Original Article

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Immunoexpression of p53 and p16 in Low and High-grade Serous Ovarian Cancer: A Cross-sectional Study

Pathology Section

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ABSTRACT

Introduction: The most common malignancy of the ovary is serous carcinoma, which can be classified as either Low-grade Serous Ovarian Carcinoma (LGSOC) or High-grade Serous Ovarian Carcinoma (HGSOC) and originates from the surface epithelium. However, the overall prognosis for both cancers is very poor. Immunohistochemical analysis of p53 and p16 expression is commonly used to detect mutations. Diffuse and strong mutations (mutant type) are almost always observed in cases of HGSOC, while focal expression (wild type) suggests the absence of mutations in HGSOC. LGSOCs are characterised by a low number or absence of genetic mutations.

Aim: To investigate the association between p53 and p16 expression in different grades and stages of Serous Ovarian Carcinoma (SOC).

Materials and Methods: This observational cross-sectional descriptive study was conducted on 62 patients diagnosed with ovarian serous carcinoma. The study focused on examining the expressions of p53 and p16 using Immunohistochemistry (IHC) in the Department of Pathology, Nil Ratan Sircar Medical College and Hospital, Kolkata, West Bengal, India, over a period of one and a half years (February 2021 to July 2022). The study parameters included clinical features, histological findings, staging, The Federation of Gynaecology and Obstetrics (FIGO), grading, p53 and p16 expression by IHC in all cases, and the association between p53 and p16 expression with the grade and stage of the cancer. Statistical analysis was performed using Analysis of Variance (ANOVA) and Statistical Package for Social Sciences (SPSS) software. A p-value of less than 0.05 was considered significant.

Results: A total of 62 cases were included in the study, with 55 cases (88.70%) classified as LGSOC and 7 cases (11.29%) as HGSOC. The mean age for LGSOC was 53.5 years, while for HGSOC it was 54 years. Among the HGSOC cases (n=55), 45 cases (81.80%) showed diffuse positive results (mutant type) for p53. In contrast, there was no diffuse p53 expression in LGSOC cases (n=7), with 5 cases (71.40%) showing focal positivity (wild type). The p-value for comparing p53 expression in both cases was significant (<0.00001). As for p16 expression, among the HGSOC cases (n=55), 31 cases (56.40%) showed diffuse positivity (mutant type), while among the LGSOC cases (n=7), most of the cases, 5 cases (71.40%), showed focal positivity (wild type). The p-value for comparing p16 expression in both cases was significant (<0.003794).

Conclusion: In conclusion, p53 along with p16 are good markers for grading SOC, and p53 is highly effective in differentiating HGSOC from LGSOC based on the positivity pattern (diffuse and strong positive for high-grade/mutant type, and focal positive for low-grade cancers). Thus, p53 has become an attractive target for the development of molecule-targeted therapies for this disease.

Keywords: High-grade serous ovarian carcinoma, Low-grade serous ovarian carcinoma, Serous ovarian carcinoma

INTRODUCTION

The most dangerous gynaecological malignancy is epithelial ovarian cancer. Worldwide, there are 140,000 annual associated deaths due to this disease. A total of 90% of ovarian cancers develop from the surface epithelium, among which serous lesions comprise 60-80%. This cancer has a poor prognosis, and the survival period is also very short [1]. The objectives of the present study were to diagnose cases of low-grade and high-grade serous ovarian cancer through histopathological examination and to study the immunoexpression of p53 and p16 in the diagnosed cases.

Due to a long, relatively asymptomatic latency period, poor awareness, and inadequate diagnostic infrastructure, most patients present late in the advanced stages of the disease, resulting in poor overall survival [1]. For many years, scientific efforts have been made to identify prognostic factors based on molecular markers. The classification of surface epithelial tumours is based on the type of tumour cell (mucinous, serous, endometrioid, clear cell) and further subclassified as benign, borderline, or malignant carcinoma. These epithelial ovarian cancers are a heterogeneous group of diseases, and their determination is relevant for prognostication and treatment prediction.

