Section: Pathology

Original Research Article

A STUDY OF UTILITY OF IMMUNOHISTOCHEMICAL MARKERS (P 53, KI-67) IN DIAGNOSIS OF SURFACE EPITHELIAL TUMORS OF OVARY

Ankita Kamthan[1], Abhishek Singh[2], Manoj Bind[3]

[1]Assistant Professor, Department of Pathology, Maharshree Vishwamitra Autonomous State Medical College, Ghazipur, Uttar Pradesh, India.
[2,3]Associate Professor, Department of Pathology, Maharshree Vishwamitra Autonomous State Medical College, Ghazipur, Uttar Pradesh, India.

Received *: 03/03/2025*
Received in revised form *: 20/04/2025*
Accepted *: 09/05/2025*

Corresponding Author:
Dr. Ankita Kamthan,
Assistant Professor, Department of Pathology, Maharshree Vishwamitra Autonomous State Medical College, Ghazipur, Uttar Pradesh, India.
Email: ankitakamthan25@gmail.com

DOI: 10.70034/ijmedph.2025.2.197

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2025; 15 (2); 1095-1100

ABSTRACT

Background: Tumors of ovary generally are more prevalent in upper socio-economic groups due to their low fertility rate and there is a racial predisposition of ovarian cancers with increased risk of Caucasians and lower risk for black women. **Objective:** To evaluate role of IHC in diagnosis of primary ovarian tumors, to study and compare the incidence of ovarian tumors and to determine whether Ki67 (LI) helps in differentiating borderlilne and malignant surface epithelial tumors

Materials and Methods: The present study was conducted among at Pathology Department of G.S.V.M. Medical College, Kanpur, India. Total 100 cases were included in the study. The patients were admitted in the Department of Surgery and Obstetrics and Gynaecology and underwent total hysterectomy with bilateral and unilateral salpingo-oophrectomy or unilateral/ bilateral salpingo-oophorectomy or oophorectomy as indicated.

Result: Benign ovarian lesions were more common (70%) than malignant ovarian lesions (30%). Surface epithelial tumors were most common tumor accounting for 64% of all ovarian lesions. Serous cystadenoma was most common tumor (60%) Serous cystadenocarcinoma was most common malignant tumor (60%). 71.42% of benign lesions were presented below the age of 40 years. Maximum incidence of malignant lesions were in age group of 41 – 50 years (46.66%). Overall 96.66% malignant lesion presented in the age group of 31 – 60 years. Increased expression of Ki-67 LI was found in malaignant surface epithelial neoplasms compared to benign and borderline tumors. High grade serous tumors had a higher percentage of P 53 expression in more than 50% of cases, in contrast to low grade serous tumor and borderline serous tumor.

Conclusion: Ki-67 is a cost effective and easily accessible immunohistochemical marker. Therefore, immunohistochemical assessment of Ki-67 expression can be included in routine histopathological report of surface epithelial tumor of ovary for better understanding of the biological behavior of the tumor and modifying treatment strategies. P53 IHC is a surrogate marker for P53 gene mutation in clinical practice.

Keywords: Immunohistochemical markers (P 53, Ki-67), diagnosis, surface epithelial tumors, ovary.

INTRODUCTION

About two thirds of ovarian tumors occurs in reproductive age group. Many risk factors are associated with increased prevalence of ovarian tumors most importantly, age, positive family history, genetic factors, hormonal and reproductive factors.[1]

Most cases are sporadic, only around 5 – 10% of ovarian cancers are hereditary. Women having

inherited mutations in BRCA-1 and BRCA-2 tumor suppressorgene are at increased risk for developing the tumor.[2]

Abdominal USG and serum CA-125 measurements were used as screening methods for diagnosis of early ovarian carcinoma. Fine needle aspiration cytology is used for primary diagnosis in a patient with advanced disease and also to monitor recurrences after treatment with an overall accuracy of differentiating benign from malignant ovarian tumor ranging from 90 – 95%.[3]

Any persistent ovarian enlargement is an immediate indication for surgical assessment and actual diagnosis rests with the histopathological examination of specimen with the histopathological examination of specimen. WHO Histological classification is used for the diagnosis of ovarian tumors. They are categorized into 3 major categories 1) Surface epithelial stromal, 2) sex cord- stromal 3) Germ cell tumors.[4]

