Mini Symposium on Changing Landscape: Original Article

A cytohistological study of p53 overexpression in ovarian neoplasms

Monisha Choudhury, Seema Goyal, Mukta Pujani, Meenu Pujani

Abstract

Background: The present study was undertaken to evaluate the diagnostic accuracy of imprint cytology in ovarian neoplasms, investigate the biological significance of p53 expression in malignant ovarian tumors and correlate it with histological type, grade and stage of tumor. **Material and Methods:** A total of 50 cases including 25 prospective and 25 retrospective cases were studied. Imprint cytology was performed on 25 ovarian tumors and compared with histopathological diagnosis. p53 immunohistochemistry was performed on all 50 cases. **Results:** On immunohistochemistry, all the benign tumors were negative for p53 while 42% of primary ovarian malignant tumors were positive. p53 expression was found to have a diagnostic value in differentiating benign from malignant tumors. p53 overexpression did not show any significant correlation with prognostic factors as stage of disease, grade of differentiation and type of tumor. **Conclusion:** The present study confirms the importance of p53 tumor suppressor gene expression as documented by immunohistochemistry in the differentiation of malignant and benign ovarian tumors.

Key words: Ovary, p53, tumors

Introduction

Rapid per operative diagnosis of ovarian masses is important. It helps the surgeon to conserve the other ovary in young patients with benign tumors as well as allows early institution of chemotherapy in advanced malignant tumors. Imprint cytology is a cost effective technique that has the potential to augment frozen section analysis, providing rapid diagnosis with higher accuracy rates as compared to frozen section used alone.[1]

p53 plays a crucial role in control of cell cycle, apoptosis and the maintenance of genomic stability. Loss of functional p53 dependent apoptotic response due to mutation may contribute to malignant transformation, tumor progression and tumor resistance to DNA damage inducing therapy. Mutation of p53 usually leads to an abnormal protein with a markedly extended half-life, resulting in accumulation of this product, that can be detected immunohistochemically.[2]

In the present study, the diagnostic accuracy of imprint cytology has been evaluated and correlated with histological diagnosis. Immunochemistry for p53 was done on imprint smears and tissue sections of benign and malignant ovarian tumors. p53 mutation was correlated with known prognostic factors for the ovarian tumors like histological type, grade and stage of the tumor.

Materials and Methods

A total of 50 cases included 25 prospective cases and 25 retrospective cases; comprising of both benign and malignant ovarian tumors. The study was approved by the institutional ethical committee.

All cases were reviewed and representative sections from the tumor selected and corresponding blocks cut into 3-5μm thick sections on poly-l-lysine coated slides. Immunostaining was performed using LSAB + technique with Monoclonal mouse antihuman p53 protein antibody (DAKO, DO7).

Twenty five cases of ovarian tumors which were operated during the study period were included. Imprint smears

Department of Pathology, Lady Hardinge Medical College and Smt. Sucheta Kriplani Hospital, New Delhi, India
Correspondence to: Dr. Mukta Pujani,
E-mail: drmuktapujani@gmail.com

were made directly from fresh unfixed specimen on plain as well as poly-l- lysine coated slides. Two slides were immediately fixed in 95% ethanol for Papanicoloau and HematoxylinandEosin stain, remaining smears were air dried and stained with Giemsa. Smears on poly-l-lysine coated slides were wrapped in aluminium foil and stored below -80°C for p53 immunocytochemistry using the peroxidase antiperoxidase technique.

Cytological diagnosis was made on imprint smears and compared with the final tissue diagnosis. Immunohistochemistry was performed for p53 on representative tissue sections. Positive p53 staining was observed as brown, granular nuclear staining. p53 immunostaining was graded and the tumor classified as positive for p53 protein if clear positive nuclear staining was found in a group of epithelial cells or >40% scattered epithelial cells.[3]

Statistical analysis

Histopathology of the tumors was used as the gold standard for final diagnosis. Statistical evaluation was done using chi-square test, student's *t test* and ANOVA F test. A *P* value of < or equal to 0.05 was considered as significant and *P* value of < or equal to 0.01 as highly significant.

Results

Out of the 50 cases, 20 (40%) were benign, 29 (58%) were malignant and one a borderline ovarian tumor. A total of the 41(82%) out of 50 tumors were histologically surface epithelial tumors, 3(6%) were sex cord stromal tumors, 4(8%) were germ cell tumors and one each, 2% were small cell carcinoma and metastatic Krukenberg tumor [Table 1].

No significant difference was observed in the mean ages of patients in benign (38.1years) and malignant tumors (38.2 years) (*P* =0.97). The difference in mean diameter of benign (11.5+9.7cm) and malignant tumors (13.9+6.9cm) was not significant (*P* =0.3). In this study, 68% of benign tumors were predominantly cystic while 35% of malignant tumors were predominantly solid and all the solid-cystic tumors were malignant, the difference being statistically significant (*P* =0.002). The sensitivity and specificity of cytology for diagnosis of malignant ovarian tumors was found to be 80 and 100% respectively. The diagnostic accuracy on cytology for malignant tumors was 96%.

All the benign tumors were negative for p53 immunostaining while 50% of the primary ovarian malignant tumors showed p53 immunoreactivity [Table 2]. On immunohistochemistry, all the benign tumors were negative while 42% of primary ovarian malignant tumors

showed positive p53 staining. One borderline tumor was negative, but the single case of Krukenberg tumor was positive for p53 [Table 3]. Difference in p53 staining in benign and malignant tumors was statistically significant both on immunocytochemistry and immunohistochemistry (*P* =0.0001). Staining for p53 was positive in 60% of stage III/IV tumors as compared to 23% of stage I/II tumors, but the difference was not statistically significant (*P* =0.08), probably because of the small sample size. Similarly, p53 was positive in 60% of poorly differentiated tumors as compared to 28% of well differentiated tumors, but the difference was not statistically significant (*P* =0.3). There was no significant correlation of p53 immunoreactivity with serous vs non serous histology of tumors.