Histologically, serous carcinoma has two grades: low-grade and high-grade. Low-grade serous carcinomas are distinguished from HGSOCs by a lesser degree of nuclear pleomorphism (less than threefold variation in nuclear size). In difficult cases, low-grade serous carcinomas show a lower mitotic rate than HGSCs (12 or fewer MF per 10 HPF) [2]. Regarding p53 immunostaining, LGSOC shows a normal pattern compared to the abnormal staining (so-called all-or-nothing patterns) present in 95% of HGSCs [2]. HGSOCs are the predominant type of SOCs, comprising 80%-90% of ovarian cancer cases. They are very aggressive in nature, with most cases being diagnosed in advanced stages and having low overall survival rates. On the other hand, LGSOCs commonly exhibit an indolent growth pattern, with better prognosis and Progression-Free Survival (PFS) rates. While important diagnostic information can be obtained from the morphology and histological grades of SOCs, IHC plays a crucial role in differential diagnosis and the evaluation of molecular features, further aiding in the characterisation of morphology and clinical behaviour [3].

The 53-KDa protein is a nuclear protein encoded by the tumour suppressor tp53 gene. It plays a role in maintaining genome integrity and promotes cellular apoptosis during Deoxyribonucleic Acid (DNA) damage. Most HGSOCs commonly exhibit tp53 gene

mutations. Increased tp53 mutations are associated with strong and diffuse nuclear expression (mutant type) or complete absence of expression (null type). Focal expression (wild type) suggests the absence of mutations in HGSOC. In the case of LGSOCs, genetic mutations are low or p53 gene mutations are almost absent in these tumours [3].

Detection of tp53 mutations has significant importance in clinical practice. Nucleotide sequencing is the most reliable technique for detecting gene mutations, but it is labour-intensive and time-consuming. Therefore, immunohistochemical analysis of p53 expression is currently preferred as a surrogate for mutational analysis [4]. Yemelyanova A et al., analysed p53 expression as a surrogate marker for mutational analysis in ovarian carcinoma [4].

The CDKN2A gene is a tumour suppressor gene that encodes p16. It functions to slow down cell progression from the G1 to S phase and regulates the cell cycle. Approximately 60%-80% of HGSOCs show diffuse p16 staining. Therefore, p16, along with p53, should be used as IHC markers in the differential diagnosis of SOCs and to distinguish between low-grade and high-grade serous ovarian cancer [3].

MATERIALS AND METHODS

It was an observational, cross-sectional descriptive study conducted in the Department of Pathology, Nil Ratan Sircar Medical College and Hospital, Kolkata, West Bengal, India, for one and a half years (February 2021 to July 2022). Special permission was obtained from the Institutional Ethical Committee (IEC number: NMC/855, dated 26.02.2021) for the present study. The study included all female patients who presented with ovarian Space Occupying Lesions (SOL) and underwent oophorectomy, salpingo-oophorectomy, and Total Abdominal Hysterectomy with Bilateral Salpingo-oophorectomy (TAH BSO).

Inclusion criteria:

1. Low-grade serous ovarian cancer {Tumour with mild to moderate cytological atypia and a lower mitotic count, usually up to 12 mitoses per 10 High Power Fields (HPFs)}.
2. High-grade serous ovarian cancer (Tumour with marked cytological atypia, presence of multinucleated cells, and a higher mitotic count, more than 12 mitoses per 10 HPFs).

Exclusion criteria:

- All other serous ovarian tumours except LGSOC and HGSOC.
- Metastasis to the ovary.

Control: For p53 positive control, a diagnosed case of HGSOC was taken. For p16 positive control, a diagnosed case of Human Papilloma Virus (HPV) positive cervical carcinoma was taken. For negative controls of p53 and p16, a case of colloid goiter was taken.

Study Procedure

The biological material was processed using a common histopathological technique with 10% formalin fixation. In each case, the standard protocol for surgical grossing of the TAH BSO specimens was followed. After a detailed examination of the gross specimen, multiple representative tissue samples were taken from the tumour and ovarian capsule. The samples were processed according to the standard protocol, embedded in paraffin, and cut into tissue blocks. The tissue blocks were then stained with Haematoxylin and Eosin (H&E). The H&E stained slides were examined to determine the tumour histology, grade, lymphovascular invasion, and capsular invasion. The tumour was staged according to the International Federation of Gynaecology and Obstetrics (FIGO) cancer staging system [1].

