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Role of Expression of P53 in Differentiating Benign, Borderline and Malignant Surface Epithelial Ovariantumors

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Abstract
Introduction*: Ovarian carcinoma is 6th most common cancer among the women worldwide. Surface epithelial ovarian tumors accounts for two third of all ovarian neoplasms and their malignant form represent about 90% of ovarian cancers. One of the most studied prognostic marker in ovarian cancer so far is overexpression of p53. Intranuclear accumulation of p53 has been detected in as many as 69% ovarian carcinomas by immunohistochemical studies.*
Method and material*: All the cases of surface epithelial ovarian tumors diagnosed during study period classified according to WHO classification. Immunohistochemical analysis was done with p53 marker.*
Results*: A total of 156 cases were studied, out of which benign tumors were the most common 117 cases (75%), followed by malignant tumors 33 cases (21%) and 6 cases (4%) of borderline malignancy. P53 immunostaining on 6 borderline surface epithelial ovarian ,out of 4 borderline serous cystadenomas 75 % were p53 positive and both borderline mucinous cystadenomas were p53 positive.*
P53 immunostaining on 33 malignant surface epithelial ovarian tumors, 78.2%serous cystadenocarcinoma were p53 positive and 71.5% mucinouscystadenocarcinoma were p53 positive . 100% Endometrioid and Adenosarcoma of ovary were p53 positive .
Conclusion*: P53 expression was high in malignant lesions compared to benign and borderline lesions, this emphasize their importance in the pathogenesis of surface epithelial ovarian cancer and suggest a relevant role in the progression to the invasive phenotype.*
In the present study correlation of p53 expression with histological type, stage and grade of tumor was found statistically insignificant. The limitations of study were restricted number of samples and using only one marker.
Key words*:-Surface epithelial ovarian tumors, P53 Immunostaining, Grade, stage and type of ovarian tumors.*

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I. Introduction

Ovarian cancer is the 6th most common cancer among the women worldwide [(1)] and second most common gynaecological malignancy [(2)].The incidence rate of ovarian cancer is 22,240 with annual deaths being 14,070. Ovarian carcinoma is usually asymptomatic in early stages and there is no standard screening test for its early detection, due to this 67% of ovarian carcinoma is diagnosed at an advanced stage.[(1)]Surface epithelial tumors of the ovary account for approximately two-third of all ovarian tumors and their malignant forms represent about 90%of ovarian cancers.

Surface epithelial tumors are classified based on tumor cell type (serous, mucinous, endometrioid, clear cell, transitional)[(4)]. Serous carcinoma is the most common type of ovarian cancer, accounting for 68% of ovarian cancers [(5)]. The distinction between borderline versus carcinoma is utmost significance for prognostic purposes [(6)]. Borderline tumors have a favorable prognosis, even in advanced stages.[(7)]One of the most studied prognostic markers in ovarian cancer so far is overexpression of p53. Intranuclear accumulation of p53 has been detected in as many as 69% ovarian carcinomas byimmunohistochemical studies.[(8)]Overexpression of mutant p53 protein is a common feature of invasive epithelial ovarian cancer and has been detected in more than half of the epithelial ovarian cancer and proposed to be a prognostic factor. [(11)]

P53 is a tumor suppressor gene. P53 links cell damage with DNA repair, cell cycle arrest, and apoptosis[(9)]. In view of these activities p53 has been rightfully called as **guardian of the genome**. With loss of function of p53, DNA damage goes unrepaired, mutations accumulate in dividing cells, and the cell marches along a one way street leading to malignant transformation. The ability of p53 to control apoptosis in response

to DNA damage has important practical therapeutic implications.Tumors that retain normal p53 are more likely to respond to irradiation and chemotherapy therapy than tumors that carry mutated alleles of the gene [(10)].Mutant p53 proteins have a prolonged half-life, accumulate in the nucleus, and can be detected by immunohistochemistry.