Based on clinicopathologicaland molecular studies, epithelial ovarian cancer (EOG) is classified as stype I or type II. Type I tumors are genetically quite stable, typically present at a low stage, and reveal distinct, norphologic differences than type II tumor.[5]

These include different histotypes- Low grade serous endometrioid, clear cell and mucinous ovariancarcinoma. Type I tumors are characterized by distinct molecular genetic profiles, such as mutation in KRAS, BRAF, PIK3CA, PTEN and erbb2, but not TP53.[5]

TP53 mutation have an important role in the prognosis and treatment of ovarian cancer. Mutations in TP53 are found in high grade and rarely in low grade serous ovarian cancers. TP53 encodes the 53 KDa nuclear protein, their mutations leading to gain or loss of function of its protein product. TP53 mutation leads either to over expression of P53 protein or complete lack of expression, while wild type P53 is associated with focal expression.

Ki-67 is an excellent immunohistochemical marker to determine proliferating cells of the tumor. It is expressed in all active phases of the cell cycle (G1,S,G2 and M phase) except in resting cells (quiescent cells – Go phase). The monoclonal Ki-67 / MIB-1 antibody reacts with the nuclear Ki-67 antigen expressed in proliferating cells.6 Its expression reflects tumor proliferation and has been found to indicate tumor aggression tumor metastasis and known to predict disease outcome in many human malignancies such as central nervous system tumors (meningioma) lymphoproliferative disease Connective tissue tumors and breast tumors.[7]

P53 is a tumor suppressor gene situated on chromosome 17.P53 gene mutation results in uncontrolled cell proliferation .Approximately 50% of malignant tumors in human have mutations in P53 gene and it is the most common tumor suppressor gene involved with human malignancies.[8]

MATERIALS AND METHODS

The present study was conducted among at Pathology Department of G.S.V.M. Medical College, Kanpur, India. Total 100 cases were included in the study. The patients were admitted in the Department of Surgery and Obstetrics and Gynaecology and underwent total hysterectomy with bilateral and unilateral salpingo-oophrectomy or unilateral/ bilateral salpingo-oophorectomy or oophorectomy as indicated. Duration of study was from July 2018 to October, 2020.

All the specimen of 100 cases were received in llabeled jar containing 10% formalin solution along with histopathological registration forms with relevant clinical information. After fixation in 10% formalin solution overnight (12 hrs), the specimens were subjected to grossing with proper gross examination and in detail demonstration of gross findings. Tissue sections were carefully dissected from representative areas following the guidelines of grossing techniques Using rotatory microtome, 3-5µ is thick sections were cut from the representative paraffin embedded blocks. The sections were then stained with hematoxylin and eosin (H and E) staining and Ki-67 and P-53 immunohistochemical staining for histopathological examination and immunohistochemistry (IHC) study, respectively.

The sections stained with routine H and E were viewed under light microscope and histopathological diagnosis was made based on the WHO classification of SEOT (Surface epithelial Ovarian Tumor). First, the tumors were broadly categorized as benign and malignant. Thereafter, it was further classified based on histological subtypes. The serous ovarian carcinoma was further classified into low grade serous carcinoma (LGSC) and high grade serous carcinoma (HGSC) based on two tier grading system.

This system is primarily based on the assessment of nuclear atypia and using the mitotic index as a secondary feature. The mitotic figure in LGSC < 12/HPF (high power field) while in HGSC it is $\geq$ 12/HPF. Staging of the malignant surface epithelial tumor of ovary was done according to the FIGO staging. It was done at the time of diagnosis based on pre-operative radiological, operative and histopathological findings.

Ki-67 Immunostaining

Ki-67 antigen immunostaining was carried out by standard IHC method and Peroxidase – antiperoxidase method using monoclonal mouse antihuman Ki-67/ MIB-1 antibody kit, code 15626 DAKO™(Gloshup, Denmark).

