Original Research Article

A Prospective Study of Expression of P53 in Ovarian Cancer and it's Correlation with Clinicopathological Parameters in a Tertiary Care Hospital

KP Umadevi[1*], Jawahar Ramasamy[2], Jeya shambavi.J[3], S Revathy[4]

[1]Associate Professor, Department of Pathology, Aarupadai Veedu Medical College & Hospital, Puducherry,India
[2]Professor, Department of Pathology, Aarupadai Veedu Medical College & Hospital, Puducherry, India
[3]Associate Professor, Department of Pathology, Aarupadai Veedu Medical College & Hospital, Puducherry,India
[4]Assistant Professor, Department of Pathology, Aarupadai Veedu Medical College & Hospital, Puducherry, India

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Abstract

Introduction: Ovarian cancer is the 6th most common cancer in women worldwide. It is the 5th leading cause of death among women in the developed countries. The incidence rate is higher in white women followed by Hispanic, Asians, Black and American Indian women.**Materials and Methods:** The present study was a single-centre, prospective study. Total 36 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.**Results:** Out of total 36 cases of serous carcinoma, 28 cases were high grade serous carcinoma and 8 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 28 HGSOC cases, 22 cases (78.57%) were diagnosed in advanced stages (FIGO Stage III and IV) while 6 out of 8(75%) cases of LGSOC were diagnosed in comparatively earlier stages (FIGO stage I and II).**Conclusion:** The significant difference of p53 expression between low 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.

Keywords: Ovarian cancer, p53 expression, CA-125.

Introduction

Ovarian cancer is the 6th most common cancer in women worldwide. It is the 5th leading cause of death among women in the developed countries[1,2].The incidence rate is higher in white women followed by Hispanic, Asians, Black and American Indian women[3].

In India, the Age Standardized incidence Rate (ASR) for ovarian Carcinoma varied from 0.9 to 8.4 per 1,00,000 person/year. Studies revealed that the peak incidence is in between the age of 55-64 years. The mean annual percentage increase in ASR ranges from 0.7 to 2.4%.

Recent molecular and genetic studies suggest that epithelial ovarian cancer can be grouped into two broad categories: Type I and Type II.[4] 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. Based on molecular and genetic studies Low grade serous carcinoma is considered as Type I and High grade serous carcinoma considered as Type II tumour[6]Most frequently mutated gene in ovarian carcinoma is p53 gene, a type of tumour suppressor gene. Studies have shown that p53 gene is mutated in about 50-80% of ovarian carcinoma[7]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[8].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.The present study was conducted to evaluate immunohistochemical expression of p53 in serous carcinoma of ovary and its correlation with clinicopathological parameters.

Materials and Methods

The present study was a single-centre, prospective study. Total 36 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

**Correspondence*
Dr.KP Umadevi
Associate Professor, Department of Pathology, Aarupadai Veedu Medical College & Hospital, Puducherry, India
E-mail: umadevi.komarla@avmc.edu

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: 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.

Results

Out of total 36 cases of serous carcinoma, 28 cases were high grade serous carcinoma and 8 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 28 HGSOC cases, 22 cases (78.57%) were diagnosed in advanced stages (FIGO Stage III and IV) while 6 out of 8(75%) cases of LGSOC were diagnosed in comparatively earlier stages (FIGO stage I and II).

Number of p53 positive cases in LGSOC was 4/8 (50%) and in HGSOC it was 28/28(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 $\rho=0.701$, $p=0.001$). 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±1460.55) than LGSOC (553.37±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 $\rho=0.695$, $p=0.000$).

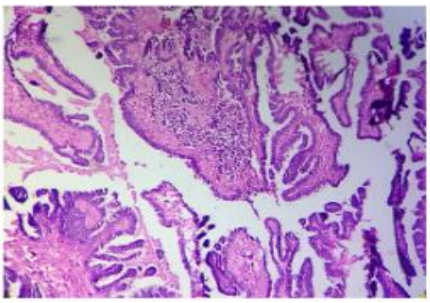

Fig 1: LGSOC: Branching papillary structure with psammoma bodies (H&EX100)

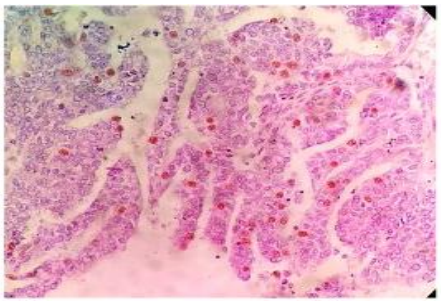

Fig 2: LGSOC: Positive p53 expression (score 2+) (IHCX100)

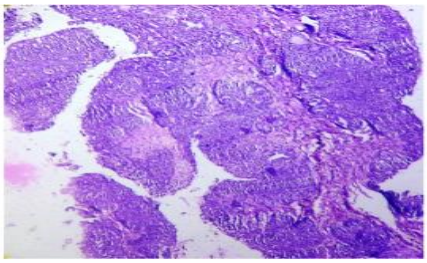

Fig 3: HGSOC: Papillary and solid pattern (H&E X100)