II. Aims And Objectives

1. To evaluate the expression of p53 in surface epithelial ovarian tumors.
2. To correlate expression of p53 with histological type, stage and grade of surface epithelial ovarian tumors.

III. Materials And Methods

In this retrospective study, histopathological slides of 156 cases of surface epithelial ovarian tumors during Sep 2016 to Aug 2018 retrieved from archieve of pathology department . The histological type was confirmed by reviewing H & E stained slides. Tumor grading was done according to the Silverberg scoring system.

The most representative section for immunohistochemistry were selected. 3-4 micrometer thick sections from each tumor blocks were obtained. IHC is done on Leica Bond Max machine by automated method with positive and negative control.All the immunostained sections were scanned randomly at 100x magnification for the most densely labelled areas. The nuclear counts were taken at 400x magnification. A total of 1000 nuclei were counted in most densely labelled microscopic fields.

The percentage of positive in each section was scored : 0 for < 5%, 1 for 5-25%, 2 for 26-75%, 3 for >75%. Then the intensity of positivity was scored : 1 for weak, 2 for moderate, 3 for severe

Statistical Analysis

The relationship between p53 expression and the clinicopathological variables was analyzed. The results were considered statistically significant if the p value was <0.05.

IV. Results And Observations

In our study on Expression of P53 in Surface Epithelial ovarian tumors we have evaluated 156 cases, aged between 10-70 years age group from September 2016 to August 2018.

Patients with surface epithelial tumors of ovary were classified into benign, borderlineand malignant group as per WHO classification[12]**Table-1**. Out of 156 cases , 117 tumors were benign (75%), 33 cases were malignant (21%), and 6 cases were of borderline type (4%). Age distribution were as per **Table-2.** Most of cases were seenin the age group of 31-40 years . The mean age was 51 for malignant tumors, 40 for borderline tumors and 32 for benign tumors.

The histomorphologicaltype of ovarian tumors were as per **Table-3.** Out of 156 patients, 117 patients had benign surface epithelial ovarian tumor while 33 patients had malignant ovarian tumor.

Since grading of ovarian carcinoma is not yet standardized, we followed the 2 tier grading system which is as follows : Type I (low grade) tumors include – low grade serous carcinoma, low grade endometrioid carcinoma and mucinous carcinoma, while Type II (high grade) tumors include High-grade serous carcinoma, High grade endometrioid carcinoma, Undifferentiated carcinoma and Carcinosarcoma.

In present study, as per Table 3 out of the 33 malignant surface epithelial ovarian tumor patients, 26 patients were of low grade and 7 patients were of high grade.

The Various Parameters affecting the stage of the tumor were noted and compared as per **Table 4.** Ovarian capsule ruptured was seen in 3% cases, Fallopian Tube implants in 18.2% cases, Serosaldeposits in6% cases, Malignant cells in ascitic fluid in 54.5% cases, Omental deposits in 24% cases.Taking all the parameters into consideration staging of surface epithelial ovarian tumors was done according to FIGO staging as per **Table 5**. In the present study maximum number of cases in stage I 70% cases

Table 1: Distribution of cases of Surface epithelial ovarian tumors.

Types of Lesion	No. of patients	% of cases
Benign Tumors	117	75%
Borderline Tumors	6	4%
Malignant Tumors	33	21%
Total	156	100%

Table 2: Age distribution of surface epithelial tumors of ovary.

Age groups (years)	Number of Patients	% of cases
11-20	6	3.8%
21-30	23	14.7%
31-40	47	30.3%
41-50	34	21.7%

51-60	26	16.6%
61-70	20	12.8%
Total	156	100%

Table 3: Histomorphological distribution of surface epithelial tumors of ovary

Histomorphological type	Differentiation of tumor	Number of patient
Serous cystadenoma	Benign	86
Serous cyst adenoma	Boderline	4
Mucinous cystadenoma	Benign	31
Mucinouscyst adenoma	Boderline	2
Serouscyst adenocarcinoma	Malignant, Low grade	17
Serous cyst adenocarcinoma	Malignant, High grade	6
Mucinous cystadenocarcinoma	Malignant, Low grade	7
Endometrioid carcinoma	Malignant, Low grade	2
Adenosarcomaof ovary	Malignant, Low grade	1
Total		156

Table 4: The Various Parameters affecting the stage of the tumor.

