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Correlation of p53 Expression with Grade and Stage of Ovarian Surface Epithelial Cancer

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Abstract

Introduction: Advanced ovarian cancer is characterized by a poor prognosis and the development of resistance to chemotherapy. In the absence of definite etiologic factors and effective tools for screening, the only possible means of improving survival would be to develop effective targeted therapy. Expression of p53 in ovarian cancer may serve as a promising therapeutic strategy to treat, prevent visceral metastasis and relapse. **Objective:** The study was carried out to find out the correlation of p53 expression with the grade and stage of ovarian cancers. **Methods:** The study had 45 cases. These cases were previously reported as ovarian surface epithelial cancers. Grading was done in all cases. Pathological staging of tumour (pT) was done in 30 cases and pathological staging of lymph node metastasis (pN) was done in 10 cases. Distant metastasis was present in only 1 case. **Results:** p53 immunostaining was found positively correlated & associated with the grade & stage of ovarian surface epithelial cancers. **Conclusion:** p53 expression may be important for prognostic outcomes or useful targets of therapeutic intervention.

Keywords: Ovarian surface epithelial cancer, p53 expression, Grade, Stage.

Introduction

Epithelial ovarian cancers account for the majority of female ovarian neoplasms. The highest incidence is in Europe and Northern America and the lowest in Africa and Asia. Pakistan has one of the highest rates of ovarian cancer among the South Asian countries which include Sri Lanka, India, Bhutan and Bangladesh.[1] But ovarian cancer rate is declining in the U.S. due to increased parity and duration of oral contraceptive use.[2]

Ovarian cancer is the deadliest gynecologic malignancy characterized by poor prognosis and the evolution of resistance to chemotherapy in the advanced stage. A woman's lifetime risk of developing ovarian cancer is 1 in 75 and her chance of dying of the disease is 1 in 100. The disease typically presents at a late stage when the five years relative survival rate is only 29%.[3] Therefore, the staging of ovarian cancer is a very important prognostic factor. Besides staging of ovarian cancer, the age of the patient, histological type, the International Federation of Gynecology and Obstetrics (FIGO) grading of tumor, presence of ascites and size of residual disease are recognized prognostic factors of ovarian cancer. Silverberg grading system instead of the FIGO grading system can also be used to stratify patients

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into low & high risk categories.[4]
Silverberg grading of ovarian cancer is correlated with survival in both early and advanced-stage disease and for all major histologic types of epithelial ovarian cancer except clear cell carcinoma.[5]

Malignant ovarian cancer, also known as carcinoma, is comprised of four main histological subtypes: serous carcinoma, mucinous carcinoma, endometrioid carcinoma and clear cell carcinoma. Serous carcinoma is the most frequent cancer among surface epithelial carcinoma of the ovary (about 75%). Endometrioid carcinoma (10%), and mucinous carcinoma (3%) are also frequently found. In developed countries, more than 90% of malignant ovarian tumors are epithelial in origin.[3]

Unfortunately, maximum cases are diagnosed with advanced disease because of the lack of specific symptoms in the early stage. Ovarian cancer shows a high response rate to first-line chemotherapy making the prognosis poor. Only a minority of patients survive beyond five years. Resistance to chemotherapy has been suggested to be due to decreased susceptibility to apoptosis. It is supposed that cell death determinants may influence the outcome of treatment.[6-8]

The p53 is the main regulator of apoptosis. It can initiate apoptosis by activating the intracellular death signal pathway.[8] The p53 protein is a transcriptional activator located on chromosome 17. It binds to specific DNA sequences in the control region of the genes, influencing their expression. This leads to the expression of specific genes necessary for the inhibition of apoptosis when damage is repairable. It arrests the cell cycle at G1 phase to allow time for DNA damage repair if it is not too great. However, p53 triggers apoptosis if the damage is large to be repaired successfully. Alteration of p53 activity, either as a result of point mutation or due to protein stabilization in the absence of obvious genetic changes allows severely damaged cells to survive and proliferate instead of undergoing apoptosis. Alteration of p53 is the most common abnormalities seen in human cancer including breast, gastric and non-small cell cancer. Accumulation of p53 protein is seen in these cases. Hence, it could be used as a prognostic marker.[7]

Given the apparent role of its expression in oncogenesis and resistance to chemotherapy, p53 expression in ovarian carcinomas has been a field of recent research interest. Besides other prognostic factors like grade and stage of tumor, histological type, age of the patient and presence of ascites, p53 expression may become a prognostic factor in the near future. The p53 expression in ovarian carcinomas may also be related to tumor progression. Consequently, p53 expression may be important for prognostic outcome or useful target of therapeutic intervention.[6]

It, therefore, appears that a correlation might be present between the expression of p53 with grade and stage of surface epithelial cancers and our study aims at investigating this correlation.

