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Correlation Of p53 Expression In Ovarian Lesions Of Different Histogenesis-An Immunohistochemical Study

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Abstract

Introduction: Mutations of the p53 gene as determined by mutation analysis and/or positive immunohistochemical (IHC) staining for p53 are common in ovarian cancer and have been associated with poor clinical outcome. However, results of the many studies on the prognostic value of p53 expression are inconclusive. Hence the present study was conducted to study immunohistochemical expression of p53 gene in various ovarian lesions. **Material and Methods:** The present study was conducted to study p53 expression in ovarian lesions of different histogenesis. Paraffin embedded hematoxylin & eosin stained tissue sections were diagnosed histologically and subsequently stained immunohistochemically with p53 antibody. Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) version 16.0. **Results:** Of 30 malignant cases, serous carcinomas were the largest group with 16 cases (53.33%) followed by mucinous with 4 cases (17%). All benign and borderline tumors were p53 negative. 86.6% of the malignancies were p53 positive and amongst which maximum number was that of serous carcinomas (50%), followed by mucinous carcinomas (10%), two cases (6.6%) each of dysgernminomas and adult granulosa cell tumor followed by one case each (3.3%) of malignant Brenner tumor, malignant mixed germ cell tumor, immature teratoma and squamous cell carcinoma of the ovary. p53 expression was high in malignant serous carcinomas cases as compared to borderline and benign (p value < 0.001) with high apoptotic and mitotic indices. ***Conclusion:*** It is concluded that strong and diffuse nuclear expression of Tp53 can be used as robust method for inferring the presence of Tp53 gene mutation in high grade serous carcinomas and can be used as surrogate marker for diagnostic workup of carcinoma ovary.

Key words: Ovary, p53, immunohistochemistry, serous, mucinous.

Introduction

Cancer of breast and cervix reign supreme among the fatal diseases in women globally. Interestingly the ovarian cancer has the highest mortality rate. This is attributed to its late clinical presentation and delayed diagnosis. Unlike the cervix and uterus, the ovaries are not clinically accessible, and therefore easy screening methods for detecting ovarian neoplasms are not available.[1]

Screening for ovarian cancer has been based on strategies using serum tumor markers or ultrasound imaging of the ovaries. However, serum CA125 is elevated in only about 50% of patients with clinically detectable early-stage ovarian carcinomas.[2] Insight into their pathogenesis requires an understanding of the genetic mutations, tumor suppressor/oncogenes, and cell cycle regulators of ovarian cancers to develop new technologies to identify other biomarkers that can be used for early detection. Mutations and/ or over expression of three oncogenes Her-2/neu, C-

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myc and K- ras and of the tumor suppressor gene p53 have frequently been observed in sporadic ovarian cancer.[2,3] p53 is one of the most commonly mutated tumor suppressor genes and it is altered in 50% of advanced cases of ovarian cancer.[4,5] The expression of p53 throws light on the prognosis of ovarian tumors. In normal cells, the wild-type p53 protein is present in low concentrations due to its rapid turnover, and hence cannot be detected immunohistochemically.[6,7] Under stress conditions which lead to DNA damage, p53 is activated and triggers at least two important events leading to growth suppression: the induction of cell cycle arrest in G1-phase allowing time for DNA repair or the promotion of apoptosis if the damage is irreparable.8 Mutations of the p53 gene as determined by mutation analysis and/or positive immunohistochemical (IHC) staining for p53 are common in ovarian cancer and have been associated with poor clinical outcome. However, results of the many studies on the prognostic value of p53 expression are inconclusive. Therefore the present study was conducted: 1) To observe the expressivity of p53 in ovarian tumors of different histological types and grades. 2) To study correlation of p53 expression, apoptotic index and mitotic index in various ovarian lesions.