Processing for Immunohistochemistry (IHC): For IHC analysis of p16 and p53, 4.0 µm paraffin sections were taken on poly-L-lysine coated slides. The sections were deparaffinised in xylene and then hydrated in descending grades of ethanol. Antigen retrieval was performed by heating the sections at 95ºC in Tris-Ethylene Diamine Tetra Acetic Acid (EDTA) buffer (pH 9.0) for three cycles of 5 minutes each. The sections were then incubated with power block (Biogenex, USA) for 10 minutes to reduce non specific antibody binding. Primary antibodies against human p16 (Dako Antihuman p16, ready to use) and human p53 (Dako Anti-human p53, ready to use) were used. The sections were incubated with the primary antibodies for one hour at 4ºC. After three washes with Trisphosphate Buffer Solution (TBS), a secondary antibody was added and incubated for 30 minutes. Following another three washes with TBS, the sections were treated with 3,3'-diaminobenzidine substrate (DAB tetrahydrochloride) for 10 minutes. The sections were then counterstained with Haematoxylin, dehydrated with ethanol and xylene, and permanently mounted with DPX. A positive control was taken from p53-positive breast carcinoma, and negative control sections were processed by omitting the primary antibody. The results were compiled and subjected to statistical analysis. Slides showing non contibutory staining were not included in the statistical analysis. The immunohistochemical results were reported as the percentage of labeled cells for each case to obtain the Positivity Index (PI). The intensity of the reaction was assessed as none, weak, intermediate, or strong [5].

Assessment of IHC staining [2]: The IHC staining was assessed using the following semi-quantitative criteria: Nuclear p53 protein expression was analysed in each case, and the percentages of cells with nuclear staining were estimated as mentioned below.

Nuclear p53 staining:

- Negative: if less than 1% of the nuclei showed staining.
- Focal positive: if 1% to 69% of the nuclei showed staining.
- Diffuse positive: if 70% or more of the nuclei showed staining.

Cytoplasmic and nuclear p16 staining:

- Negative: if less than 10% of the cells showed staining, or if the staining was absent or of low intensity.
- Focal positive: if 10% to less than 89% of the cells showed staining.
- Diffuse positive: if 90% or more of the cells showed staining.

Laboratory investigations and parameters studied: In the present study, detailed history and clinical findings of the patients were collected with their consent. After TAH and BSO operation, careful macroscopic examination was performed, and representative sections were taken for grading the SOC as low-grade or high-grade using a binocular microscope. The FIGO staging [1] was determined through microscopic examination. Immunohistochemical examination was conducted on each case to assess the expression of p53 and p16 in low-grade and high-grade serous carcinomas. Finally, the p-value was calculated to determine the association of these immunomarkers with the different grades and stages of carcinoma.

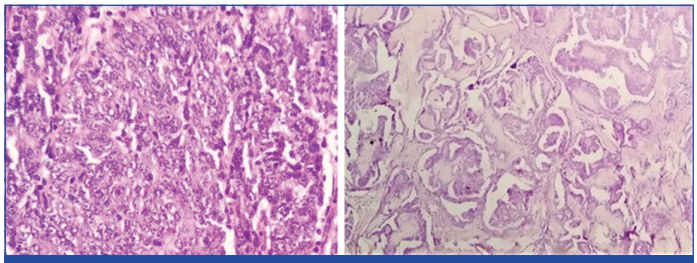
STATISTICAL ANALYSIS

The images were acquired using a Nikon Eclipse E600 microscope and Lucia 5 software program. Statistical analysis was conducted using ANOVA and Pearson tests with SPSS 10 software. The data was tabulated in Microsoft Excel and analysed with appropriate statistical methods, including the chi-square test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

In the present study, all 62 collected specimens (100%) were TAH and BSO. Among them, 55 cases (88.70%) were HGSOC [Table/Fig-1], and 7 cases (11.30%) were LGSOC [Table/Fig-2]. The mean age for HGSOC was 54 years, while the mean age for LGSOC was 53.5 years. The majority of HGSOC cases, 34 (61.80%), occurred between 56-65 years [Table/Fig-3]. Most of the