S.No.	Parameters	No. of cases positive	No. of cases negative
1	Ovarian capsule ruptured	3%(1)	96.9%(32)
2	Fallopian Tube implants	18.2%(6)	81.8%(27)
3	Serosal deposits	6%(2)	93.9(31)
4	Malignant cells in ascitic fluid	54.5%(18)	45.5%(15)
5	Omental deposit of malignant cells	24%(8)	75.7%(25)

p value <0.0001 (S)

Table 5: Staging of surface epithelial ovarian tumors done

S.No.	Stage	No. of cases
1	Stage I	23(70%)
2	Stage II	2(6%)
3	Stage III	8(24%)
4	Stage IV	0

P53 IHC was done on all the cases of borderline and Malignant surface epithelial ovarian tumors. In our study, benign tumors were excluded because review of literature strongly shows normal expression of p53 in benign lesions of ovary.

P53 IHC on borderline and malignant tumors as per **Table 6**. Out of 6 borderline cases 5 were positive. Of these 5 positive cases, 3 cases were of borderline serous and 2 cases of borderline mucinous. Out of 33 malignant cases 26 were positive. Of these 26 positive cases, 18 were of serous cystadenocarcinoma, 5 cases of mucinous cystadenocarcinoma, 2 cases of endometrioid, and 1 cases of adenosarcoma ovary.Comparision of p53 expression of in low and high grade tumors as per **Table 7**. P53 was positive in 19 of 26 cases of low grade tumors and in all of the 7 cases of high grade surface epithelial ovarian tumors.The intensity of p53 immunostaining in malignant surface epithelial ovarian tumors was as per **Table 8**

Table 6: Results of p53immunostaining in Borderline and Malignant tumors

Types of Tumor	P53+ve	P53-ve	Total
Borderline Serous	3(75%)	1(25%)	4
Borderline mucinous	2(100%)	0	2
Serous cystadenocarcinoma	18(78.2%)	5(21.7%)	23
Mucinouscystadenocarcinoma	5(71.5%)	2(28.5%)	7
Endometrioid adenocarcinoma	2(100%)	0	2
Adenosarcoma ovary	1(100%)	0	1

p value of borderline tumors 1.000 (NS)
p value of malignant tumors 0.792 (NS)

Table 7 Comparision of expression of p53 in Low grade and High grade malignant tumors

Grade of tumor	Total		P53 +ve		P53 –ve	
	No.	%	No.	%	No.	%
Low grade tumors	26	78.8%	19	73%	7	100%
High grade tumors	7	21.2%	7	26.9%	0	0%
Total tumors	33	100%	26	78.8%	7	21.2%

p value 0.272 (NS)

Table 8: Intensity of p53 immunostaining in Malignant Surface Epithelial ovarian tumors

Intensity of p53staining	Serous cystadenocarcinoma	Mucinous cystadenocarcinoma	Endometrioid Adenocarcinoma	Adenosarcoma of ovary
Negative	5(21.7%)	2(28.5%)	0	0
+1	1(4.3%)	0	0	0
+2	7(30.4%)	4(57.1%)	0	1(100%)
+3	10(43.4%)	1(14.2%)	2(100%)	0

p value 0.572 (NS)