Material and methods

The present cross sectional study was conducted on 60 specimens of ovarian neoplasms received in the Department of Pathology over a period of 18 months from August 2021- February 2022. A total of 60 patients showing ovarian neoplasm on histology were studied. Ethical approval from Institutional Ethics Committee (IEC) was obtained for this study. Written consent was taken from all patients and data was used in unidentified manner. **Inclusion criteria:** All the ovarian tumour specimens, clinically diagnosed as ovarian neoplasms with definite histopathological diagnosis were considered. **Exclusion criteria:** Non neoplastic lesions and ovarian tumours treated with neo-adjuvant chemotherapy or radiotherapy was excluded. Conventional hematoxylin and eosin stained sections prepared from the specimens were examined for final histological diagnosis and apoptotic and mitotic indices were calculated. Histologic grading was done according to the degree of differentiation. They were graded as I to III (well, moderately, and poorly differentiated, respectively) on the basis of cellular atypia, architectural complexity and invasive propensity. The representative sections were stained immunohistochemically for p53 expression. Primary monoclonal mouse anti-p53 antibody was procured from the Novacastra. Antigen retrieval was done as per specification of kit. Slides were immersed in citrate buffer and put in pressure cooker for 4 cycles of 5 minute each. Known cases of colorectal adenocarcinoma were chosen as positive control and negative control sections were provided by omission of primary antibody. p53 positivity was seen as a brown colored product staining the nuclei. Scoring of p53 expression in tumor cell nuclei was evaluated in accordance with the scoring system devised by Chan et al[3] both quantitatively and qualitatively. Quantitative scoring was done as, 0 = <5%; 1 = 5-25%; 2 = 25-50%; 3 = 50-75%; 4 = >75% tumor cells nuclei showing positivity. An intensity score was made on the basis of the average intensity of staining: 0 = negative; 1 = weak; 2 = medium; 3 = strong. The final score for each section was obtained by multiplying the percentage score by the intensity score.

Apoptotic index (AI) was done by counting apoptotic bodies counted among 1000 tumor cells using 40X objective. It was measured as number of apoptotic cells and bodies among 100 cells.

Mitotic index (MI) is the rate of proliferation of cells. Similarly, mitotic index (MI) was counted among 1000 tumor cells using 40X objective and measured as number of cells in mitosis among 100 cells.

Statistical analysis: Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) version 16.0. Statistical evaluation was done using chi-square test, student's t test and ANOVA F test. A P value of > or equal to 0.05 was considered as significant and P value of > or equal to 0.01 as highly significant.

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Results

Majority of patients with benign ovarian neoplasms included in the study was in the age group of 21-40 years with mean age of 31.60 ± 10.3. The maximum number of patients with malignant ovarian neoplasms was in 31-70 years of age group with mean age of 43.93 ± 18.3 supporting the fact that cancers occur in the higher age group.[1,2] Among these specimens, 20 cases (33.33%) were of benign, 10 (16.66%) borderline and 30 (50%) cases were of malignant nature. Benign neoplasms comprised of serous cystadenoma (11) and mucinous cystadenoma (9) ***Fig.1 & 2 (a, b, c, d)***. Among borderline category, 5 cases each were of borderline serous and mucinous tumors of ovary. Of 30 malignant cases, 16 cases (53.33%) were of serous papillary cystadenocarcinoma, four were of mucinous cystadenocarcinoma (13.33%), one case each (3.3%) of endometroid carcinoma, malignant Brenner's tumor and clear cell carcinoma, two cases each of (6.6%) of dysgerminoma, adult granulosa cell tumor and one case (3.3%) was of immature teratoma, malignant mixed germ cell tumor and squamous cell carcinoma each. Out of 20 benign cases two cases were bilateral (10%). Out of 30 malignant cases, nine cases were bilateral (30.0 %). Among nine bilateral cases, seven cases were of serous cystadenocarcinoma and two were of mucinous cystadenocarcinoma. CA 125 levels were mean=90IU/ml±10.4 for borderline and 290IU/ml±30.9 for malignant serous carcinomas. CA-125 levels were significantly higher in malignant, serous, advanced stage, grade 3 and p53 positive tumors. **Apoptotic index (AI):** The morphological features which were considered to be typical to apoptosis were chromatin condensation on the periphery of the nucleus, and a heavily stained nucleus. The apoptotic indices were defined as the mean percentage of apoptotic cells and were low for benign ovarian tumors. Specimens with apoptotic indices >1 were found only in malignant tumors. The AI ranged from 0-0.01 with mean of 0.00 ± 0.003 in benign and borderline cases while it ranged from 0.01 to 0.10 with mean of 0.04 ± 0.02 in malignant cases. Majority of the benign and borderline cases (90%) did not show any apoptosis while all the malignant cases showed some degree of apoptosis. AI was higher in the malignant cases as compared to the borderline and benign cases with highly significant p value. It was seen that in 81.8% of Grade I cases, AI ranged from 0.02 to 0.03 (mean 0.03 ± 0.010), in 81.5% cases of Grade II from 0.03 to 0.05 (mean 0.04 ± 0.011) while it rose to 0.08 to 0.10 (mean 0.09 ± 0.010) in 100% of Grade III cases. Therefore, there was a gradual increase in AI with increase in grade with a highly significant p value ($p < 0.001$).