SOC cases, 49 (79.00%), occurred at postmenopausal age. Among the low-grade cases (n=7), 3 cases (42.8%) were associated with endometriosis. All high-grade and low-grade cases had a common clinical presentation of abdominal distension and fatigue. Among other symptoms of high-grade cases, ascites was present in 41 cases (74.5%), low back pain in 41 cases (74.5%), and weight loss in 31 cases (56.4%). Most low-grade cases experienced constipation and pelvic discomfort.

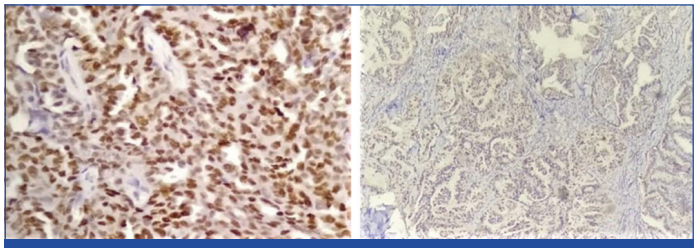


[Table/Fig-1]: HGSOC at high power, H&E staining, 400X.
[Table/Fig-2]: LGSOC at high power, H&E staining, 400X. (Images from left to right)

Histological-grade	Age-wise distribution of serous ovarian carcinoma							
	35-45 (Years)		46-55 (Years)		56-65 (Years)		66-75 (Years)	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
Low-grade	02	28.60%	0	0	03	42.80%	02	28.60%
High-grade	11	20.00%	0	0	34	61.80%	10	18.20%

[Table/Fig-3]: Age wise distribution of LGSOC and HGSOC patients.

In all SOC cases (n=62), 45 cases (72.60%) showed diffuse p53 expression, 9 cases (14.50%) showed focal positivity, and 8 cases (12.90%) were negative. For p16 expression, 33 cases (53.20%) showed diffuse expression, 14 cases (22.60%) showed focal positivity, and 15 cases (24.20%) were negative. Among the 55 HGSOC cases, 45 cases (81.80%) showed diffuse p53 expression [Table/Fig-4], 6 cases (10.90%) showed negative expression, and 4 cases (7.30%) showed focal positivity. Among the 7 LGSOC cases, there was no diffuse p53 expression, 5 cases (71.40%) showed focal positivity [Table/Fig-5], and 2 cases (28.60%) showed negative expression [Table/Fig-6].



[Table/Fig-4]: Diffuse nuclear p53 expression in HGSOC IHC, 400X.
[Table/Fig-5]: Focal nuclear expression of p53 in LGSOC IHC 400X. (Images from left to right)

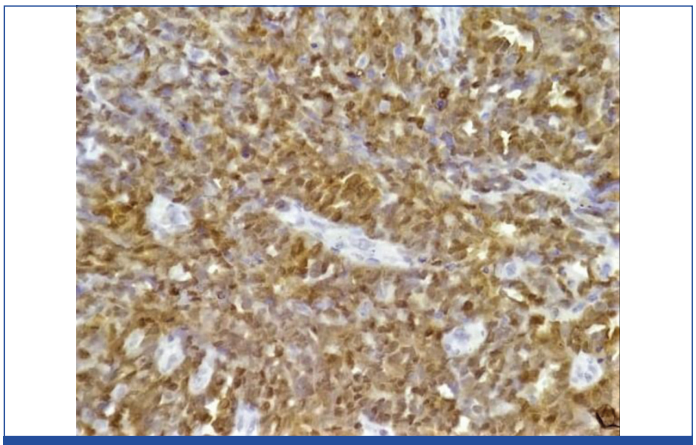
Histological-grade	Expression of p53					
	Negative		Focal		Diffuse	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
Low (07)	02	28.60%	05	71.40%	0	0
High (55)	06	10.90%	04	07.30%	45	81.80%

[Table/Fig-6]: Comparison of p53 expression between LGSOC and HGSOC.

