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Expression of P53 Protein in Neoplastic and Non Neoplastic Ovarian Lesions

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Abstract

Background Ovarian cancer is one of the most common causes of gynecologic neoplasm all over the world.

Objective The objective is to shed light on the role of p53 protein and patient`s age in the pathogenesis of ovarian lesions.

Methods Paraffin embedded blocks of 62 patients with ovarian lesions were studied. Thirty-five cases of surface epithelial ovarian tumors, (31 cases of invasive surface epithelial ovarian tumors, and 4 cases of borderline intermediate malignancy cases of neoplastic ovarian cystic lesions). In addition, eighteen cases of benign neoplastic ovarian cystic lesions and 9 cases of non- neoplastic functional one were enrolled in this study. All of cases included, were stained with p53 by immunohistochemistry.

Results Immunohistochemistry for p53 showed that malignant cases were positive for p53 while all benign cases were negative for p53 and the borderline cases were also negative for p53. The non-neoplastic cases were negative for p53. There is a significant statistical difference in P53 expression in malignant cases compared to other groups ($P < 0.001$). A significant difference in mean age of malignant and border line cases in comparison with benign and non-neoplastic cases; ($P < 0.001$).

Conclusion Protein p53 may play a role in the pathogenesis of malignant ovarian cancer but not in benign lesions. The age of the patient has a role as a risk factor in ovarian lesions.

Keywords Ovarian lesion, ovarian cancer, p53, immunohistochemistry.

Introduction

Ovarian cancer is one of the most common causes of gynecologic neoplasm and is the fifth cause of cancer mortality in women. The high mortality rate in women with ovarian cancer is due to its detection at advanced stages.

Even though there have been improvements in surgical techniques and treatment options, five-year survival for ovarian cancer still remain at approximately 45% [(1)].

Ovarian tumors are heterogenous. Insight into their pathogenesis requires understanding of the mutations involved, overexpression of oncogenes and role of cell cycle regulators. There have been persistent efforts in the investigation of molecular markers in epithelial ovarian tumors, but the results are controversial [(2)].

Among the most common genetic alterations in human ovarian cancer are p*53* mutations. Defects in this tumor suppressor pathway are

present in over eighty percent of human cancers [3,4] and have been associated with poor prognosis in ovarian carcinomas [5,6].

Mutation in the p53 gene is the most common single genetic alteration in human ovarian cancer. Either loss of wild type p53 protein function, gain of oncogenic function or the ability to activate p53 protein inappropriately severely compromises the capacity for controlled cellular proliferation and cellular growth [7].

A number of studies that have paid particular attention to histological criteria of malignancy of serous tumors have found that p53 mutations are strongly associated with high-grade serous carcinomas, but are rare in low grade or borderline serous carcinomas [8-11]. The p53 protein plays a key role in cell cycle regulation and suppression of tumor development. DNA damage results in increased levels of p53, which lead to cell cycle arrest in G1 phase, followed by DNA repair or apoptosis. Mutations of the p53 gene as determined by mutation analysis and/or positive immunohistochemical (IHC) staining for p53 are common in ovarian cancer and have been associated with poor clinical outcome [12].

The aim of the current study is to evaluate the expression of p53 by immunohistochemistry (IHC) and to compare it with clinicopathologic prognostic factors of ovarian tumors namely age and malignancy.

Methods

In this study, 62 ovarian cystic lesions were involved. Specimens belong to the period from June 2011 to March 2012 were collected from private laboratory in Baghdad. According to the hematoxylin and eosin staining, the patients were grouped into:

- Thirty five cases of surface epithelial ovarian tumors, (31 cases of invasive surface epithelial ovarian tumors, and 4 cases of borderline intermediate malignancy cases of neoplastic ovarian cystic lesions).
- Eighteen cases of benign neoplastic ovarian cystic lesions .
- Nine cases of non- neoplastic functional one.

The diagnosis of these tissue blocks was based on the obtained pathological records of these cases from laboratory records. Following processing of these tissue blocks, a confirmatory histopathological re-examination of the slides was done by consultant histopathologist in Department of Pathology, College of Medicine, Al-Nahrain University.

Sections were made from each of the paraffin embedded blocks as follows: one section 4 µm thick sections were made on ordinary slides to be subjected to haematoxylin and eosin stain. This was conducted to confirm the diagnosis and tumor grade. Another section, 4 µm thick sections was made on positively charged slide for detection of p53 by immunohistochemistry using monoclonal mouse Anti human p53 protein. This technique is done the Department of Pathology and Department of Microbiology College of Medicine Al-Nahrain University. It is based on the detection of the product of gene expression (protein) in malignant and normal cells using specific monoclonal antibodies, i.e., primary antibody for specific epitope, which binds to nuclear targeted protein. The bound primary antibody is then detected by secondary antibody (usually rabbit or goat anti-mouse), which contains specific label (in this context we used peroxidase labeled polymer conjugated to goat-anti mouse immunoglobulin). The substrate is peroxidase (H_2O_2) in diaminobenzedine (DAB) of chromogen solution then stained with hematoxlyin as a counter stain. Positive reaction will result in a brown colored precipitate at the antigen site in tested tissue [13] shown in figure 1.

