Original Research Article

Immunohistochemical expression of p53 in serous carcinoma of ovary and its correlation with clinicopathological parameters

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A R T I C L E   I N F O

A B S T R A C T

Introduction: Ovarian carcinoma is one of the most prevalent causes of mortality associated with carcinoma in women. More than 90% of ovarian cancer originates from epithelial cells. Multiple oncogene and tumour suppressor genes are involved in ovarian carcinogenesis among which p53 gene is found to be most frequently mutated.

Aim: To study the immunohistochemical expression of p53 in serous carcinoma of ovary and its correlation with clinico pathological parameters.

Materials and Methods: This study was carried over 18 histopathologically confirmed cases of serous carcinoma of ovary in VSSIMSR, Burla through a period of 1 year. Analysis of p53 expression was done immunohistochemically.

Results: Out of total 18 cases of serous carcinoma, 14 cases were high grade and 4 cases were low grade. While 78.57% (11) cases of HGSOC were diagnosed in advanced stages (FIGO Stage III and IV); advanced stage (FIGO Stage III) case reported for LGSOC was limited to just 25% (1). Further while only 50% cases of LGSOC were positive for p53 immunostaining, it was 100% in case of HGSOC. Mean value of preoperative CA-125 was high in HGSOC as compared to LGSOC.

Conclusion: The significant difference in p53 expression between HGSOC and LGSOC, indicates an altogether different pathogenesis of these tumours. The aggressive nature of this tumour is also suggested by the higher preoperative CA-125 values in case of HGSOC and diagnosis at a time when the tumour has already advanced.

1. Introduction

Ovarian cancer accounts for 2.5% of all malignancies among females and 5% of female cancer deaths because of low survival rates due to late diagnosis.[1] Age, family history of ovarian or breast cancer and inheritance of BRCA-1, BRCA-2 gene mutations are significant risk factors associated with increased pre valence of ovarian tumours. Ovarian cancer comprises of group of malignancies differentiated by cell or site of their origin, pathologic grade, risk factors, prognosis, and treatment.[2,3] Epithelial tumours of ovary constitute two third of all the ovarian neoplasm and their malignant forms represent about 90% of all the ovarian cancers.[4] Recent molecular and genetic studies suggest that epithelial ovarian cancer can be grouped into two broad categories: Type I and Type II. With clinical differences, there are also notable genetic differences. While, Type 1 cancers are associated with mutations in ARID1A, KRAS, PIK3CA, BRAF and PTEN ; the majority of Type 2 cancers are associated with mutations in p 53.[5] Based on recent two-tier binary grading system Serous carcinoma of ovary is now classified into low grade and high grade carcinoma and further suggests that high grade and low grade carcinoma does not represent two different grades of the same tumour but represent two different tumour types instead.[6] Based on molecular and genetic studies Low grade serous carcinoma is considered as Type I and High grade serous carcinoma

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considered as Type II tumour.

Most frequently mutated gene in ovarian carcinoma is p53 gene, a type of tumour suppressor gene.[7] Studies have shown that p53 gene is mutated in about 50-80% of ovarian carcinoma.[8]

LGSOC lacks p53 gene mutations and is considered to arise from borderline tumours. In contrast HGSOC arises as denovo and it has been suggested that 100% of HGSOC are in fact p53 mutated.[5,9]

Though, it is considered that Nucleotide sequencing is the most reliable technique to detect gene mutation, but due to time and effort involved, it is rarely used as a diagnostic tool. Therefore Immunohistochemical analysis of p53 expression is commonly used as a mimic for mutational analysis.[10–12]

The present study was conducted to evaluate immunohistochemical expression of p53 in serous carcinoma of ovary and its correlation with clinicopathological parameters.

2. Materials and Methods

The present study was a single-centre, prospective study. Total 18 histopathologically diagnosed cases of serous carcinoma were considered in the present study. Based on recent two-tier binary grading system, Serous ovarian carcinoma was histologically classified into low grade and high grade. All the relevant clinical history, investigation findings and serum CA-125 value were collected from patient case file. Staging was performed according to FIGO criteria. Immunohistochemical staining was done for p53 with Dako Flex monoclonal mouse anti-human p53 protein clone DO-7 over FFPE tissue sections which were mounted on poly-L-Lysine coated slides. Further sections were deparaffinised and microwave method was used for antigen retrieval. After that endogenous peroxidas e blocking was performed. Then, incubation with monoclonal primary antibody was done for 30 minutes in humidifying chamber. Then, secondary antibody conjugated with horseradish peroxidase enzyme was applied for 30 minutes. Thereafter, freshly prepared di-amino benzidine (DAB) was applied for 10 minutes. Finally haematoxylin was used for counterstaining and slides were then dehydrated and mounted.