Mitotic index (MI): The MI ranged from 0 to 0.01 with a mean of 0.00 ± 0.003 in benign and borderline cases while it ranged from 0.01 to 0.09 with a mean of 0.03 ± 0.021in malignant cases. MI was higher in the malignant tumors as compared to the borderline and benign with highly significant p value. This implies that mitotic activity increases with increase in the tumor aggressiveness. It is seen that in 90.90% of Grade I cases MI ranged from 0.01 to 0.03 (mean 0.02 ± 0.009), in 81.25% cases of Grade II from 0.02 to 0.04 (0.03 ± 0.014) while it rose to 0.08 to 0.09 (mean 0.08 ± 0.006) in 100% of Grade III cases. Therefore, there was a gradual increase in MI with increase in grade. This correlation was found to be highly significant with a p value of 0.001.

p53 immunohistochemistry:

Results were interpreted in terms of p53 nuclear staining both quantitatively (i.e. percentage positivity) as well as qualitatively (i.e. staining intensity), using scoring system devised by Chan WY et al.[3] Final p53 score was calculated by multiplication of quantitative as well as qualitative results. 86.6% of the malignancies were p53 positive and amongst which maximum number was that of serous carcinomas (50%), followed by mucinous carcinomas (10%), two cases (6.6%) each of dysgernminomas and AGCT followed by one case each (3.3%) of malignant Brenner tumor, malignant mixed germ cell tumor, immature teratoma and squamous cell carcinoma of the ovary. Amongst serous carcinomas, fifteen cases showed (15/16, 94%), diffuse strong intensity and one case (6%) was negative. Among, borderline serous cases, the rate and intensity of positivity was less (1/10, 10%). All the benign cases were negative for p53 expression. Among four cases of mucinous carcinomas studied, three cases showed p53 positivity (75%), two cases had weak expression and one had strong expression of p53 protein. One case each of malignant Brenner's tumor and squamous cell carcinomas of the ovary also showed medium staining intensity in 5-50% of the nuclei ***Fig. 3(a, b,***

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c, d). Similarly some germ cell tumors and sex cord stromal tumors also showed p53 positivity but rate and intensity of positivity was less as compared to surface epithelial ovarian tumors (SEOTs). Hence p53 is a more specific marker for SEOTs especially for serous carcinoma of the ovary.

It is observed that in 86.66% of the malignant cases the total score ranged from 6-12 while in borderline cases the score ranged from 3-5. All the benign cases were negative for p53 staining. The score was high in malignant cases as compared to benign and borderline cases with a significant p value <0.001. The apoptotic index, mitotic index and p53 score were maximum in malignant cases as compared to benign and borderline cases. There was a highly significant positive correlation between p53 expression, apoptotic and mitotic index (p<0.001) in malignant cases. ***Table 1 and Table 2 show clinico-pathological and immunohistochemical features of the cases.***

So, after performing IHC staining with nuclear marker p53, on benign, borderline and malignant ovarian lesions, it was noteworthy to observe that p53 expression, both qualitatively and quantitatively was significantly high in malignant biopsies as compared to benign and borderline cases.