Data were analyzed using SPSS version 16 and Microsoft Office Excel 2007. Nominal data were expressed as frequency and percentage. Numeric data were expressed as mean $\pm$SEM (Standard error of mean). Chi-square test was used to assess relation between nominal data, while ANOVA test and student t-test were used to analyze difference among the mean of numeric data. *P*-value (< 0.05) was considered significant.

Results

The current study included four major categories: malignant, borderline, benign and non-neoplastic cases. The non-neoplastic cases include 4 (50%) corpus luteum cysts and 4 (50%) follicular cysts.

The benign cases include: 9 (50%) serous adenoma, 9 (50%) mucinous adenoma. The borderline cases include: 2 (50%) serous tumors, 1 (25%) mucinous tumors and 1 (25%) endometroid tumors. The malignant cases include: 26 (83.87%) serous tumors, 2 (6.45%) mucinous tumors and 3 (9.68%) endometroid tumors as seen in table 1.

Table 1. Neoplastic cases

Type	Benign		Borderline		Malignant		Total	
	No.	%	No.	%	No.	%	No.	%
Serous	9	50	2	50	26	83.87	37	69.81
Mucinous	9	50	1	25	2	6.45	12	22.64
Endometroid	0	0	1	25	3	9.68	4	7.54
Total	18	100	4	100	31	100	53	100

The mean age of non-neoplastic cases is (34.0±4.46) years. While, the mean age of benign cases is (31.44±1.96) years. In addition, the mean age of borderline cases is (51.75±4.3) years and the mean age of malignant cases is (50.06±1.86). There is a significant difference in mean age of malignant and borderline cases in comparison with benign and non-neoplastic cases; ($P < 0.001$) as shown in table 2.

Table 2. Comparison of mean age among the neoplastic and non-neoplastic cases

Group	No.	Mean Age	SEM
Non neoplastic	9	34.00	4.46
Benign	18	31.44	1.96
Borderline	4	51.75	4.30
Malignant	31	50.06	1.86

$P = < 0.001$

Malignant cases showed positive result in 30 cases out of 31 for P53. While the benign cases (18 cases) were negative for P53. Moreover, the borderline cases (4 cases) were negative for p53 and the non-neoplastic cases (9 cases) were also negative for p53, (Table 3).

Table 3. Immunohistochemical expression of P53

P53 Results	Non neoplastic		Benign		Borderline		Malignant	
	No.	%	No.	%	No.	%	No.	%
Positive p53	0	0	0	0	0	0	30	96.8
Negative p53	9	100	18	100	4	100	1	3.2
Total	9	100	18	100	4	100	31	100

$P = < 0.001$

There is a significant statistical difference in p53 expression in malignant cases compared to other groups ($P < 0.001$).

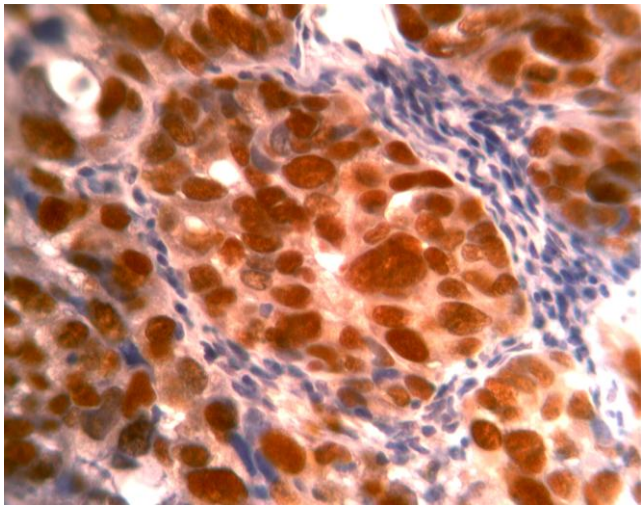

Figure 1: Moderately differentiated ovarian serous cystadenocarcinoma showing intense nuclear P53 staining reaction DAB staining (200 X)

Discussion

Ovarian cancer is the seventh most common cancer in women worldwide, with nearly a quarter of a million women diagnosed every year. 5-year survival is just 30%, a figure that has not changed for the past 30 years [14]. The current study showed that p53 was negative in all benign tumors but positive in 96.8% of malignant cases. Previous studies showed mutation in or inactivation of p53 in 57% [2], 46% [15] of invasive ovarian tumors, but in only 8% of borderline tumors and nonexistent in benign tumors [16]. This difference with the results of the current study may be due to genetic background differences, samples size or methodology variations.