Table 1: Distribution of type of tumor in relation to total cases

Type of cases	No. of cases (%)
Benign	
Serous cystadenoma	8 (16)
Mucinous cystadenoma	9 (18)
Fibroma	2 (4)
Thecoma	1 (2)
Total	20 (40)
Malignant	
Serous cystadenocarcinoma	17 (34)
Mucssinous cystadenocarcinoma	6 (12)
Dysgerminoma	2 (4)
Yolk sac tumor	2 (4)
Small cell carcinoma	1 (2)
Krukenberg tumor	1 (2)
Total	29 (58)
Borderline mucinous tumor	1 (2)
Total	50 (100)

Table 2: Comparative analysis of p53 immunocytochemistry in benign and malignant ovarian tumors

p53 (*n*=24)	Benign	Malignant
Positive	0	2
Negative	20	2
Total	20	4

Note: *n*=24, one case of Krukenberg tumor was excluded from prospective cases for analysis of p53 in ovarian tumors (*P*<0.0001)

Table 3: Comparative analysis of p53 immunohistochemistry in benign and malignant ovarian tumors

p53 (*n*=48)	Benign	Malignant
Positive	0	12
Negative	20	16
Total	20	28

Note: *n*=48, one case of Krukenberg tumor and one borderline tumor was excluded (*P*<.0001)

Discussion

Of the total of 50 cases of ovarian tumors, 20 (40%) were benign, 29 (58%) malignant and one borderline ovarian tumor. There were 41 (82%) surface epithelial tumors, 4 (8%) germ cell tumors, 3 (6%) sex cord stromal tumors, one case each of small cell carcinoma and Krukenberg tumor. No significant difference was observed in the mean age of patients and tumor size between benign and malignant tumors. Imprint cytology performed on 25 cases comprised of 20 benign and 4 malignant tumors. Correct diagnosis on cytology of benignity and malignancy of the tumor was possible in 24(96%) out of 25 cases. Cytology could not offer the correct diagnosis in a case of Krukenberg tumor because the smears werehypocellularwith presence of only spindle shaped fibroblast like cells alongwith few scattered epithelial cells. However, immunostaining showed p53 positivity even in the few scattered representative cells. Diagnostic accuracy for malignant lesions in this study was 96% which was higher as compared to Nagai *et al*.[4] who found 83.6% accuracy. Correct specific diagnosis was possible in 88% cases.

A total of 12 (42%) out of 28 primary ovarian tumors and one case of Krukenberg tumor was positive for p53. All the 20 benign tumors and one borderline tumor were p53 negative. Out of the 23 surface epithelial tumors, 11(47%) cases were p53 positive and only 1 out of 4 germ cell tumors showed p53 positivity. The positivity rates for p53 overexpression in epithelial cancers ranged from 38% as reported by Inoue *et al*.[5] to 62% reported by Hartmann *et al*.[6] This variability in the p53 positivity maybe attributed to differences in the antibodies used, staining techniques and variable sample size in different studies. There was a complete correlation between immunocytochemistry and immunohistochemistry in all the 24 cases where both were put up [Table 4].

In the present study, the difference in p53 positivity in benign and malignant tumors was highly significant both by immunocytochemistry and immunohistochemistry. This finding was in concordance with other studies.[5,7,8]

p53 was positive in 60% of stage III/IV tumors as compared to 23% of stage I/II tumors, but the difference was not statistically significant ($P =0.08$). This is in close agreement with other studies.[3,6,7] However, other authors[9,10] have demonstrated a significantly higher rate of p53 positivity in advanced stage epithelial cancers as compared to early stage cancer. In this study, 60% of the poorly differentiated tumors showed p53 positivity which is higher in comparison to 28% positivity in well differentiated tumors, but the difference was not statistically significant (probably due to small sample size). This is in concordance with Kohler *et al*.[11] and Marks *et al*.[7] However, others studies[3,6,8,9] have demonstrated a significant correlation between p53 overexpression and higher grade of tumor. In the present study, no significant correlation of p53 positivity with serous vs non serous type histology was found which is consistent with the study of Hartmann *et al*.[6] There was a significant correlation between immunocytochemistry and immunohistochemistry for p53 in all the 25 prospective cases. Min KW *et al*. also reported that the expression of p53 correlated with the grade and type of ovarian cancer. p53 was absent in the borderline ovarian tumors, whereas ovarian carcinoma showed expression of p53.[12]

Using conventional immunohistochemistry, assessment of the prognostic value of p53 protein levels is limited by the non-quantitative nature of the method. Psyrri *et al*. used an immunofluorescence-based method of automated in situ quantitative measurement of protein analysis (AQUA).[13] Anderson KS *et al*. examined the value of serum p53 autoantibodies (p53-AAb) as detection and prognostic biomarkers in ovarian cancer. Antibodies to p53 were detected in the sera of 42% of patients with advanced serous ovarian cancer.[14]

This study thus indicates that cancers with p53 mutations are more aggressive than cancers without p53 mutation. p53 may be used as a marker to predict aggressive behavior and poor differentiation in malignant tumors of the ovary.

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Table 4: Correlation of p53 immunocytochemistry with immunohistochemistry

Immunocytochemistry (*n*=24)	Immunohistochemistry		Total
	Negative	Positive	
Negative	22	-	22
Positive	-	2	2
Total	22	2	24

Note: *n*=24; one case of Krukenberg tumor showed positivity in only few scattered epithelial cells

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