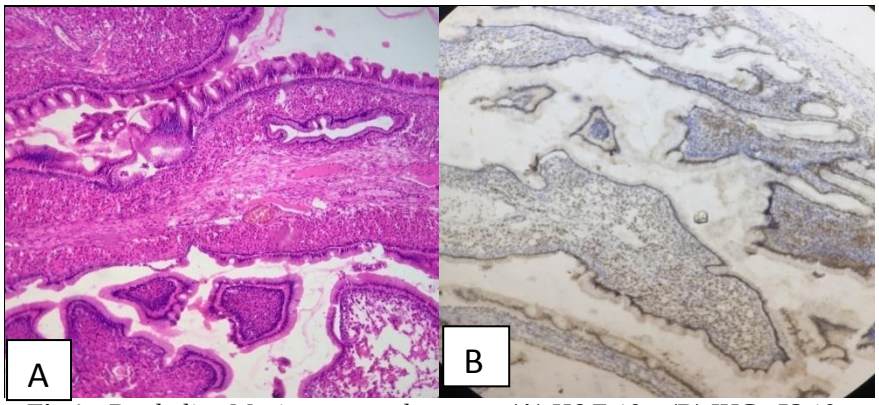

Fig 1:- Borderline Mucinous cystadenoma – (A) H&E 10x. (B) IHC p53 10x

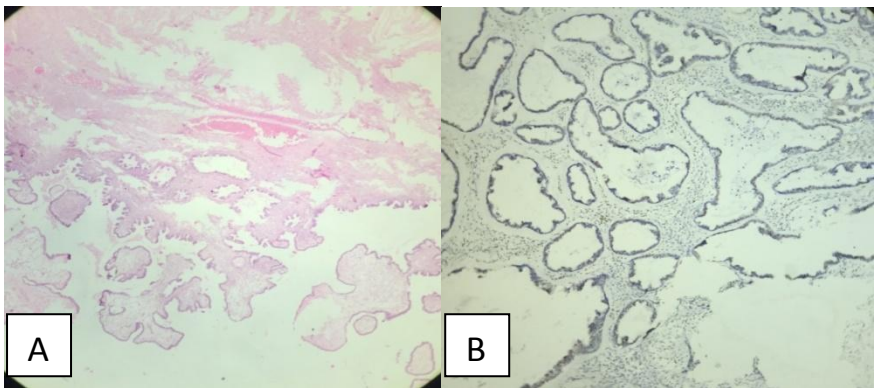

Fig 2:- Borderline Serous cystadenoma-(A) H&E 4x (B) IHC P53 10x

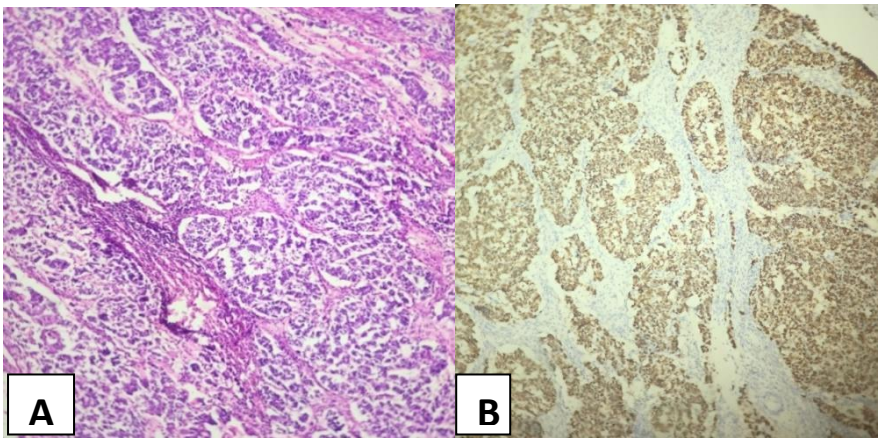

Fig 3:- High Grade Serous cystadenocarcinoma-(A) H&E10x (B) IHC p53 10x

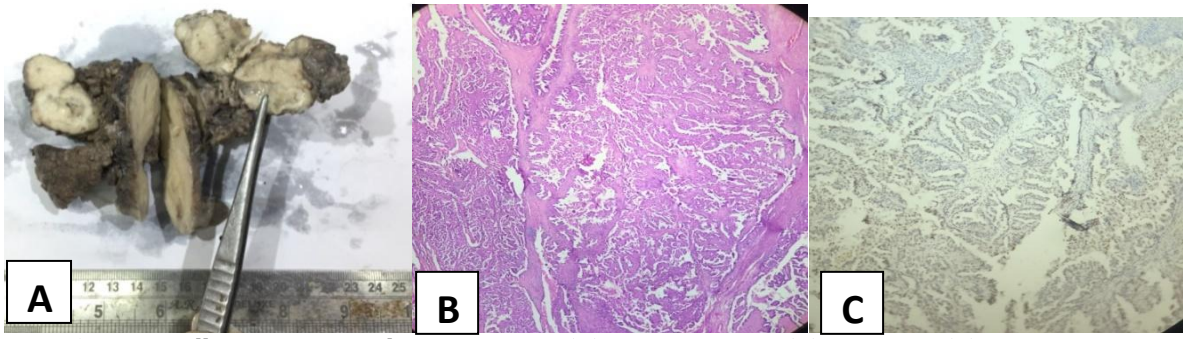

Fig. 4:- Papillary serous cystadenocarcinoma - (A) Gross specimen (B) H&E 10x. (C) IHC p53 10x

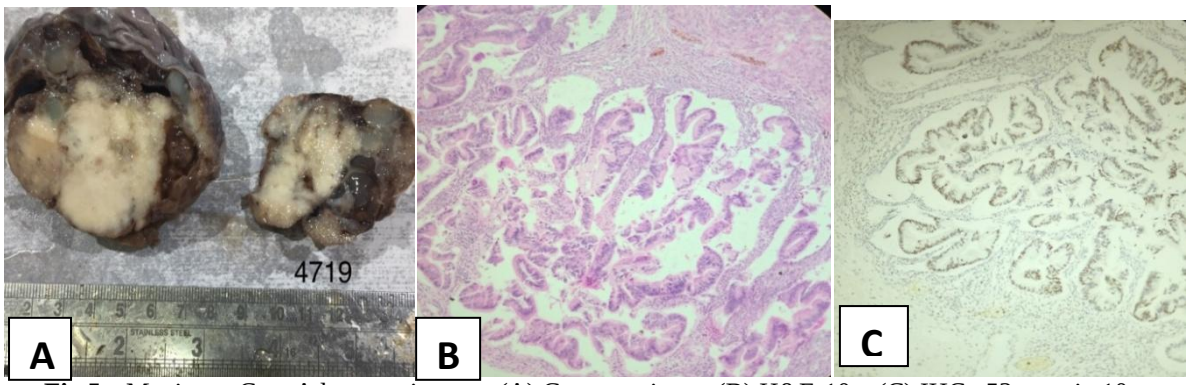

Fig 5:- Mucinous Cyst Adenocarcinoma - (A) Goss specimen. (B) H&E-10x. (C) IHC p53 protein 10x