Nuclear staining was acknowledged as a positive reaction. Scoring for p53 was based on proportion of cells in a given tumour specimen showing distinct nuclear positivity. p53 scoring result were done as follows:[13,14] 0 (negative or occasional positive), 1+($<$10% cells positive), 2+ (10-25% cells positive),3+ (26-50 % cells positive), 4+ (51-75% cells positive), 5+($>$ 75% cells positive).All the statistical analysis were done using IBM SPSS statistical software version 23.

3. Results

Out of total 18 cases of serous carcinoma, 14 cases were high grade serous carcinoma and 4 cases were low grade serous carcinoma (Table 1). All the cases were in the age group of 40 to 60 years of age group.

In the present study (Table 2), it was observed that, out of 14 HGSOC cases, 11 cases (78.57%) were diagnosed in advanced stages (FIGO Stage III and IV) while 3 out of 4 (75%) cases of LGSOC were diagnosed in comparatively earlier stages (FIGO stage I and II).

Number of p53 positive cases in LGSOC was 2/4 (50%) and in HGSOC it was 14/14(100%), showing a statistically significant difference (p$<$0.05) (Table 3). Correlation analysis shows a strong positive correlation of p53 expression with grade of serous carcinoma (Spearman's rho correlation ρ=0.701, p =0.001). Higher level (3+,4+, and 5+) and diffuse and patchy expression of p53 was expressed in HGSOC (Figure 5). The expression of p53 in LGSOC was focal reflected in low expression score(1+ and 2+)(Figure 2).

Mean value of CA -125 was significantly higher in HGSOC (2059$\pm$1460.55) than LGSOC (553.37$\pm$278.52) with the difference being statistically significant (p$<$0.01). On correlation analysis, preoperative CA-125 levels had strong positive correlation with grade of serous carcinoma. (Spearman's rho correlation ρ=0.695, p=0.000).

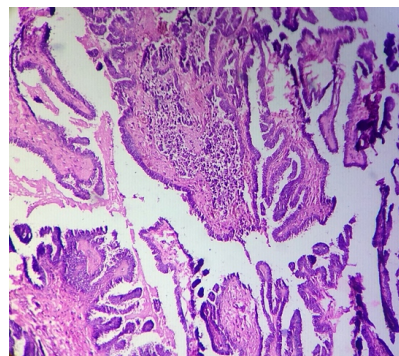**Fig. 1:** LGSOC: Branching papillary structure with psammoma bodies (H&EX100)

4. Discussion

Low grade and high grade serous carcinoma harbour different molecular abnormalities and have different clinical courses. LGSOC are slow growing tumours and have mild to moderate nuclear atypia with occasional mitotic figure. Micropapillary architecture is typical, necrosis is unusual and psammoma bodies are much more frequent (Figure 1). They arise from adenofibroma, atypical proliferative serous

Table 1: Distribution of Serous carcinoma according to two-tier binary grading system

Grade	Number	Percentage (%)
Low	4	22.22
High	14	77.78
Total	18	100

Table 2: Distribution of Low grade serous carcinoma and High grade serous carcinoma according to FIGO staging system

| | FIGO Stage | | | |
	Stage 1	Stage II	Stage III	Stage IV
Low grade serous carcinoma	1	2	1	–
High grade serous carcinoma	0	3	7	4

**Table 3:** Pattern of p53 expression in low and high grade serous carcinoma of ovary

| S.No. | Histotype | Total No | Positive cases | | Negative cases | | p-value |
			Number	Percentage (%)	Number	Percentage (%)	
1	Low grade serous carcinoma	4	2	50	2	50	<0.05
2	High grade serous carcinoma	14	14	100	0	0	

Table 4: Comparative analysis of p53 expression in low and high grade serous carcinoma of ovary with other studies