Materials and methods

This was a cross-sectional type of observational study carried out at the Department of Pathology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh; from January 2017 to July 2019. The sample size was 45. The study material was representative paraffin blocks of tumor tissue, histologically diagnosed as ovarian surface epithelial cancers. The cases were taken from the department of Pathology, BSMMU.

Histomorphological and IHC analysis:
Hematoxylin and eosin-stained sections of each case were reviewed to confirm the histological diagnosis of surface epithelial cancers of the ovary. From paraffin-embedded blocks, 5 micrometer thick sections were taken that were deparaffinized with xylene and rehydrated through a graded series of alcohol subsequently. For antigen retrieval, the samples were treated with Dako Target Retrieval solution. Solutions were taken in a Coplin jar and were heated in a water bath at 46^0C for 30-40 minutes. Then the

sections were stained with p53. Immunostaining was done manually following the avidin-biotin-peroxidase staining method.

The morphological parameters including tumour type, number of mitoses in per ten high power field and p53 immunoreactivity among different grades and stages of surface epithelial cancers of the ovary were recorded.

For immunohistochemistry, p53 expression of the submitted blocks was evaluated using Dako Autostainer Plus at the immunohistochemistry laboratory, Department. of Pathology, BSMMU. A known case of p53-positive serous carcinoma of the ovary was used as control of p53 immunostaining.

The expression of p53 was analyzed in epithelial cells under the microscope using the following 5-point scoring system. Nuclear staining was interpreted as positive for p53. P53 staining was assessed as percent staining from each section and scored as having either >1% or <1% positive cells. Sections having <1% positive cells were given no score.

Evaluation of Immunopositive cell: [8]

Immunoreactive cell percentage p53	Score
<1%	0
1-10%	1
11-25%	2
25-50%	3
>50%	4

Grading of surface epithelial cancers of the ovary:
Silverberg grading system is based on combined scores for architecture, nuclear atypia and mitotic activity. Tumours composed of well-differentiated cells having no clumped chromatin and no nucleoli were considered nuclear grade I. If both clumped chromatin and nucleoli were present, then it was considered as nuclear grade III. Cells having intermediate morphology between nuclear grade I and III were considered as nuclear grade II.[9]

Score	Architecture	Nuclear grade	Mitotic activity/10
1	Glandular	Grade-1	0-9
2	Papillary	Grade-2	10-24
3	Solid	Grade-3	>24

Total score	Silverberg grade
3-5	1
6-7	2
8-9	3

Staging of surface epithelial cancers of the ovary:
For staging, the TNM staging system was followed.[10]

Observations and results

A total number of 45 cases were selected for the study. Out of these 45 cases, the majority belonged to the age range of 31-40 years and 41-50 years; each group having 12 (26.7%) cases. The minimum age was 18 years and the maximum age was 70 years. The mean age was 44.4 years with a standard deviation (SD) of 12.3 (Table 01). Out of 45 cases, 29 (64.4%) were serous, 13 (28.9%) were mucinous and 3 (6.7%) were endometrioid (Table 02). Grading was done in all the cases. Among 45 cases, 18 (40%) cases were well differentiated, 17 (37.8%) cases were moderately differentiated & 10 (22.2%) cases were poorly differentiated (Table 03).

