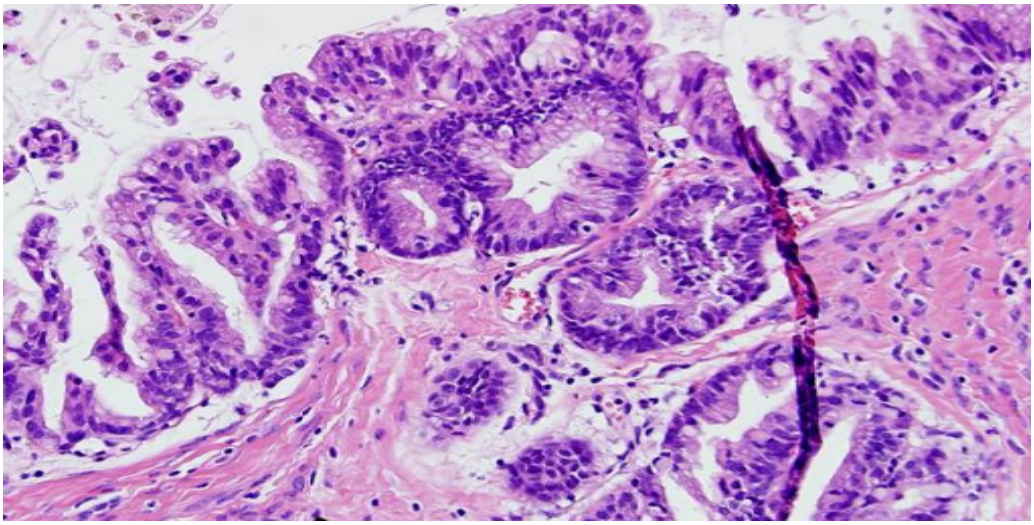

Figure 5: Mucinous adenocarcinoma showing goblet cells and stratification in epithelium with expansile invasion and packed glands (400x)

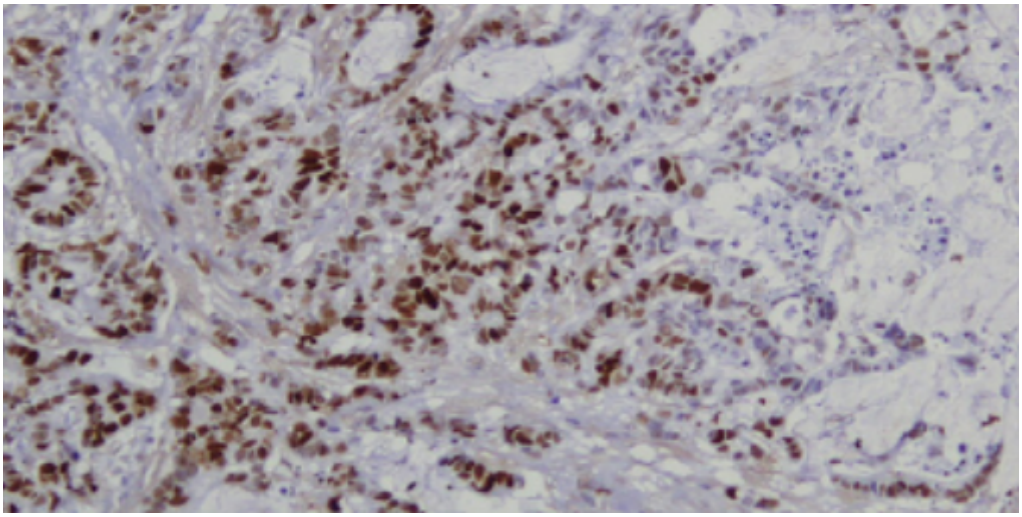

Figure 6: Section of mucinous adenocarcinoma showing strong p-53 expression, nuclear positivity (400x)

Histological classification was done in accordance with WHO classification of ovarian tumors. Serous tumors (63%) were the most frequent tumors encountered in the study followed by Mucinous tumors (33%). Case of Endometrioid tumors (2%) , Brenner tumor and Clear cell tumor accounted for (1%) case in each group.