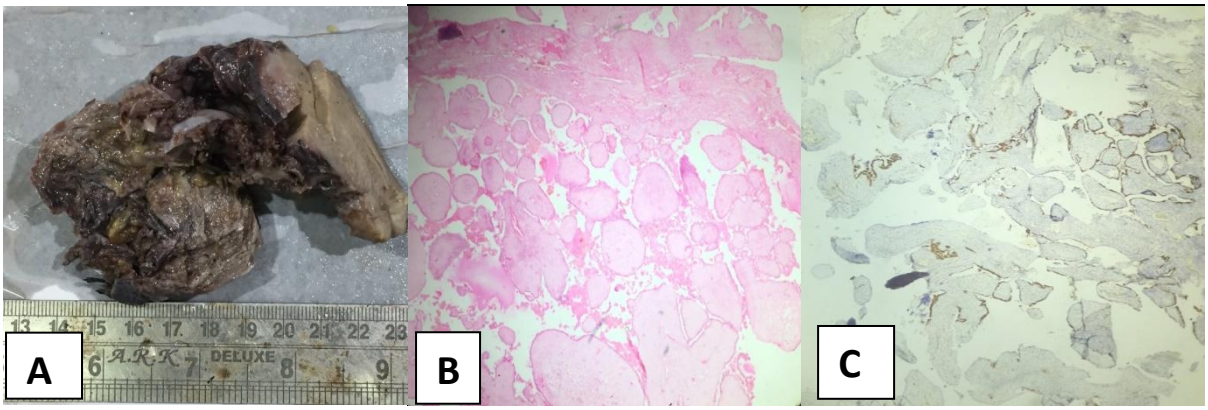

Fig.6:-Serous cystadenofibrocarcinoma(A) Goss specimen (B) H&E10x. (C) IHC p53 10x