Table 01: Distribution of the study subjects by age (n=45)

Age (in years)	Number of patient	Percentage
≤ 20	1	2.2
21-30	6	13.3
31-40	12	26.7
41-50	12	26.7
51-60	10	22.2
>60	3	6.7
Mean±SD		44.4±12.3

Table 02: Distribution of the cases according to the type of tumour (n=45)

Type of tumour	Number of patients	Percentage
Serous carcinoma	29	64.4
Mucinous carcinoma	13	28.9
Endometrioid carcinoma	3	6.7

Table 03: Distribution of the cases according to grade (n=45)

Grade	Number of patients	Percentage
Well differentiated	18	40.0
Moderate differentiated	17	37.8
Poorly differentiated	10	22.2

Out of 45 cases, tumour stage (pT) was done in 30 cases. Among 30 cases, T1 was found in 17 (56.7%) cases, T2 was found in 3 (10%) cases. Nodal metastasis (pN) was assessed in 10 cases. Out of these 10 cases, nodal metastasis was found in 2 (20%) cases. Distant metastasis (pM) was present in only 1 case (Table 04). A total of 23 (51.1%) cases showed p53 immunoreactivity in <1% of cells. Also, 12 (26.7%) cases showed the presence of p53 immunoreactivity in between 11 to 25% of cells & 9 (20%) cases showed p53 immunoreactivity in between 25-50% of cells (Table 05).

Table 04: Distribution of the cases according to TNM stage (n=45)

TNM stage	Number of patients	Percentage
Stage pT		
T1	17	56.7%
T2	3	10%
T3	10	33.3%
Not done	15	
Stage pN		
N0	8	80%
N1	2	20%
Not done	35	
M stage X		
Bladder	1	100%
Not done	44	

Table 05: Distribution of the study patients according to p53 immunoreactivity (n=45)

p53 immunoreactivity	Number of patients	Percentage
<1% cell	36	51.1
11-25% cell	1	2.2
25-50% cell	9	20.0
>50% cell	12	26.7

Association between p53 immunoreactivity with tumour grade was found after doing statistical analysis by chi-square test. The p-value was found significant (0.011). It appears that the percentage of p53 expression increases with a higher grade of tumour, which is statistically significant ($p<0.05$). (Table 06). Association between p53 immunoreactivity with tumour stage (pT) was found after doing statistical analysis by chi-square test. The p-value is found significant (0.011). It appears that the percentage of p53 expression increases in the advanced stage, which is statistically significant ($p<0.05$). (Table 07).

Table 06: Association between p53 immunoreactivity with grade (n=45)

Grade	p53 immunoreactivity				p-value
	Positive (n=22)		Negative (n=23)		
	n	%	n	%	
Well differentiated	4	18.2	14	60.9	
Moderate differentiated	11	50.0	6	26.1	0.013[s]
Poorly differentiated	7	31.8	3	13.0	

s=significant
(P value reached from chi-square test)

Table 07: Association between p53 immunoreactivity with TNM stage

Grade	p53 immunoreactivity				p-value
	Positive (N=22)		Negative (N=23)		
	n	%	n	%	
Stage pT					
T1	8	36.4	9	39.1	
T2	0	0.0	3	13.0	0.011[s]
T3	9	40.9	1	4.3	
Not done	5	22.7	10	43.5	

After doing Spearman's rank correlation test p53 immunostaining was found positively correlated & associated with grade & stage of ovarian surface epithelial cancers (Figure 01, 02 & 03).

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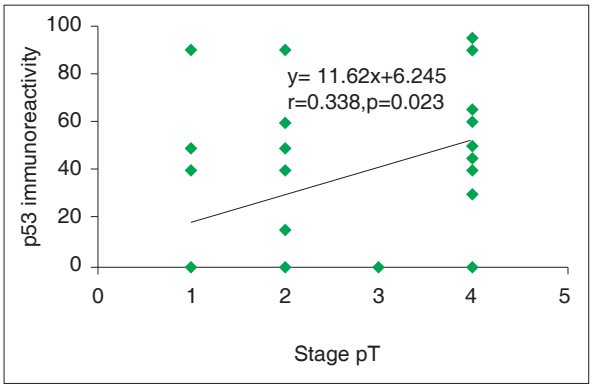

Figure 01: Scatter diagram showing positive correlation (r=0.338; p=0.023) between stage pT and p53 immunoreactivity.