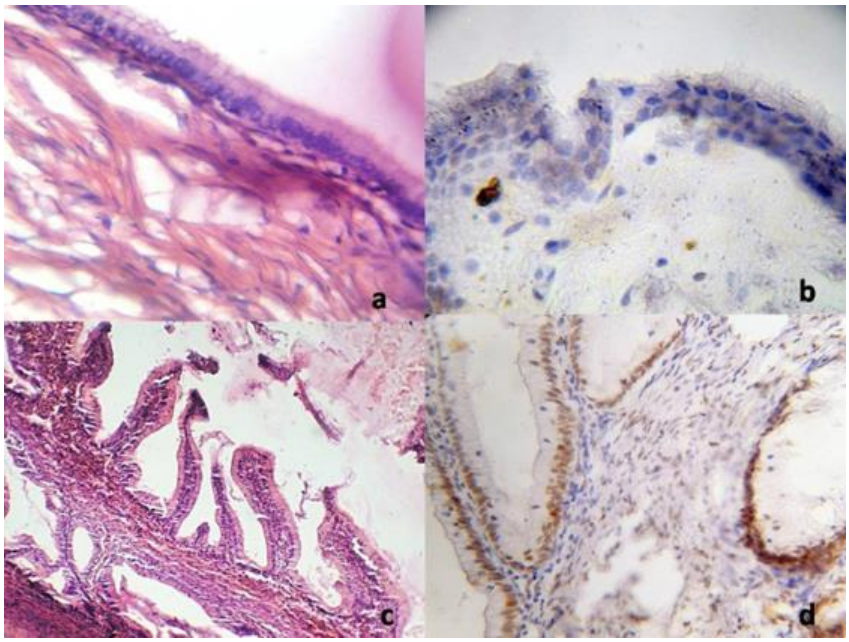

Figure.1. (a) Mucinous cystadenoma of ovary (H&E, 400x) (b) Mucinous cystadenoma showing negative p53 immunostaining (IHC, 400x) (c) Borderline mucinous tumor (H&E, 100x) (d) Borderline mucinous tumor of ovary showing p53 score 6 (IHC, 400x).

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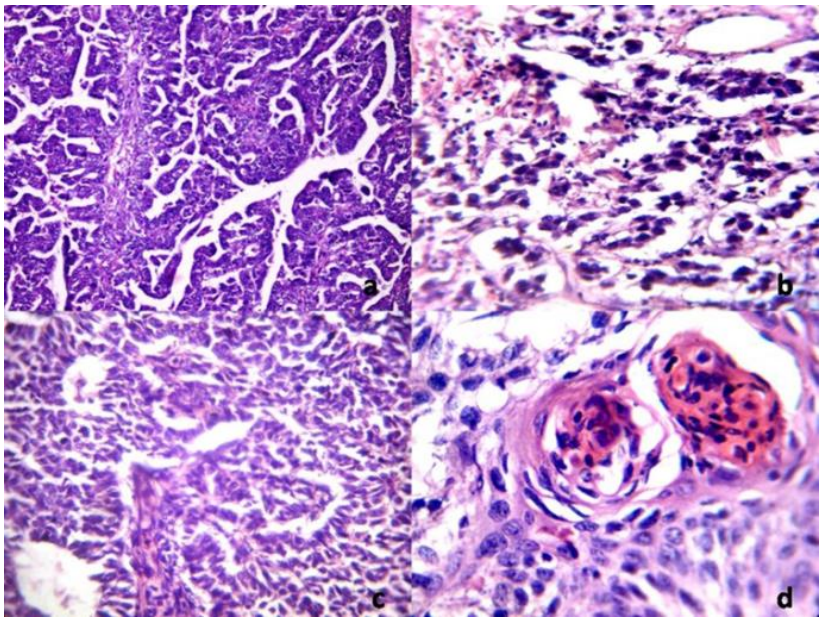

Figure.2. Morphological spectrum of tumors (a) Serous papillary cystadenocarcinoma, High grade (b) Dysgerminoma (c) Granulosa cell tumor (d) Squamous cell carcinoma of ovary (H&E, 400X).