Tumors were further categorized as benign, borderline and malignant based upon their histological features. Majority of tumors were benign (49%) followed by malignant (37%) and borderline tumors (14%). Serous cystadenomas 28/49 (57.2%) constituted the predominant group among the benign tumors followed by mucinous cystadenomas 17/49 (34.7%) serous cystadenofibroma 3/49 (6.1%) and Brenner tumor 1/49 (2%). Majority of benign tumors presented in premenopausal patients.

Borderline tumors accounted for 14 cases and comprised of 9/14 (64.3%) Borderline serous tumors followed by 5/14(35.7%) Borderline mucious tumors.

Amongst malignant tumors, Serous carcinoma constituted of total malignant cases 23/37(62.2%) followed by Mucinous carcinoma 11/37(29.8%), Endometrioid tumor 2/37(5.3%) and Clear cell type tumor 1/37 (2.7%).

Among 23 cases of serous carcinoma malignant tumors were graded according to “two tier grading system” as low and high grade tumors. Among them 4 cases (17.4%) were low grade while 19 cases (82.6%) were high grade serous carcinoma. Out of malignant surface epithelial tumors other than malignant serous tumors 4 cases were Grade 1, 10 were Grade 2 and 5 were Grade 3 tumors.

p-53 immunostaining was performed on all 100 cases of SEOT, 48 cases (48%) out of 100 SEOT showed brown colour positive nuclear staining for p53 immunostaining. Among 48/100 cases with positive p53 expression 20/48 (41.7%) showed +3 IHC score followed by 17/48 (35.4%) cases with +2 IHC score. Only 11/48 (22.9%) cases revealed +1 IHC score. +3 IHC score was observed mainly revealed in malignant tumors. +2 IHC score was observed in 10 malignant cases, 4 cases of borderline tumors and 3 cases of benign tumors. Whereas +1 IHC score observed in 4 cases of benign and malignant lesion, 3 cases of borderline lesion. While 52/100 cases were show negative expression for p-53 immunostaining majority of were benign lesions i.e. 42 cases. In our study p53 expression was found to be more in malignant tumors 33/37 (89.2%) as compared to borderline 8/14 (57.1%) and benign tumors 7/49 (14.3%). Tumor type showed statistical significant correlation with p53 positivity (p=0.000014). Association of p-53 staining with tumor stage 58.3% cases of stage I, 66.6%, 84.6%, and 100% cases of stage II, III, IV respectively revealed positive expression. Hence p-53 positive expression more in high stage tumors.

Ki-67 immunostaining was performed on all 100 cases of SEOT, 53 cases(53%) out of 100 SEOT showed brown colour positive nuclear staining for Ki-67 immunostaining. Among 53 cases with positive Ki-67 expression 21/53 (39.6%) showed +3 IHC score followed by 20/53 (37.7%) cases with +2 IHC score. Only 12/53 (22.7%) cases revealed +1 IHC score. +3 IHC score was observed mainly in malignant tumors. +2 IHC score was observed in 12 malignant cases, 6 cases of borderline tumors and 2 cases of benign tumors. Whereas +1 IHC score observed in 3 cases of benign and 4 cases of malignant lesion, 5 cases of borderline lesion. 47 cases were show negative expression for Ki-67 immunostaining majority of were benign lesions i.e. 44 cases. In our study Ki-67 expression was found to be more in malignant tumors 35/37 (94.6%) as compared to borderline

13/14 (92.8%) and benign tumors 5/49 (10.2%). Tumor type showed statistical significant correlation with Ki-67 positivity (p=0.000012). Hence Ki-67 positivity more towards the malignant lesions. Association of Ki-67 staining with tumor stage 91.7% cases of stage I, 100%, 92.2%, and 100% cases of stage II, III, IV respectively revealed positive expression. Hence Ki-67 positive expression more in high stage tumors.