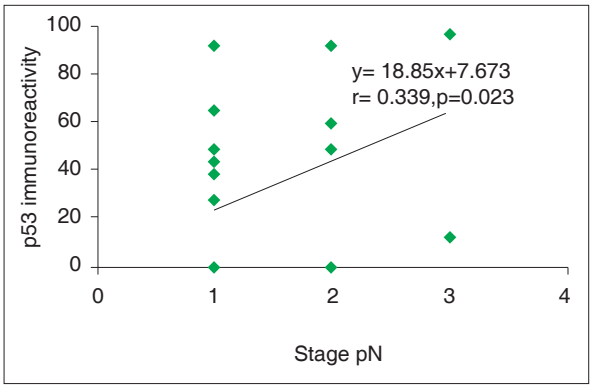

Figure 02: Scatter diagram showing positive correlation (r=0.339; p=0.023) between stage pN and p53 immunoreactivity.

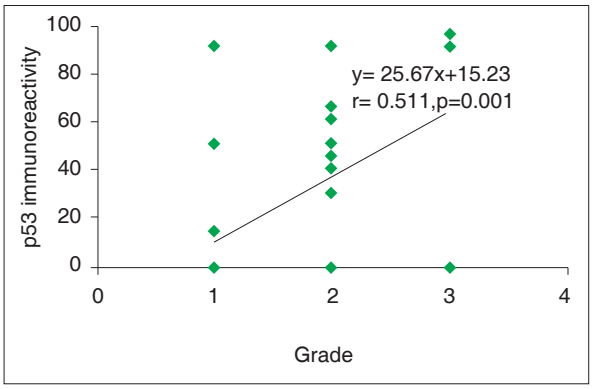

Figure 03: Scatter diagram showing positive correlation (r= -0.054; p=0.724) between tumor grade and p53 immunoreactivity.

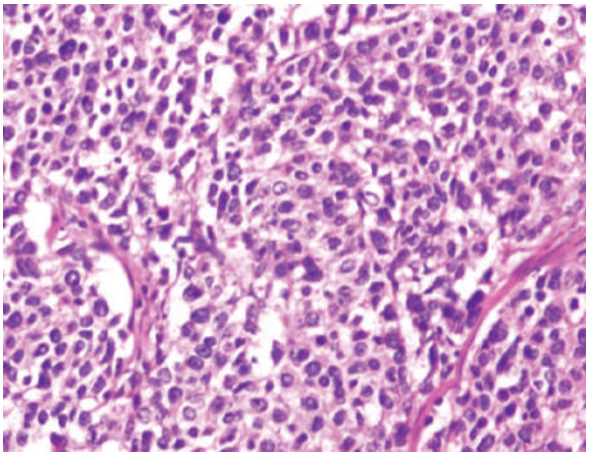

Figure 04: Photomicrograph of a case of serous carcinoma (H/E stain, 400X)

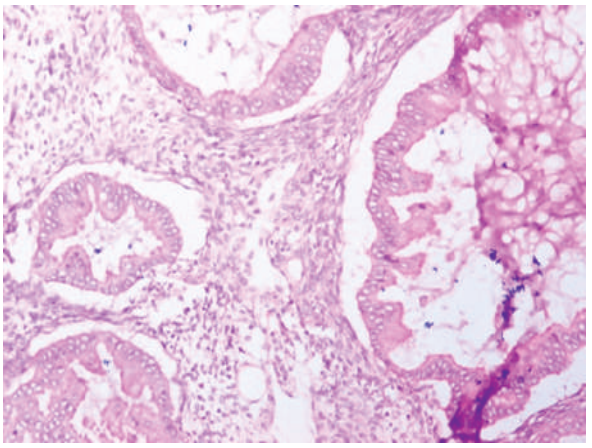

Figure 05: Photomicrograph of a case of mucinos carcinoma. (H/Estain, ×200)