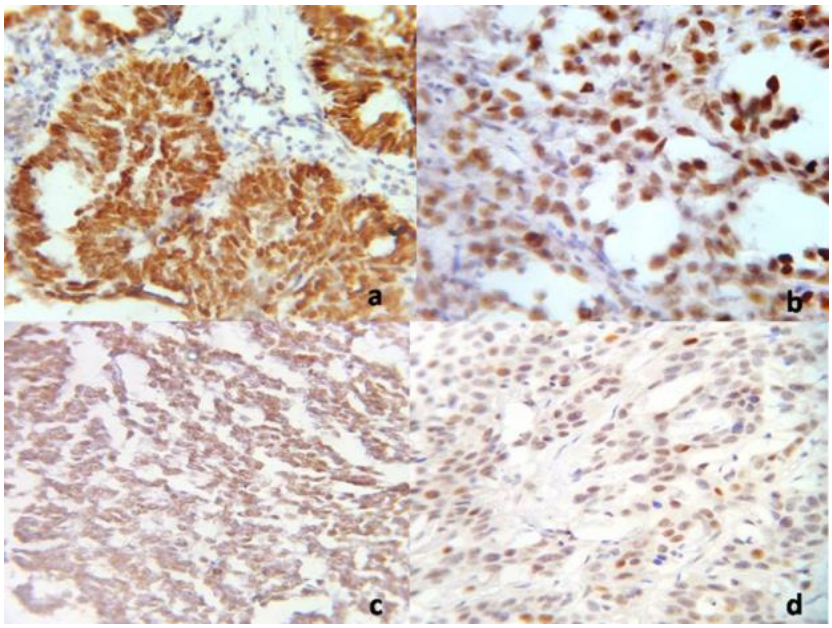

Figure.3. (a) Serous papillary cystadenocarcinoma showing p53 score 12 (b) Dysgerminoma showing p53 score 6 (c) Granulosa cell tumor with p53 score 9 (d) Squamous cell carcinoma showing p53 score 3 (Immunoperoxidase stain, 400X).

Follow up: Follow up was available in 16 cases of Malignant SEOTs with mean period of 1.5±0.4 years. Five cases of high grade serous carcinomas (HGSCs) died after completing chemotherapy. These cases had diffuse strong expression of p53 immunostaining.

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Table 1. Clincopathological characteristics of the patients.

Parameters	Benign (n=20)	Borderline (n=10)	Malignant (n=30)
Age, years	31.60 ± 10.3	32.77 ± 11.3	43.93 ± 18.3
Histological Types	Serous cystadenoma=11 Mucinous cystadenoma=9	Borderline serous=5 Borderline mucinous=5	Serous cystadenocarcinoma=16 Mucinous cystadenocarcinoma=4 Endometroid carcinoma=1 Clear cell carcinoma=1 Malignant Brenner tumor=1 Granulosa cell tumor=2 Mixed germ cell tumor=1 Dysgerminoma=2 Immature teratoma=1 Squamous cell carcinoma=1
Laterality	16=unilateral, 4=bilateral	All unilateral	21=unilateral, 9=bilateral
Apoptotic index (Mean±SD) p value <0.001; Highly significant	0.00 ± 0.003	0.01 ± 0.005	0.04 ± 0.021
Mitotic index (Mean±SD) p value <0.001; Highly significant	0.00 ± 0.003	0.02 ± 0.003	0.03 ± 0.021
Total p53 score (Mean Rank) Mann Whitney Z value 4.509 p value <0.001; Highly Significant	0.00	6.50	LGSC 8.12 HGSC 25.17
p53 score and apoptotic index Spearman Correlation Coefficient	0.365; p =0.299; Not Significant	0.366; p =0.299; Not Significant	0.439; p = 0.015; Significant

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p53 score and mitotic index Spearman Correlation Coefficient	0.079; p =0.03; Not Significant	0.178; p =0.04; Not Significant	0.600; p < 0.001; Highly Significant

Table 2: p53 positivity in Ovarian tumor of different histogenesis.