Ki-67 immunopositivity was observed as brown granular nuclear staining for Ki-67 scoring, the most immunopositive area of the tumor was selected avoiding foci of inflammation. The number of immunopositive nuclei is counted in 1000 tumor cells in atleast 10 HPF (x 40). the percentage of immunopositive cells is referred to as Labeling

index (LI)/ proliferative index. The average of three counts of Ki-67 immunopositive cells over the same slide was taken and expressed as the percentage of Ki-67 immunopositive cells in the tumor[9]. Ki-67 expression was quantitatively assessed and regarded as negative (if Ki-67 LI < 1%) and positive (if Ki-67 LI≥1%). [10]

P53 Immunostaining

Immunohistochemistry was performed to measure the protein expression of P53 monoclonal antibodies in ovarian carcinoma cases.

Both the quantity of nuclear positivity and the staining intensity were measured. The intensity of staining was reported as negative weak, moderate or strong (0,1+,2+,3+)

An HIC score of P53 staining intensity was categorized as :

0 → for none (no brownish color seen using X 40 magnification.

+1→ for weak (brownish color seen using X 10 and X
40)

+2 for moderate (brownish color seen using X 10 magnification)

+3 for strong (brownish color staining testing X 4 Magnification

The percentage of positive tumor cells was quantified by counting cells manually in atleast 100 cells in 10 high power field averged and categorized as –

≥75% of cells considered as high overexpression

≤ 75 – 50% of cells considered as – moderate expression

≤50% of cells considered as focal expression

Only 75 – 100% positive tumor cells with moderate and strong staining intensity considered as positive results.

RESULTS

Total number of cases studied were 100. Of 100 cases, 70(70%) were of benign lesions and 30 (30%) were of malignant lesions.

Serous cyst adenoma was most common benign lesion, 42 cases (60%) followed by mucinous cystadenoma 21 (30%) cases.

Table 1: Distribution of malignant lesions according to histological type(N=30)

Category	Histological type	No. of cases	Percentage
Surface Epithelial tumor	Serous adenocarcinoma	18	60
	Mucinous adenocarcinoma	06	20
	Endometrioid adenocarcinoma	03	10
	Transitional cell ca	02	6.66
	Seromucinous carcinoma	01	3.33

Serous adenocarcinoma was most common, 18 cases (60%) of total 30 malignant lesions.

Table 2: Overall age incidence in the study group (N=100)

Age (Yrs)	No. of cases	Percentage
11 – 20	05	05%
21 – 30	21	21%
31 – 40	34	34%
41 – 50	32	32%
51 – 60	07	07%
61 – 70	01	01%
Total	100	100

Most common age incidence 66 cases (66%) including both benign and malignant lesion in age group 31 – 50 years. Maximum number of cases 87% presented in age group of 21 – 50 years. 05 cases (05%) presented in the age group of less than 20 years. Youngest patient was a 15 year female and oldest was 61 year female.

Benign lesions were most common 24 cases 34.28% in age group of 31 – 40 years. Out of 70 benign lesion 50 cases (71.42%) were presented below the age of 40 years. Maximum number of malignant lesions 14 cases (46.6%) were reported in the age group of 41 – 50 years followed by 33.33% in age group of 31 – 40 years and 16.66% in age group of 51 – 60 years. Overall of 30 malignant lesions, 29 cases 96.66% presented in the age group of 31 – 60 years. Serous cyst adenoma was most commonly 36 cases (85.71%) seen in the age group of 21 – 50 years with a mean age of 35.5 years.. Mucinous cyst adenoma was most commonly 14 cases (66.66%) seen in the age group of 31 – 50 years with a mean age of 40.5 years.

Table 3: Age distribution according to histological diagnosis in malignant lesions (n=30)

Lesions	Age group (Years)						Total
	11-20	21-30	31-40	41-50	51-60	61-70	
Serous cyst adeno carcinoma	0	0	6	9	2	1	18
Mucinous cyst adeno carcinoma	0	0	1	3	2	2	6
Endometrioid carcinoma	0	0	2	0	1	0	3
Transitional carcinoma	0	0	1	1	0	0	2

Seromucinous carcinoma	0	0	0	1	0	0	1

Serous carcinoma was most commonly seen, 15 cases (75%) in the age group of 31 – 50 years with a mean age of 40.5 years. All mucinous CA i.e. 6 cases (100%) was detected in the age group of 31 – 60 years with a mean age of 45.5 years.