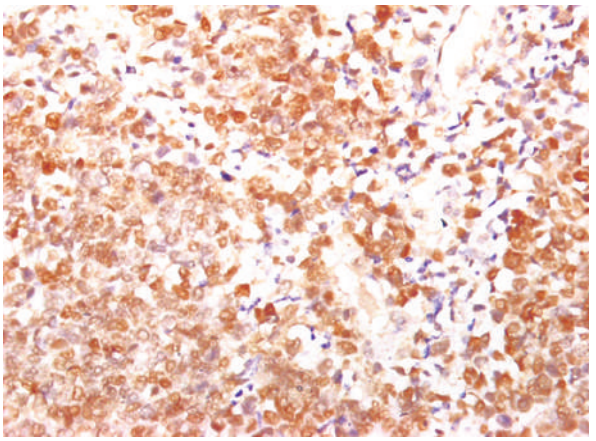

Figure 06: Photomicrograph showing p53 positivity in serous carcinoma of ovary (p53 immunostain, 400X)

Discussion

Ovarian cancer is a lethal gynecological malignancy. It shows no specific symptoms in the early stage. So, the presentation of ovarian cancer in the advanced stage is more common. The advanced stage of ovarian cancer is associated with the evolution of resistance to chemotherapy.[6,7] Therefore, staging remains the most important factor of ovarian cancer.

Besides staging of ovarian cancer age of the patient, histological type, FIGO grading of tumour, presence of ascites and size of residual disease are recognized prognostic factors of ovarian cancer.[11] Silverberg grading system instead of FIGO grading system can also be used to stratify patients into low & high risk categories.[4]

Silverberg grading system is based on combined scores for architecture, nuclear atypia & mitotic activity. This grading system correlates with the stage of cancer also.[5] Therefore, Silverberg grading system is also a prognostic factor of ovarian cancer.

The p53 is the cell death determinant as it is one of the main regulators of apoptosis. It is supposed that cell death determinants may influence the outcome of treatment.

IHC offers a diagnostic prediction for p53 gene expression when it is overexpressed.[12] This cross-sectional study was carried out to correlate p53 expression with the grade & stage of ovarian cancer. Immunostaining of p53 was done in histologically reported 45 cases of ovarian cancer.

In the present study of a total of 45 cases serous carcinoma was found as the most common surface epithelial cancer of the ovary. It accounted for 64.4% of the total number of surface epithelial cancers of ovary. Serous carcinoma was followed by mucinous carcinoma (28.9%) & endometrioid carcinoma (6.7%). No other histological type is included in this study.

Tumor staging (pT) was done in 30 cases out of 45 cases. Ten cases (33.3%) were found in the advanced stage (pT3). Out of 10 advanced-stage ovarian cancer cases, 9 cases showed immunoreactivity for p53. Tumour stage (pT) is found significantly associated & positively correlated after doing the chi-square test & spearman rank correlation test respectively.

The p53 is positively correlated with lymph node staging (pN). Out of 10 cases where nodal metastasis were assessed 2 cases showed lymph node metastasis. Both 2 cases were immunoreactive for p53.

Out of 45 cases, 40% of cases were well differentiated, 37.8% of cases were moderately differentiated & 22.2% cases were poorly differentiated. Out of 29 serous carcinomas, 8 were poorly differentiated & out of 3 endometrioid carcinomas, 1 was poorly differentiated. No poorly differentiated mucinous carcinoma was found.

The p53 immunoreactivity showed an association & positive correlation with tumour grade. Maximum well differentiated tumours (60.9%) were negative for p53 immunoreactivity. Poorly differentiated serous carcinomas showed either diffuse positivity (7 out of 8 cases) for p53 or absence of immunoreactivity for p53 (1 out of 8 cases). The absence of immunoreactivity in poorly differentiated serous carcinomas may be explained by the non-sense mutation of p53. Well & moderately differentiated serous carcinomas either showed focal immunoreactivity for p53 or absence of immunoreactivity for p53. Mucinous carcinomas & Endometrioid carcinomas also showed either focal p53 immunoreactivity or the absence of p53 immunoreactivity. Positive correlation between p53 immunoreactivity & tumour grade indicates that p53 tends to accumulate in higher graded ovarian carcinoma. But, two histologically diagnosed mucinous carcinoma showed diffuse immunoreactivity for p53. One of them was well differentiated and another one was moderately differentiated. It indicates that genetic lesions may be higher in morphologically categorized low grade carcinoma.

Limitations

There were a few limitations of this study.

1. Small sample size
2. Only three histologic types of surface epithelial tumor were included.

Conclusion

The p53 expression showed a positive correlation with increasing grade & stage of surface epithelial cancers.

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