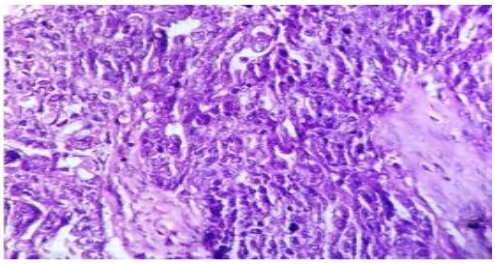

Fig 4: HGSOC: Pleomorphic tumour cells, high mitotic index and abnormal mitotic figure (H&E X400)

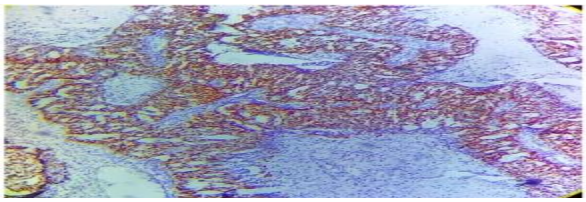

Fig 5: HGSOC: Positive p53 expression (score 5+) (IHC X100)

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

S. No	Grade	Number	Percentage (%)
1	Low	8	22.22
2	High	28	77.78
3	Total	36	100

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

	FIGO Stage			
	Stage I	**Stage II**	**Stage III**	**Stage IV**
Low grade serous carcinoma	2	4	2	-
High grade serous carcinoma	0	6	14	8

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

S. No	Histotype	Total No	Positive cases Number	Percentage	Negative cases number	Percentage	P-Value
1	Low grade serous carcinoma	8	4	50%	4	50%	<0.05
2	High grade serous carcinoma	28	28	100%	0	0	

Table 4: 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<0.001

Discussion

Low grade and high grade serous carcinoma harbor 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 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. 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.HGSOC cases are associated with higher CA-125 values and have worse prognosis when compared with LGSOC. 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 al18 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)[9]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) (p<0.001) and Sallum et al (2018) (p<0.001). Sylvia et al (2012) and Cooper et al (2002) (p<0.001) in their study also observed that, median CA-125 levels were significantly increased in high grade tumours[10]

Conclusion

The significant difference of p53 expression between low 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.

Conflict of Interest: Nil Source of support:Nil

References

1. Malpica A, Deavers MT, Lu K, Bodurka DC, Atkinson EN et al. Grading ovarian serous carcinoma using a two-tier system. Am J Surg Pathol. 2004; 28(4):496–504.
2. Khandakar B, Mathur SR, Kumar L, Kumar S, Gupta SD et al. Tissue biomarkers in prognostication of serous ovarian cancer following neoadjuvant chemotherapy. Biomed Res Int. 2014.
3. Herod JJ, Eliopoulos AG, Warwick J, Young NG, Kerr LS, DJ. The prognostic significance of Bcl-2 and p53 expression in ovarian carcinoma. Cancer Res . 1996; 56(9):2178–2184.
4. Vang R. Molecular Alterations of TP53 are a Defining Feature of Ovarian High-Grade Serous Carcinoma: A Rereview of Cases Lacking TP53 Mutations in The Cancer Genome Atlas Ovarian Study. Int J Gynecol Pathol. 2016; 35:48–55.
5. Yemelyanova A, Vang R, Kshirsagar M. Immunohistochemical staining patterns of p53 can serve as a surrogate marker for TP53 mutations in ovarian carcinoma: an immunohis-tochemical and nucleotide sequencing analysis. Mod Pathol. 2011; 24:1248–1253.
6. Murny´ak B, Hortob´agyi T. Immunohistochemical correlates of TP53 somatic mutations in cancer. Oncotarget. 2016; 7(40):64910–64920.
7. Cole AJ, Dwight T, Gill AJ. Assessing mutant p53 in primary high-grade serous ovarian cancer using immunohistochemistry and massively parallel sequencing. Sci Rep. 2016; 6:26191.
8. O'neill CJ, Deavers MT, Malpica A. An immunohistochemical comparison between low-grade and high-grade ovarian serous carcinomas: significantly higher expression of p53, MIB1, BCL2, HER-2/neu, and C-KIT in high-grade neoplasms. Am J Surg Pathol. 2005; 29:1034–1041.
9. Tiwari RK, Saha K, Mukhopadhyay D, Datta C, Chatterjee U, Ghosh TK. Evaluation of Preoperative Serum Levels of CA 125 and Expression of p53 in Ovarian Neoplasms: A Prospective Clinicopathological Study in a Tertiary Care Hospital. J Obstet Gynaecol India. 2014; 66(2):107–114.
10. Kurman RJ, Carcangiu ML, Herrington CS. WHO Classification of Tumours of Female Reproductive Organs. In WHO Classification of Tumours. 4. Aufl. Lyon: WHO Press. WHO Press, 2014.