Surface epithelial lesions	**Total no. of cases**	**No. of cases showing p53 positivity**	**Staining intensity and pattern**	**%**	**Peritoneal deposits**	**CA-125 Levels**	**Follow Mean 1.5±0.4 Years**
Benign							
Serous	11	0.0	Negative	0.0%	Not seen	WNL	A
Mucinous	09	0.0					
Borderline	10						
Serous	5	01	Weak in <5% of the nuclei	3.3 %	Not seen	Mildly elevated (Mean=90IU/ml±10.4)	A
Mucionous	5	01	Weak in 5-25% nuclei				
Malignant							
LGSC	04	03	Medium in 25-50% of nuclei.	5%	Not seen	Highly Elevated (Mean=290IU/ml±30.9)	A
HGSC	12	12	Strong diffuse in >75% of nuclei.	20%	08 Cases +		5=D 1=A 6= NA
Mucinous carcinoma	04	03	Weak in 5-25% of nuclei in 2 cases and Strong in 25-50% of nuclei in	5%	02 Cases+	Elevated (Mean=180IU/ml±20)	2= D 2= A

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			one case.				
Endometroid carcinoma	01	0.0	Negative	0.0%	Not seen	NA	A
Clear cell carcinoma	01	0.0	Negative	0.0%	Not seen	NA	D
Malignant Brenner tumor	01	01	Medium, in 25-50% nuceli	1.6%	Not seen	NA	NA
Squamous cell carcinoma	01	01	Medium, in 5-25% of nuclei	1.6%	Not seen	NA	NA
Malignant germ cell tumor			Weak-medium, in		Not seen	WNL	
Dysgerminoma	02	02	20-50% of nuclei	6.6%			A
Malignant mixed germ cell tumor	01	01	Medium in 5-25% of the nuclei				D
Immature teratoma	01	01	Weak in 5-25% of the nuclei				A
Sex cord stromal tumors Adult Granulosa cell tumor	02	01 01	Medium, in 25-50% of nuclei Strong, Diffuse in >75 nuclei	3.3%	Not seen	NA	LAMA

Abbreviations: NA=Not avaialable, A=Alive after chemotherapy, D= Dead, CT=Chemotherapy, LAMA=Left against medical advise

Discussion

Ovaries are the source of female fertility, and at the same time the origin of many of the most complex as well as lethal neoplasms.[1,2]

Epithelial ovarian cancer comprises the majority of malignant ovarian tumors in adult women. These neoplasms are classified into distinct morphologic categories based on the appearance of the epithelium into tumors of serous, mucinous, endometrioid, clear cell, transitional, squamous, mixed and undifferentiated type.[3-6] Current data indicate

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that each of these histologic subtypes is associated with distinct morphologic and molecular genetic alterations. High-grade serous and possibly endometrioid carcinomas most probably arise from surface epithelial inclusion glands with TP53 mutations and dysfunction of BRCA1 and/ or BRCA2.[7-11] Low-grade serous carcinomas probably arise in a stepwise fashion in an adenoma-borderline tumor-carcinoma sequence from typical to micropapillary borderline tumors to low-grade invasive serous carcinoma via activation of the RAS-RAF signaling pathway secondary to mutations in KRAS and BRAF.[12,13]

Mucinous carcinomas arise via an adenoma-borderline tumor-carcinoma sequence with mutations in KRAS. Low-grade endometrioid carcinomas arise from endometriosis via mutations in CTNNB1 (the gene encoding beta-catenin) and PTEN.[14] Although the morphologic data strongly support an origin of clear cell carcinoma from endometriosis, there is limited data on the genetic alterations in these uncommon tumors.[15-18]

The age range of the patients with benign ovarian neoplasms included in the study was 21-40 years with mean age of 31.60 ± 10.3 and malignant ovarian neoplasms was in 31-70 years of age group with mean age of 43.93 ± 18.3 supporting the fact that cancers occur in the higher age group.[19] Of total 20 benign cases, two cases of serous cystadenoma was bilateral. Of total 30 malignant cases, nine cases were bilateral which included seven cases of serous adenocarcinoma and two of mucinous adenocarcinoma. Similarly, Arik et al studied 28 malignant serous tumors, out of which five were unilateral and 23 were bilateral supporting that bilaterality is a common feature of serous neoplasms.[13]

Out of 30 cases, maximum number of cases was of serous cystadenocarcinoma (53.33%) followed by mucinous cystadenocarcinoma (13.33%), dysgerminoma (6.6%), granulosa cell tumor (6.6%) and one case (3.3%) each of endometroid carcinoma, clear cell carcinoma, immature teratoma, mixed germ cell tumor and squamous cell carcinoma. In all studies including ours, maximum number of cases was of serous cystadenocarcinoma. Dietl et al studied seven cases of dysgerminomas.[14] Similarly Ala-Fossi et al studied exclusively granulosa cell tumour of the ovary.[15] But the present study considered tumors of various origins i.e. surface epithelial, germ cell tumours and sex cord stromal tumors. The difference in results if found could be due to different histogenetic origin of the neoplasms studied.