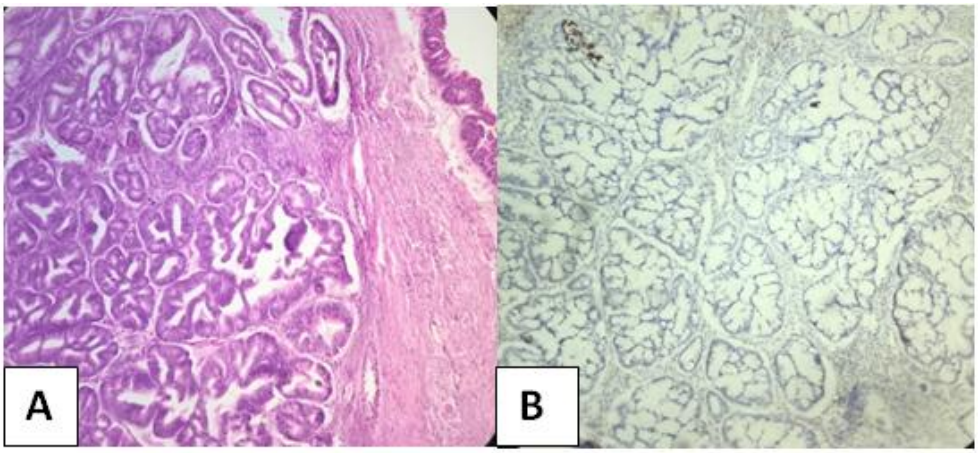

Fig 7:- Mucinous cystadenocarcinoma- H&E-40X (B) IHC p53 10x

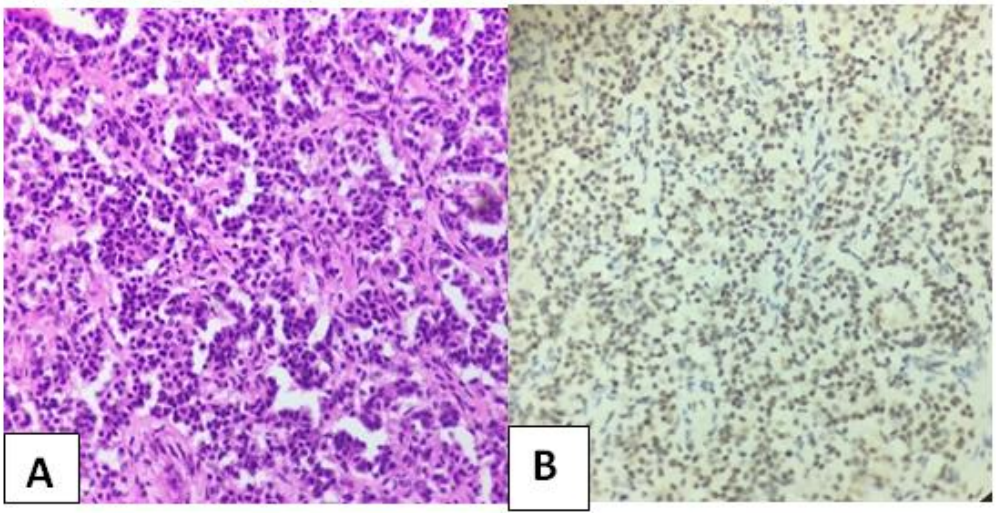

Fig 8: EndometrioidAdenocarcinom–(A) H&E -40x. (B) IHC p53 40x

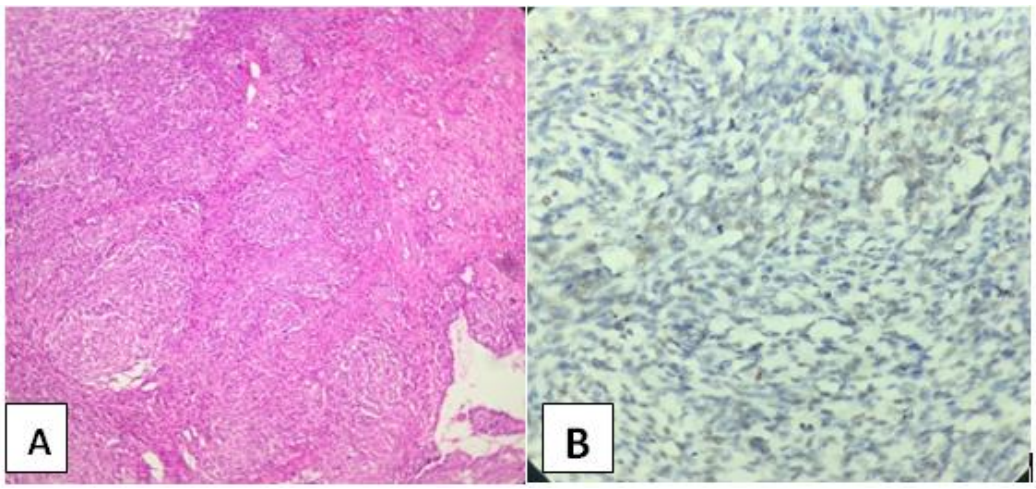

Fig 9:- Adenosarcoma of ovary – (A) H&E 10x. (B)IHC p5340x

V. Discussion

A total of 156 cases were studied, out of which benign tumors were the most common (75%), followed by malignant tumors (21 %) and borderline tumors(4%) . Most of the benign tumors were unilateral bilateral cases were mostly malignant. The maximum patientwas in the age group of 31-40 years. The youngest patient was 15 years old and the oldest was 69 years old. The present study was in concordance with both studies as per **Table -9**, where most of the cases were seen between 30-50 years of life [13,14].

In Benign tumors serous cyst adenoma was the most common neoplasm found and accounted for 86 cases (74%) , followed by mucinous cystadenoma, which accounted for 31 cases (26%).In borderline tumors, 4 cases are of borderline serous tumors (66.5%) and 2 borderline mucinous tumors (33.4%). Serous cystadenocarcinoma (70%) were most common malignancy followed by mucinous cystadenocarcinoma(21%).Comparative analysis of benign and malignant lesions with other studies [13,14] was as per **Table-10**. Comparative analysis of histomorphological types with other authors [13-15] showed the similar results as per **Table -11.**