	Low grade	High grade	P value
Giurgea et al	1/8	9/16	p=0.06
Naik et al	1/2	7/7	p<0.05
O´Neil et al	30/47	4/22	p<0.05
Marinas et al	5/8	12/12	p=0.000
Sallum et al	4/22	59/85	p<0.0001
Present study	2/4	14/14	<0.00001

Table 5: Comparative analysis of preoperative serum CA-125 (median value in U/mL) in low and high grade serous carcinoma of ovary with other studies

Grade	Fader et al	Sallum et al	Present study
Low grade	119.1	98	588.25
High grade	246.7	954	1583
p value	p <0.001	p<0.001	p<0.01
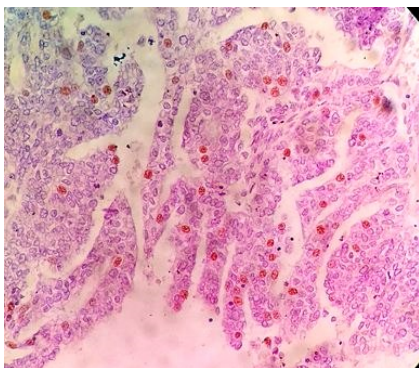
Fig. 2: LGSOC: Positive p53 expression (score 2+) (IHCX100)

tumour or serous borderline tumours and associated with frequent mutations of the KRAS, BRAF or ERBB2 genes. They rarely harbour p 53 mutations and are genetically stable.[2,4,15] In contrast, HGSOC is rapidly growing, highly aggressive and diagnosed mostly at advanced stages. They are typically composed of solid masses of cells with slit like spaces. Papillary areas are common. Nuclei are large, hyperchromatic and Pleomorphic. Multinucleation is common and mitosis is frequent (Figures 3 and 4). Recent studies suggest that a significant number of HGSOC cases originate from the intraepithelial carcinoma in the fallopian tube.[2,4,15–17]
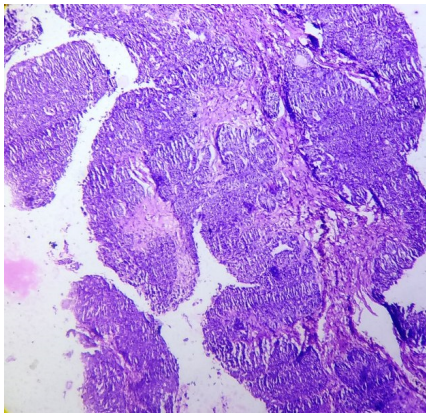
Fig. 3: HGSOC: Papillary and solid pattern (H&E X100)
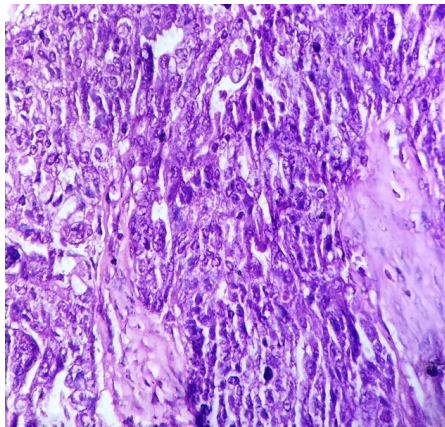

Fig. 4: HGSOC: Pleomorphic tumour cells, high mitotic index and abnormal mitotic figure(H&E X400)
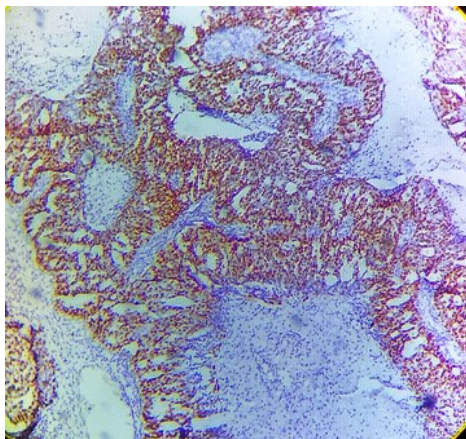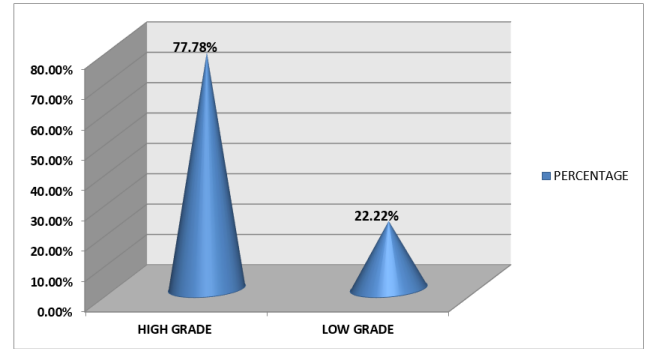