Expression of P53 in ovarian tumors of different histological types:

In present study, out of 20 benign ovarian neoplasms, all the cases were negative for p53 staining. Among 10 borderline cases, two (20%) were positive for p53 staining, out of which one serous cystadenoma was weakly positive and one case was of mucinous cystadenoma showed medium staining intensity. In a study done by Chan et al[3], two out of 11 benign cases (18.0%) were weakly positive for p53 staining. Kohler et al and Klemi et al included seventeen and six benign cases in their study respectively, out of which, none (0.0%) was positive.[16,17] Marks et al examined p53 expression in 107 epithelial ovarian cancers by IHC and observed high nuclear expression p53 protein in the malignant epithelium in 54 (50%) cases and expression of p53 protein was undetectable in 13 benign gynecological tissues.[18]

In current study, of all malignant ovarian neoplasms, the surface epithelial tumors showed highest p53 expressivity as compared to sex-cord stromal or germ cells tumors. This difference could be because of different histogenetic origin of ovarian neoplasms included in the present study implicating a possible association of p53 mutation in tumors of diverse origin. In this study, p53 expression was mainly found in high grade serous cystadenocarcinomas (HGSC). All low grade serous carcinomas (LGSC) were negative or showed weak p53 staining. The present study observed 86.6% positivity of p53 among malignant lesions which is well within the range reported in the literature i.e. 47%-80%.19-25 Chan et al observed strong expression of p53 proteins in 54% (25/46) of the malignant ovarian tumors.[3] Yemelyanova et al observed medium-strong staining intensity in 30 cases (52.63%).[23] In the present study, of total 24 surface epithelial (SEOTs) malignant cases, serous carcinomas showed strong diffuse staining pattern in 12 cases followed by medium staining in three cases. Among mucinous carcinomas (total 4 cases) only one (1/4) case showed strong-diffuse

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staining pattern followed by weak staining in two cases. Both endometroid and clear cell carcinoma were negative for p53 expression. Malignant Brenner's tumor and squamous cell carcinomas also showed medium staining intensity. Expression of p53 increases as one proceeds from benign to malignant end of the spectrum and this association was statistically found to be highly significant i.e. p <0.001 in the present study. Chan et al observed that among malignant ovarian tumors, p53 expression was more prevalent in high grade tumors than in low grade tumors, implicating a possible association of p53 expression with malignancy of ovarian cancers.[3] In our study, the intensity and quantity p53 immunostaining was more in high-grade serous tumors as compared to low-grade surface epithelial tumors. But it did not show statistically significant association (p = 0.092) between the histological grades of the tumors. It is possibly due to small number of high grade cases included in the study that we could not find a statistical significance in our study. Similarly Marks et al and Berker et al were unable to demonstrate a significant association between p53 expression between different histological grades of the tumors.[18,20] Teh and Lee examined both benign and borderline malignant /malignant mucinous neoplasms for p53 protein accumulation by the means of an anti-human p53 protein monoclonal antibody on paraffin sections.[24] Results showed that p53 protein accumulation is associated to a similar degree with both malignant mucinous cystadenocarcinomas and mucinous cystadenomas of borderline malignancy. This suggests that p53 mutations may play an important and early role in malignant transformations of 1/3rd or more of mucinous ovarian neoplasms.[24] In the present study also high grade mucinous carcinomas showed medium staining in >75% of the tumor cell nuclei.

p53 immunopositivity was also observed in adult granulosa cell tumor and malignant germ cell tumor of the ovary. Dietl et al investigated the immunohistochemical expression of p53 in seven cases of dysgerminomas of the ovary and concluded that overexpression of p53 thus appears to be very common in dysgerminoma, as it is in epithelial ovarian cancer.[14]