Among the 55 HGSOC cases, 31 cases (56.40%) showed diffuse p16 expression [Table/Fig-7], 15 cases (27.30%) showed negative expression, and 9 cases (16.30%) showed focal positive expression. Among the 7 LGSOC cases, 2 cases (28.6%) showed diffuse p16 expression, while 5 cases (71.40%) showed focal positivity, and there was no negative expression [Table/Fig-8]. Out of 62 cases, the highest number, 30 cases (48.4%), were Stage III according to FIGO staging, followed by FIGO I with 21 cases (33.90%) and FIGO II with 11 cases (17.70%). No cases of Stage IV were found in the present study. Diffuse expression of p53 in FIGO I, II, and III was observed in 9 cases (42.90%), 9 cases (81.80%), and 27 cases (90.00%), respectively. No cases of Stage IV were found in our study [Table/Fig-9]. The focal expression of p53 (wild p53) was highest in 7 cases (33.33%) under FIGO I stage. Diffuse p16 immunoexpression was highest in 11 cases (100%) under FIGO Stage II, while focal p16 expression was highest in 14 cases (66.70%) under FIGO Stage I [Table/Fig-10].

DISCUSSION

Ovarian serous cancer, whether low-grade or high-grade, is a lethal malignancy in adult females. HGSOC is the predominant variety of ovarian surface epithelial cancer and is known for its aggressive nature. Only 30% of these patients survive for a maximum of five years [1]. A binary pathway for the development of this cancer has been proposed based on the tumour grade. HGSOC is believed to directly arise from the ovarian surface epithelium, the fimbrial end of the fallopian tube, or the epithelium of cortical inclusion cysts. These tumours do not yet have a definitive precursor lesion and are



[Table/Fig-7]: Diffuse and strong p16 (cytoplasmic and nuclear) in HGSOC IHC, 400X.

Histological-grade	Expression of p16					
	Negative		Focal		Diffuse	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
Low (07)	0	0	05	71.40%	02	28.60%
High (55)	15	27.30%	09	16.30%	31	56.40%

[Table/Fig-8]: Comparison of p16 expression between LGSOC and HGSOC.

FIGO staging	Negative		Focal		Diffuse	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
FIGO-I (21)	05	23.80%	07	33.30%	09	42.90%
FIGO-II (11)	0	0	02	18.20%	09	81.80%
FIGO-III (30)	03	10%	0	0	27	90%
FIGO-IV (0)	0	0	0	0	0	0

[Table/Fig-9]: Association of p53 immunoexpression with FIGO staging.

FIGO staging	Negative		Focal		Diffuse	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
FIGO-I (21)	03	14.30%	14	66.70%	04	19%
FIGO-II (11)	0	0	0	0	11	100%

FIGO-III (30)	12	40%	0	0	18	60%
FIGO-IV (0)	0	0	0	0	00	0

[Table/Fig-10]: Association of p16 immunoexpression with FIGO staging.

strongly associated with mutations in the p53 tumour suppressor gene. On the other hand, LGSOC seems to evolve from benign cyst adenoma through the stage of a borderline serous tumour in a sequential step-wise fashion. LGSOC has more mutations in the BRAF and KRAS genes compared to the p53 gene, which is more commonly mutated in HGSOC. Therefore, different genes and pathways seem to be involved in the development of different grades of ovarian serous carcinoma [1].

Comparing to HGSOC, LGSOCs have an indolent growth pattern. Although they are not very responsive to chemotherapy, their prognosis is better than HGSOC. They have longer disease PFS rates. While valuable research and publications have been done worldwide regarding p53 and p16 expression in low-grade and high-grade serous ovarian cancer, research in India is quite limited [1]. There is no published record of any such study done in West Bengal. Consequently, the therapeutic implications, such as the development of targeted chemotherapeutics and immunotherapies, as well as their clinical trials, relevance, economic feasibility, and practical feasibility, remain largely unexplored.