Table 4: Immunohistochemistry :Ki-67 labelling index

S.NO.	HPE DIAGNOSIS	KI-67 (LI)
1.	Low Grade Serous Carcinoma	24%
2.	Low Grade Serous Carcinoma	19%
.3.	Low Grade Serous Carcinoma	26%
4.	Low Grade Serous Carcinoma	28%
5.	Low Grade Serous Carcinoma	22%
6.	High Grade Serous Carcinoma	52%
7.	High Grade Serous Carcinoma	47%
8.	High Grade Serous Carcinoma	44%
9.	High Grade Serous Carcinoma	43%
10.	High Grade Serous Carcinoma	39%
11.	High Grade Serous Carcinoma	42%
12.	High Grade Serous Carcinoma	33%
13.	High Grade Serous Carcinoma	46%
14.	High Grade Serous Carcinoma	41%
15.	High Grade Serous Carcinoma	48%
16.	High Grade Serous Carcinoma	40%
17.	High Grade Serous Carcinoma	38%
18.	High Grade Serous Carcinoma	49%

There are about 18 cases of serous carcinoma of which 3 cases showed Ki-67 LI between 0-25 and 14 cases showed Ki-67 LI between 26 – 50 and 1 case showed Ki-67 LI above 50. Highest level of Ki-67 LI was found to be in high grade serous carcinoma. Among all, highest Ki-67 LI was found 52% and least Ki-67 LI was found to be 19%.

TABLE 5: Immunohistochemistry: Ki-67 Labelling Index

S.No.	HPE Diagnosis	Ki-67(LI)
1	Mucinous carcinoma	28%
2	Mucinous carcinoma	24%
3	Mucinous carcinoma	26%
4	Mucinous carcinoma	30%
5	Mucinous carcinoma	18%
6	Mucinous carcinoma	23%
7	Mucinous carcinoma	29%
8	Mucinous carcinoma	31%
9	Mucinous carcinoma	23%
10	Mucinous carcinoma	36%
11	Mucinous carcinoma	42%
12	Mucinous carcinoma	40%

There were about 12 mucinous tumor. There are about 12 cases of mucinous carcinoma of which 4 cases (33.33%) showed Ki-67 (LI) between 0-25 and 8 cases (66.66%) shows Ki-67 (LI) between 26 – 50. Among all highest Ki-67 LI was found to be 42% and least Ki-67 LI was 18%.

TABLE 6: P53 IMMUNOHISTOCHEMISTRY

S.No.	HPE Diagnosis	P53 HIC
1	High Grade serous carcinoma	Positive
2	High Grade serous carcinoma	Positive
3	High Grade serous carcinoma	Positive
4	Low Grade serous carcinoma	Positive
5	Low Grade serous carcinoma	Negative
6	High Grade serous carcinoma	Positive
7	Low Grade serous carcinoma	Negative
8	High Grade serous carcinoma	Positive
9	Low Grade serous carcinoma	Negative
10	High Grade serous carcinoma	Negative
11	Mucinous carcinoma	Positive
12	Mucinous carcinoma	Negative
13	Mucinous carcinoma	Negative
14	Mucinous carcinoma	Negative

P53 Immunohistochemistry staining was applied to all (14) cases of malignant tumors including low grade and high grade serous carcinoma and mucinous carcinoma. Resusltswere interpreted as

positive when cells show diffuse and intense nuclear staining.

About 3 cases (75%) of low grade serous carcinoma belonging to type I pathway of tumorigenesis showed negative for P53 immunohistochemical staining whether about 5 cases (83%) of high grade serous carcinoma which are included in the type II pathway of tumorigenesis showed positivity for P53 staining.

DISCUSSION

A total 100 cases of benign and malignant lesions of ovary along with 3 immunomarker relevant for various ovarian carcinomas were studied.