Fig. 5: HGSOC: Positive p53 expression (score 5+) (IHC X100)

Graph 1: Distribution of serous carcinoma according to two-tier binary grading system)
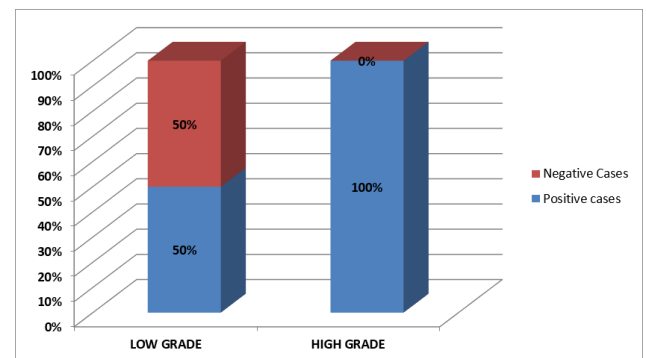

Fig. 6: Expression of p53 in low grade serous carcinoma and high grade serous carcinoma of ovary

HGSOC cases are associated with higher CA-125 values and have worse prognosis when compared with LGSOC.[18] The two types of serous ovarian carcinoma harbour different molecular abnormalities and have different clinical courses. These data from the literature were confirmed in our study.

Sallum et al[18] in their study observed that women with HGSOC accounted for a significantly higher proportion of advanced stage disease (80% vs 42.9%,p<0.001) compared with women having LGSOC. Similar findings were observed in the present study with cases in advanced stages accounting for HGSOC being much higher than LGSOC (78.57% vs 25%, p<0.001).

Similar to other studies reported in literature (Table 4) we too observed that HGSOC was associated with higher and diffuse p53 expression. In the study conducted by Sallum et al (2018)[18] (p<0.0001), Naik et al (2015)[19](p<0.05), O´Neil et al(2005)[13] (p<0.05) and Marinas et al(2012)[20] (p=0.000), there was statistically significant difference between p53 expression and grade of serous carcinoma, well correlated with our study(p<0.00001).   Whereas Giurgea et al (2012)[21] (p= 0.06) found no significant correlation between p53 immunostaining and grade of serous carcinoma.   Sallum et al (2018)[18] in their study observed that, p53 expression was diffuse in 68.2% of

cases, completely absent in 30.6% and was focal in 1.2% of HGSOC, compared with LGSOC, which showed diffuse expression in 9.5%, complete absence in 81.0% and focal expression in 9.5% (p < 0.0001). In the present study, we too observed that higher staining of p53 (4+ and 5+) was present in HGSOC. In contrast, in LGSOC 0 to 2+ staining pattern was observed. 100% cases of HGSOC in contrast to 50% cases of LGSOC showed positive immunostaining for p53 and the difference was statistically significant(p<0.00001).

In the present study we observed that median value of preoperative CA-125 in the low grade serous carcinoma (588.25U/mL) was significantly lower than those with high-grade serous carcinoma (1583U/mL; p < 0.01), similar to other studies (Table 5) conducted by Fader et al (2013)[22] (p<0.001) and Sallum et al (2018)[18] (p<0.001). Sylvia et al (2012)[23] and Cooper et al (2002)[24](p<0.001) in their study also observed that, median CA-125 levels were significantly increased in high grade tumours.

5. Limitation of our study

Sample size was small.

6. Conclusion

The significant difference of p53 expression between l ow grade and high grade serous carcinoma of ovary strongly suggests that the underlying pathogenesis of these two tumours is different. Higher p53 expression mainly in high grade carcinoma suggests its prominent role in the pathogenesis of high grade serous carcinoma of ovary. Further association of high grade serous ovarian carcinoma with advanced FIGO stages and higher CA-125 value suggests aggressive nature of this tumour.

7. Source of funding

None.

8. Conflict of interest

None.

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