Ala-Fossi et al investigated p53 expression in 30 cases of granulosa cell tumours of ovary and found that eleven tumors were positive for p53 and 19 were negative. The association between p53 immunoreactivity and stage was statistically significant (P = 0.026). They showed that expression of mutated p53 in ovarian granulosa cell tumours seems to be associated with unfavorable prognosis.[15] In 2007, Rajesh et al[25] noticed that expression p53 was high in surface epithelial tumours, especially the serous cystadenocarcinoma as compared to the benign and borderline tumours. Further the expression of p53 in tumours arising from germinal cells, sex-cord stromal cells are observed to be very low. The expression of p53 and its correlation to the morphology enhances the prognostic significance. ***Table 3*** shows comparative studies on p53 expression in SEOTs. Sylvia et al, Gursarn et al, Naik et al and Amanullah et al observed that p53 were negative in benign and higher in malignant, serous, grade 3, and tumors with ascites.[31-34].

From the above discussion, it is observed that different authors have found different p53 expression rate among the various histological grades. Many factors influence p53 study results. The different tissues used in different studies can give different results. The p53 antigen is reported to be better presented in fresh tissue studied by frozen section. The different enzymes used for staining and the microwave procedure can also have a marked effect on the staining results. Another reason for the different reported results is the different 'cut off' values.[27-34]

Table 3: Comparative study of p53 positivity in Benign, Borderline and Malignant Surface Epithelial ovarian tumors (SEOTs)

Studies	**Benign**		**Borderline**		**Malignant**	
	Total cases	% positivity	Total cases	% positivity	Total cases	% positivity
Marks et al	13	0 (0.0%)	NA	NA	107	56 (52.3%)

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(1991)						
Klemi et al (1994)	6	0 (0.0%)	10	0 (0.0%)	45	24 (53%)
Hartmann et al (1994)	NS	-	NS	-	284	177 (62%)
Chan et al (2000)	11	2 (18.0%)	27	5/27 (19%)	46	25 (54%)
Berker et al (2002)	NS	-	NS	-	50	33 (66%)
Sagarra et al (2002)	NS	-	NS	-	90	42 (47%)
Malamou-Misti et al (2007)	NS	-	NS	-	95	67 (70.5%)
Gursan et at (2009)	30	0(0.0%)	10	0.00%	35	40.00%
Sylvia et al (2010	17	0(0.0%)	10	20.00%	33	57.60%
Yemelyanova et al (2011)	NS	-	NS	-	57	40 (80%)
Naik et al (2015)	82	6.10%	12	75%	16	81.25%
Amanullah, et al (2020)	30	0 (0.0%)	7	0 (0.0%)	23	65.2%
Present Study	20	0 (0.0%)	10	2 (20%)	30	86.6%

Relation of P53 expression with apoptotic and mitotic indices:

It was found that in normal human ovaries and benign tumors, only a small number of apoptotic cells were found to scatter among surface epithelial cells reflecting low apoptotic activity. Malignant tumors exhibited a slightly greater average apoptotic index, and apoptotic cells were most frequently seen in grade II and grade III malignant tumors, as indicated by the highest apoptotic indices among all of the ovarian samples examined.[26] Diebold et al reported similar observations that apoptosis was particularly prominent in high grade tumors, suggesting that although malignant tumors show high proliferative activity, relatively high apoptotic activity counteracts, leading to high cellular turnover in these tumors.[24] It has been shown that when the proliferative activity of the malignant tumors exceeds apoptotic cell death, an accumulation of tumor cells results.

In the present study, a significant positive correlation was seen between p53 score and AI and MI i.e. high apoptosis and mitosis was seen in tumors with high p53 score and vice versa. Similar results have been shown by various other

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studies.[31-34] High proliferation rate with associated TP53 alteration indicate poor prognosis, but the association between p53 expression and patient prognosis is controversial.

The main limitation of the study is less number of cases included of different histological origin. More cases of germ cell and sex cord stromal tumors needs to be studied to draw valid conclusions.

Conclusion: It is concluded that strong and diffuse nuclear expression of Tp53 can be used as robust method for inferring the presence of Tp53 gene mutation in high grade serous carcinomas and can be used as surrogate marker for diagnostic workup of carcinoma ovary. Its correlation with mitotic and apoptotic indices with increasing grade of malignancy can further help in differentiating benign from malignant tumor.

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