As shown in table I , out of 100 cases, benign cases (70%) were far more common than malignant lesions (30%). This is in concordance with Mankar DV, Jain GK et al (2015), studied 257 cases of ovarian tumors and found that 180 (70%) were benign and 77 (30%) were malignanat.[11]

Serous cyst adenoma was most common benign lesion (60%) followed by mucinous cyst adenoma was responsible for 30% of benign cases. In study conducted by Modi D et al (2016) serous cyst adenoma was the most frequent tumors and comprised of 52.7%. Mucinous cyst adenomas constituted 28.4% of all the ovarian tumors.[12]

Of total 30 malignant lesions, serous carcinoma was most common 18 cases (60%) followed by mucinous carcinoma 6 cases (20%) and endometrioid carcinoma 3 cases (10%). This is in concordance with Monika Malli et al (2014) who reported that serous carcinomas account for 44.4% of all ovarian malignancy.[13]

The maximum number of 34 cases (34%) was presented in the age groups of 31 – 40 years followed by 32 cases (32%) in 41 – 50 years age group and 14 cases (14%) in 21 – 30 years age group. Overall 77% cases presented in the age between 21 – 50 years. Youngest patient was a 15 years female and oldelst was 61 years female. Scully et al (1998) reported that about two third of ovarian tumors occurs in women between the age of 20 – 50 years and 80 – 90% of them in women between the ages of 20 – 65 years.[14] They also mentioned that 60 – 70% of benign tumors occurs in women under the age of 40 years in contrast, 80 – 90% of ovarian malignant tumors are detected after 40 years. In a study on 270 ovarian tumors Kuldeep AVK et al (2011) concluded that benign neoplasm were seen more commonly in younger age group that is below 40 years of age. The results of present study are within expected range of above mentioned study except that in present study carcinomas are detected one decade earlier. This could be attributed to lower sample size of our study or lower life expectancy in our country than Western countries.

Serous cyst adenoma was most commonly 31 cases (73.80%) seen in the age group of 21 – 50 years. Mucinous cyst adenoma was most commonly 14 cases (66.66%) seen in the age group of 31 – 50 years. This is in close concordance with Kooning et al (1989) who mentioned that benign serous tumors occurs at any age but are most common in women in reproductive age group. Scully (1993) also described that mucinous cystadenomas can occur at any age but are diagnosed most often in women in the fourth to sixth decades.

Serous carcinoma was most commonly seen, 15 cases (83.33%) in the age group of 31 - 50 years with a mean age of 45.77 years. No case was detected below the age of 30 years. All cases of mucinous carcinoma i.e. 6 cases (100%) were detected in the age group of 31 - 60 years with a mean age of 45.5 years. This is in concordance with Kooning et al (1989) who described that serous carcinoma is rare before the age of 20 years but incidence increases thereafter, with an average patient age of 46 years.[15] Scully (1993) mentioned that mucinous carcinomas generally occurs in older women, with mean ages of 51 to 52 years..Fortunately in our study also, these cases presented before the age of 20 years.[14]

We have observed that only malignant tumors were positive for Ki-67 expression (Ki-67 LI > 1%). Similarly in the study conduced by Verma et al, positive Ki-67 expression was observed only in the malignant tumors 1HC expression of P53 and Ki 67 markers in epithelial ovarian neoplasms .

In the present study, Ki-67 LI was higher in serouscystademocarcinoma than mucinous cystodenocarcinoma signifying that Ki-67 expression is higher in malignant serous ovarian tumors. Similarly, in the study of Mahadevappa et al, the mean Ki-67 LIof serous carcinoma was 65.03 +1.67, which was higher than that of mucinous carcinoma 60.24 + 21.91and prognostic significance of Ki-67 immunohistochemical expression in surface epithelial ovarian carcinoma.

Among serous cystadenocarcinoma. The median Ki-67 Li of HGSC was 45% and Ki-67 Li of LGSC was 24%, which signifies that there is higher Ki-67 expression in HGSC as compared to LGSC.

Similar association was found in the studies of Kobel et al,[16] and Chen et al. In Kobel et al, the median Ki-67 LI of LGSC was 25% and that of HGSC was 22.4%. In Mahadevappa et al., the median Ki-67 LI of LGSC was 37.96% and that of HGSC was 65.34% Ki-67 expression is higher in HGSC than LGSC because HGSC owing to its grade has more cellular proliferative activity and nuclear atypia along with higher mitotic activity than LGSC. (Chen M, Yao S, Cao Q, Xia M LiUJ, HeM et al. The prognostic value of Ki-67 in ovarian high grade serous carcinoma.