Statistically significant correlation was observed between immune expression of both p-53 and Ki-67 markers for diagnosis of epithelial tumors (p value=0.000012); as with high p-53 over expression, there was a tendency towards a higher expression of Ki-67. There co-expression was seen in 46 cases out of 100 cases of SEOT.

Discussion:

Ovarian cancer is a wide-ranging term that groups together a diverse set of neoplasm originating from the ovary, with carcinomas comprising 90% of ovarian cancer. Based upon morphological criteria, SEOTs are classified as serous, mucinous, endometrioid, Brenner, clear cell type, seromucinous and others [18]. The etiology of SEOTs is poorly understood and although several risk factors have been identified, their direct involvement remains largely unaddressed. Somatic and germline genetic mutations in one the important risk factors in ovarian carcinogenesis [19].

p-53 is the most frequently altered gene in human cancers and loss of functional p-53 protein occurs in most epithelial ovarian cancers [20]. Ki-67 is a proliferation markers helpful in predicting disease outcome in many types of malignancies including ovarian neoplasm [21]. Hence there is a need to study p-53 and Ki-67 IHC in an Indian cohort considering all the technical factors that could potentially affect the staining (antibody clone, IHC technique, interpretation of staining, etc.) The study evaluated the histomorphological spectrum of SEOTs and expression of p-53 and Ki-67 by IHC in the different histological types and grades of SEOTs.

In the present study 100 cases of SEOTs were distributed in age range of 20-80 years. It can be observed that benign tumors mainly occurs in younger age group while malignant SEOTs occur in the sixth and seventh decades. The age wise distribution of various tumor types is comparable with Mohapatra I et al. (2021) [17] and Qasim et al. (2017) [22].

We found 48% of total cases were positive for p-53 expression. Singh A et al.(2021) [23], Mohapatra I et al. (2021) [17], Hamdi et al. (2012) [24], and Tiwari et al.(2016) [25] also found positive expression for p-53 in 55.7% 44.7%, 43.3% and 51.2% of their total cases respectively.

In present study p53 expression was found to be more in malignant tumors (89.2%) as compared to borderline (57.1%) and benign tumors (14.3%) While 85.7% benign tumors showed negative expression. Abbassi F. et al. (2016) [26], Qasim et(2017) [22] also observed majority of malignant tumors revealing p-53 expression.

In our study we found that p53 expression was significantly higher (+3 IHC score) in malignant tumors (51.3%) while none of benign tumors show +3 IHC score. (p value = 0.000014). which is comparable with the study of Mohapatra I et al. (2021) [17] who studied that 52.6% malignant cases revealed (+3 IHC score) while none of benign cases revealed (+3 IHC score).

On comparing p-53 expression in different grades of malignant tumors a higher positivity (100%) was noted in high grade serous carcinoma and Grade 3 tumors in non-serous tumors. Naik et al.(2015) [21] and Singh M et al. (2021) [27] also found result for high grade serous tumors

positive expression for p-53 as 100% and 88.8% respectively. [28]

Table 1: Comparison of Results- P53 Expression And Tumor Types

Authors	Year	No. of cases	Benign	Borderline	Malignant
Naik et al.	2015	110	6.1%	75%	75%
Abbasi F. et al.	2016	109	14.9%	50%	55.6%
Qasim et al	2017	60	35%	20%	90%
Singh A et al.	2021	76	21.05%	50%	71.9%
Mohapatra I et al.	2021	52	14.3%	75%	89.5%
Present study	2022	100	14.3%	57.2%	89.2%

In present study 47.6% serous tumors showed p-53 positivity wile mucinous tumors revealed positivity in 45.5% cases. Endometrioid tumors and clear cell tumours showed 100% positivity for p-53 because all of them were malignant lesions i.e. comparable with the study of Naik et al. (2015) [21], Tiwari et al (2016), Mohapatra I et al. (2021) [17], Singh A et al. (2021) [23]