Table 9: Comparative analysis of age incidence of Surface Epithelial tumors with other studies

Age group	Kae, et al. [13]	Jha, eta al. [14]	Present study
11-20	0%	2%	3.8%
21-30	28%	30%	14.7%
31-40	13%	11%	30.3%
41-50	38%	25%	21.7%
51-60	15%	20%	16.6%
61-70	6%	12%	12.8%
Total	100%	100%	100%

Table 10: Comparative analysis of Benign/Malignant lesions with other study

	Kar, et al [13]	Jha, et al.[14]	Present study
Benign	57%	79%	75%
Borderline	9%	0%	4%
Malignant	34%	21%	21%
Total	100%	100%	100%

Table 11: Comparative analysis of the various histological types with other studied

Histological types	Kar, et al [13]	Jha, et al [14]	Maheshwari, et al [15]	Present study
Serous tumors	70%	68%	58%	72.4%
Mucinous tumors	24%	32%	36%	25.6%
Total	100%	100%	100%	100%

In the present study, we have studied the expression of p53 in borderline and malignant surface epithelial ovarian tumors by IHC using monoclonal antibody against p53 protein (clone DO-7; Dako)

Present study observes that p53 staining of malignant surface epithelial ovarian tumors was statistically significantly higher than that of benign tumors .