P53 positivity was observed in 50% of EOC cases and higher positivity is found in HGS(83.3%). This result agreed with those of Tan etal., who found that 64.18% (43/67) of HGS samples analyzed over expressed P53. Harris etal., found that in 274 cases, 68% of tumors were characterized as P53 mutant (n=186) and P53 mutant tumors were more likely to

be HGS (72%) with significant association between P53 expression and histology and grade of tumor. Kobel et al showed P53 marker over expressed in 69% (118/171) of HGS cases.

The present study as in table XIII showed that P53 marker was expressed in (83.33%) of type II, and (25%) of type I. This result agreed with Caster etal., 2018, who reported that P53 was highly expressed in type II EOC (68.8%) than type I (33.3%).

CONCLUSION

Ki-67 is a cost effective and easily accessible immunohistochemical marker. Therefore, immunohistochemical assessment of Ki-67 expression can be included in routine histopathological report of surface epithelial tumor of ovary for better understanding of the biological behavior of the tumor and modifying treatment strategies.

P53 IHC is a surrogate marker for P53 gene mutation in clinical practice, Detecting P53 mutations by immunohistochemistry helps in correct classification and further research of morphologically confusing EOT.

REFERENCES

1. Jaffrey T. Quirk et al. risk factors for invasive epithelial ovarian carcinoma by histological subtype 2004, vol.3
2. Ferlay et al : risk factors and age distributions in Ireland 1995 – 2007
3. Lee G et al . Fine Needle Aspiration Cytology in the diagnosis and classification of recurrent and metastatic gynaecological malignancies 1988 vol. 29, 375 – 378.
4. Jeffrey SD, Russell P., Robert KJ: Surface epithelial tumors. In Robert Kurman J. Blausteins Pathology of female genital tract. Fifth Eddition 2002: 791 – 881.
5. Chen LY, Huang RL, ChanMW, et al: TETI reprograms the epithelial ovarian cancer epigenome and reveal case in Kinase 2X as a therapeutic target. Pathol. 2019; 248(3): 363 – 76.
6. Cattoretti G, Becker MH, Key G, Duchrow M, Schluter C, Galle J et al : Monoclonal antibodies against recombinant parts of the Ki-67 antigen (MIB1and MIB3) detect proliferating cells in microwave processed formalin fixed paraffin sections. J Pathol 1992; 168; 357 – 63.
7. Gursan N, Sipal S, Calik M, Gundogdu . P53, Bcl-2,Ki-67 Li (labeling index) status in benign, proliferative and malignant ovarian surface epithelial neoplasms. Eurasian J Med. 2009; 41 : 10 – 4.
8. Nesringursan et al P53, Bcl- 2, Ki- 67, status in benign borderline, Malignant ovarian surface epithelial neoplasm. Eurasian journal of Medicine April 2009, Vol.41.
9. Choudhury M., Goyal S, Piyani M, Acytohistological study of Ki-67 expression in ovarian tumors. Indian J PatholMicrobiol 2011; 54 : 21 – 4.
10. Qasim YA, Saeed SZ, Rashid IM :Immunohistochemical study of P53 and Ki-67 expression in surface epithelial a tumor of the ovary. Saudi Journal of Pathology and Microbiology. 2017; 2 : 8.
11. Mankar DV, Jain GK. Histopathological profile of ovarian tumor : a twelve year institutional experience. Muller J Med Sci. Res. 2015; 6 : 107 – 11.
12. Modi D, Rathore GB, Delwadia KN, Gosmani HM: Histopathological pattern of neoplastic ovarian lesion. IAIM 2016; 3(1): 51-57.
13. Malli M, Vyas B, Gupta S, Desai H, A Histopathological study of tumors in different age groups. Int. J. Med. Sci. Public Health 2014; 3 : 338 – 341.
14. Scully RE. Ovary In : Henson DE, AlboresSaavedra J, eds; The pathology of incipient Neoplasia. Philadelphia: WB Saunders, 1998: 283 – 300.
15. Kooning PP, Campbell K, Mishell DR Jr. Grimes DA: Relative frequency of primary ovarian neoplasm. a 10 years review obstet&gynaecol. 1989; 74 : 921 – 6.
16. Kobel M, Piskorz AM, Lee S, et al. Optimized P53 immunohistochemistry is an accurate predictor of TP53. Mutation in ovarian carcinoma J Pathol. Clin Res 2016; 2(4): 247 -58.