Table 2: Comparison of Results- P53 Expression And Histological Subtypes

Author	Naik et al.	Tiwari et al.	Singh A et al.	Mohapatra I et al.	Present study
Year	2015	2016	2021	2021	2022
No. of cases	110	86	76	52	100
Serous tumors	22.4%	80%	45.2%	36.3%	47.6%
Mucinous tumors	13.3%	43.8%	29.2%	66.6%	45.5%
Endometrioid	80%	100%	100%	80%	100%
Brenner tumors	100%	0%	75%	66.7%	0%
Clear cell type	100%	100%	100%	100%	100%
Seromucinous tumors	-	-	0%	-	-

On comparing p53 expression in different stage of malignant SEOT according to FIGO staging p-53 expression increases with stage of tumor as Stage IV tumors show 100% positive expression while in stage I and stage II tumors 66.6% lesion showed positive expression. This is comparable with the study of Amanullah et al. (2020) [28] who found that in Stage IV and stage I tumours 88.5% and 36% cases show positive expression respectively.

Ki-67 was express in pattern of SEOT. Total 53% cases were revealed positive immunoexpression for Ki-67. Highest Ki-67 LI (+3) was significantly associated with 94.6% (35/37) of positive malignant SEOT 51.3% (19/37) while majority of the cases 89.8%(44/49) with negative immunoexpression belonged to benign histological type. That was found to be statistically significant (0.000012) which is comparable with the study of Mohapatra I et al. (2021) [17] who studied that 52.6% malignant cases revealed (+3 IHC score) while none of benign cases revealed (+3 IHC score).

In present study 50.8% serous tumors showed Ki-67 positivity wile mucinous tumors revealed positivity in 54.6% cases. Endometrioid tumors and clear cell tumours showed 100% positivity for Ki-67 i.e. comparable with the study of Naik et al. (2015) [21], Mahadevappa A et al(2017) [29] and Mohapatra I et al. (2021) [17].

Table 3: Comparison of Results- Ki-67 Expression and Histological Subtypes

Author	Naik et al.	Mahadevappa A et al	Mohapatra I et al.	Present study
Year	2015	2017	2021	2022
No. of cases	110	36	52	100
Serous tumors	25.8%	65.03%	50%	50.8%
Mucinous tumors	13.3%	60.24%	66.6%	54.6%
Endometrioid	100%	-	60%	100%
Brenner tumors	100%	100%	100%	0%
Clear cell type	100%	53.35%	33.3%	100%
Seromucinous tumors	-	-	-	-

The FIGO staging has been recognized as a salient independent prognostic factor with higher stage reflecting more aggressive tumor biology. In present study we found that stage IV and stage II lesions show 100% positive immunoexpression with Ki-67. While Stage I lesions there were 93.3% lesions showed positive immunoexpression with Ki-67. Which is in parallel to study done by Mahadevappa A et al (2017) [29] a total of 75% were high grade tumors. High Ki-67 was associated with high grade tumors (69.9%), high grade serous tumors (65.3%) and advanced FIGO staging (70.6%). [30]

The higher frequency rate of co-expression (46%) was found between these two immunomarkers. This pattern of association and accordance may indicate that these markers may run aligned in relation to tumour behavior and similar result has been observed by Mohapatra I et al. (2021) [17] and Gursan et al [15].

Table 4:

	Ki-67 negative	Ki-67 positive	Total
p-53 negative	45	7	52
p-53 positive	2	46	48
Total	47	53	100

Correlation between P-53 and Ki-67 Immunoexpression for Surface Epithelial Ovarian Tumors:

Chi square statistic is 44.1065. The P value is <0.05 (0.000012)

Conclusion

Hence, we conclude that p-53 and Ki-67 expression increases across the spectrum of SEOTs from benign to borderline to malignant lesions and are useful diagnostic adjuvant. This aids in early diagnosis, risk assessment, prediction of aggressive behavior of SEOTs and can serve as useful biomarker to assess malignant potential specially in borderline lesions which is helpful in management and provide appropriate therapy in these patients.

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