Among the serous type, 18 cases (78.2%) were positive and among the mucinous type, 5 cases (71.5%) were positive. Positivity rates in our study are high as compared to Pde Graff, et al. [17] and J.R.Mark, et al. [19] (Table - 12). The reason for this variation are unknown. However possible source of variation may be attributed to[22]

a. interobserver variability in interpretation of slides and technical problems with antigen retrieval.
b. The properties of different antibodies.
c. The scoring method applied to p53 immunoreactivity

Table 12: Comparative analysis of the results of p53 immunostaining with other studies

Author	Total cases	Method of evaluation	No. +ve cases	% of +ve cases
Pyrii, et al. [16]	120	AQUA	98	81.6
Pde Graff, et al. [17]	476	IHC OF TMA	248	52.1
Ayadi,et al. [18]	57	IHC	42	73.6
J.R.Marks, et al. [19]	107	IHC	54	50.4
Kuprijanczyk,etal. [20]	38	IHC, SSCP	26	68
Reles, et al. [21]	178	IHC	110	62
Present study	33	IHC	26	78.7%

Among the malignant surface epithelial ovarian tumors, positivity was high in serous type compared to the mucinous tumors. Positivity rates varied with histologic type, grade and stage of the tumor.

P53 expression in relation to age

P53 expression was mainly found in the 6th decade of life (30.9%). This may be related to the accumulation of somatic mutations [108]. It is known that loss of heterozygosity on chromosome 17 increases with age.[23] The promoter of MDM2 (Murine Double Minut 2) and p53 interaction partner contains a functional estrogen receptor signal in the DNA. [24]Therefore, the effect of the p53 on risk of cancer in women could depend on menopausal status.Statistically, there was no significant correlation between p53 overexpression and age of the patients.

P53 expression in relation to histological type of tumors

Our study showed p53 expression in carcinomas mainly, this was reported by others who found that malignant surface epithelial tumors, especially serous cystadenocarcinomas of the ovary showed high expression of p53 compared to the benign and borderline tumor[25]

Statistically, there was no significant correlation between p53 expression and histological type of tumors. Review of literature showed conflicting results; some with no significant relation andothers with significant relationship.Gursan et al [25]found most significant in serous carcinoma. In the study of Ayadi et al[26], there was no significant difference in expression of P53 between serous and non-serous tumors (p=0.84).

P53 expression in relation to grade of tumors

In the recent years, a two tier grading system has been proposed by malpica A etal for malignant surface epithelial tumors of the ovary, such as, Low-grade(Type I) and High grade(Type II)

In the present study P53 expression was mainly found in invasive serous cystadenocarcinoma tumor (47.6%). However, the expression of p53 in relation to grade was not significant statistically and this is also

shown in three other studies [27-29]. According to Malpica et al, in applying 2 - tier grading system, the survival of patients with low grade tumors were significantly higher than high grade tumors. They observed that median survival was 1.7 years for patients with high-grade tumors compared to 4.2 years for patients with low grade tumors. [30]

VI. Conclusion

Ovarian carcinoma is 6th most common cancer among the women worldwide and second leading cause of cancer related deaths . P53 expression was high in malignant lesions compared to benign and borderline lesions, this emphasize their importance in the pathogenesis of surface epithelial ovarian cancer and suggest a relevant role in the progression to the invasive phenotype and therefore immunohistochemistry is a good screening method that can be used to predict malignant versus proliferative tumors. P53 protein immunostaining is associated with several other prognostic factors , it may not have individual prognostic value. In the present study correlation of p53 expression with histological type, stage and grade of tumor was found statistically insignificant. The limitations of study were restricted number of samples and using only one marker.

Conflict of Interest - None
Source of funding- None

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