The present study was conducted on 62 cases of serous ovarian cancer, with 55 cases (88.70%) being high-grade and seven cases (11.30%) being low-grade. The received specimens mainly consisted of total abdominal hysterectomy with bilateral salpingo-oophorectomy in all the cases. There were no salpingo-oophorectomy or oophorectomy specimens, indicating that the clinicians and surgeons took precautions to avoid the chance of recurrence, poor prognosis, or further spread. Overall, the mean age for the present study population was 53.5 years. The lowest and highest ages for the cases were 40 and 68 years, respectively. This signifies that the incidence of serous ovarian cancer increases with age. Elderly patients with serous carcinomas are more likely to experience early recurrence, recalcitrance, and platinum resistance than younger patients [1].

Grossly, serous carcinoma presents as a large solid cystic mass with an irregular outer surface. The cut surface is also multicystic to solid and contains haemorrhagic areas. Sometimes, a gritty sensation can be felt during grossing due to calcified psammoma bodies. Microscopically, HGSOC cases are composed of solid areas with occasional papillary projections, severe cellular atypia, tumour giant cells, a high mitotic count, and plenty of psammoma bodies in a background of haemorrhage and necrosis [Table/Fig-1] [1]. On the other hand, LGSOC cases show multicystic masses with fine papillary projections, mild to moderate cellular atypia, low mitotic figures, and occasional psammoma bodies [Table/Fig-2] [1]. The majority of HGSOC cases (61.80%) occurred between 56-65 years, while the majority of LGSOC cases (42.80%) occurred between 56-65 years [Table/Fig-3]. The mean age for low-grade and high-grade cases was 53.5 years and 54 years, respectively, similar to the findings of Jha R and Karki S, (mean age 51 years) and Deng F et al., (mean age 56 years) [6,7]. They stated that compared with younger patients, elderly patients with SOC are commonly characterised by high tumour grade, poor performance status, and undertreatment. In our study, the majority of SOC cases (79.00%) occurred at postmenopausal age. The Chi-square statistic was 0.2753, and the p-value was 0.599793. This result was not significant at $p<0.05$, similar to the findings of Qasim YA et al., (p-value was 0.6234) [8].

All of the high-grade and low-grade cases in the present study had a common clinical presentation, which included abdominal distension and fatigue. Among other symptoms, ascites, low back pain, and weight loss were present in 41 cases (74.50%), 41 cases (74.50%), and 31 cases (56.40%) of high-grade cases, respectively. Most of the low-grade cases experienced constipation and pelvic discomfort, similar to the findings of Hunn J and Rodriguez GC, (n=54 cases, HGSOC- 47, LGOC-07 cases). Among high-grade cases, ascites was present in 30 cases (63.82%), and weight loss in 25 cases (53.19%) [9].

In the case of high-grade and high-stage cancer, tumour growth involves the capsule and can spread through lymphovascular invasion and peritoneal spread. The inflammatory reactions due to metastatic deposits cause the accumulation of serous fluid, which is manifested by ascites. Endometriosis was the predominant cause of LGSOC in the present study, supported by the findings of Hjerpe E et al., [10]. However, no definite cause was found in most of the high-grade cases. The exact cause or biomolecular pathway is still under continuous research.

Among all the SOC cases (n=62), 45 cases (72.60%) showed diffuse p53 expression, and 33 cases (53.20%) showed diffuse p16 expression. Therefore, p53 associated with p16 are commonly used as IHC markers in the differential diagnosis of SOC. Among all the HGSOC cases (n=55), 45 cases (81.80%) showed diffuse p53 expression (mutant type) [Table/Fig-4], 6 cases (10.90%) were negative (null type), and 4 cases (07.30%) showed focal positivity. Therefore, diffuse and null type (51 cases) made up a total of 92.7% of p53 expression, similar to the findings of Marinaş MC et al., (n=20, 18 HGSOC cases showed diffuse positivity) and Amaral JD et al., (n=50, 44 HGSOC cases showed diffuse positivity) [1,11]. They concluded that diffuse p53 mutations were present in nearly 100% of high-grade serous ovarian cancer. However, Köbel M et al., found that p53 was completely absent in 153 (30.30%) cases, focally expressed in 62 (12.00%) cases, and overexpressed in 287 (57.70%) cases of 502 HGSOC cases. They also found an association between the complete absence of p53 and an unfavourable outcome [12].

In the case of LGSOC (n=07), there was no diffuse p53 expression, while five cases (71.40%) showed focal positivity (wild type) [Table/Fig-5], and two cases (28.60%) had negative expression. The p-value for the comparison of p53 expression in high-grade and low-grade cases was <0.00001, indicating a significant difference. This finding is similar to the study by Cole A et al., where all LGSOC cases (100%) showed wild type mutation [13].

Out of the 62 cases, diffuse expression of p53 was noticed in 9 (42.90%), 9 (81.80%), and 27 (90.00%) cases under FIGO stages I, II, and III, respectively. No cases of Stage IV were found. The focal expression of p53 (wild type) was highest in 07 cases (33.33%) under FIGO stage I. The Chi-square statistic was 17.0701, and the p-value was significant (0.001873). This finding is similar to the studies by Kmet LM et al., (n=51, diffuse expression in 21 (90.00%) under Stage III cancer) and Arik D and Kulacoglu S, (n=28, diffuse expression in 17 (87.00%) under Stage III cancer). They found that p53 abnormalities were positively associated with increasing tumour grade and stage [14,15].

Among all the HGSOC cases (n=55), 31 cases (56.40%) showed diffuse p16 expression, 15 cases (27.30%) were negative, and 09 cases (16.30%) showed focal positivity. This finding is similar to the study by Rambau PF et al., where p16 block expression was most frequent in 4334 HGSOC cases (56.00%) out of 6525 cases [16]. Phillips V et al., also demonstrated that p16 was highly expressed in 38 cases (49.35%) of HGSOC (n=77 cases) [17].

Among the LGSOC cases (n=7), 02 cases (28.60%) showed diffuse p16 expression, while 05 cases (71.40%) showed focal positivity. There were no cases with negative p16 expression. The Chi-square statistic for the comparison of p16 expression in high-grade and low-grade cases was 11.1486, and the p-value was significant (<0.003794). Most low-grade cases exhibit an indolent growth pattern and take more time to spread. Therefore, they are often identified at a lower stage. In the present study, most of the LGSOC cases were found at FIGO Stage I. On the contrary, high-grade cases are rapid growers and spread quickly, often presenting with non

specific and vague symptoms. This is why the maximum number of HGSOC cases was found at FIGO Stage III in the present study.

Out of the 62 cases, diffuse p16 immunoexpression was highest at FIGO Stage II (n=11), which was 100%. Focal p16 expression was highest in 14 cases (66.70%) under FIGO Stage I (n=21). The chi-square statistic was 43.3338, and the p-value was significant (<0.00001). This finding is similar to the study by Shandiz FH et al., where diffuse p16 immunoexpression was highest at FIGO Stage II (n=30 cases, 63.82%), and focal p16 expression was highest under FIGO Stage I (n=12 cases, 25.53%) [18].

Out of the 62 cases, the maximum number of cases (30, 48.40%) were found at Stage III according to FIGO staging, followed by FIGO I with 21 cases (33.90%) and FIGO II with 11 cases (17.70%). This finding is similar to the study by Seidman JD et al., where out of 113 cases, FIGO I had 30 cases (26.54%), FIGO II had 33 cases (29.20%), and FIGO III had 50 cases (44.24%) [19]. No cases of Stage IV were found in the present study.

Limitation(s)

The present study included only a small study population of 62 patients who attended a tertiary care hospital. Since, the present study is single-centered and conducted in Kolkata, West Bengal, India the results cannot be generalised to all regions.

CONCLUSION(S)

So, authors concluded from the study that p53 is highly effective in differentiating HGSOC from LGSOC based on the pattern of positivity. Extensive diffuse and strong nuclear positivity in high-grade cases signifies increased tp53 gene mutations (mutant type). Zero diffuse positivity in low-grade confirms that tp53 gene mutation is never present in low-grade cases. p16 can also differentiate between high-grade (56.40% diffuse expression) and low-grade (28.60% diffuse expression) SOC. However, p53 has been shown to be a robust and more effective immunomarker than p16 for differentiating these SOCs. All investigations and procedures were conducted in a government hospital, and the cost was covered by the hospital.

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