Alterations of p*53* occur via a variety of mechanisms, such as mutations and deletions, or protein stabilization without any obvious genetic changes. Point mutations often result in a dominant-negative inhibition of the function of the wild type allele and/or gain of novel functions. Most of these mutant p53 proteins have a prolonged half-life, accumulate in the nucleus and can be detected by immunohistochemistry [17] whilst 26% to 81% of ovarian cancers have been reported to have mutations or overexpression of p*53* [18].

In ovarian cancer, the age of the patient considered an important risk factor. Patients older than 69 years of age exhibited significantly poorer survival than those younger [18]. In the current study, there was a significant difference in mean age of malignant and borderline cases in comparison with benign and non-neoplastic cases.

Different studies [12,19-21] showed over-expression of p53 detected by immunohistochemistry, as Kerbel *et al* [20] showed that p53 overexpression was detected in 43.3% of serous ovarian cancer while none of the normal ovarian tissues revealed immunohistochemical expression for p53, with significant level of expression between malignant and benign tissues ($p< 0.001$). p53 overexpression was reported more frequently in higher grades of differentiation with significant level of expression ($P < 0.05$) this indicates that serous ovarian tumors with positive p53 expression are biologically bearing more aggressive behavior and patient`s age both can be used as a prognostic markers in patient with ovarian cancer [20].

Another study done in Mosul/IRAQ by Hamdi and Saleem [21] in 2012, showed that, p53 expression was not significantly related to the age of the patients, grade, or to the histological type of the tumors. It was mainly found in malignant serous tumors (50%), in the poorly differentiated tumors (47.6%), and in the 6th decade of age (30.8%) [21].

In conclusion, p53 may play a role in the pathogenesis of ovarian cancer, in addition to patient`s age.

References

1. Jemal A, Siegel R, Ward E, et al. "Cancer statistics, 2007" *Ca-A.* Cancer J Clin. 2007; 57(1):43-66.

2. Sylvia MT, Kumar S. and Dasari P. The expression of immunohistochemical markers estrogen receptor, progesterone receptor, Her-2-neu, p53 and Ki-67 in epithelial ovarian tumors and its correlation with clinicopathologic variables. Indian J Pathol Microbiol. 2012; 55:33-7
3. Hahn WC, Weinberg RA. Modeling the molecular circuitry of cancer. Nat Rev Cancer. 2002; 2:331-41.
4. Sherr CJ, McCormick F. The RB and p53 pathways in cancer. Cancer Cell. 2002; 2: 103-12.
5. Bali A, O'Brien PM, Edwards LS, et al. Cyclin D1, p53, and p21Waf1/Cip1 expression is predictive of poor clinical outcome in serous epithelial ovarian cancer. Clin Cancer Res. 2004; 10:5168-77.
6. Tachibana M, Watanabe J, Matsushima Y, et al. Independence of the prognostic value of tumor suppressor protein expression in ovarian adenocarcinomas: A multivariate analysis of expression of p53, retinoblastoma, and related proteins. Int J Gynecol Cancer. 2003; 13:598-606.
7. Corney DC, Flesken-Nikitin A, Choi J, et al. Role of p53 and Rb in Ovarian Cancer. Adv Exp Med Biol. 2008; 622:99-117.
8. Kupryjanczyk J, Thor AD, Beauchamp R, et al. p53 gene mutations and protein accumulation in human ovarian cancer. Proc Natl Acad Sci USA. 1993; 90:4961-5.
9. Kupryjanczyk J, Bell DA, Dimeo D, et al. p53 gene analysis of ovarian borderline tumors and stage I carcinomas. Hum Pathol. 1995; 26:387-92.
10. Skomedal H, Kristensen GB, Abeler VM, et al. P53 protein accumulation and gene mutation in relation to overexpression of MDM2 protein in ovarian borderline tumors and stage I carcinomas. J Pathol. 1997; 181: 158-65.
11. Zheng J, Benedict WF, Xu HJ, et al. Genetic disparity between morphologically benign cysts contiguous to ovarian carcinomas and solitary cystadenomas. J Natl Cancer Inst. 1995; 87:1146-53.
12. de Graeff P, Hall J, Crijns APG. Factors influencing p53 expression in ovarian cancer as a biomarker of clinical outcome in multicentre studies. Br J Cancer. 2006; 95: 627-33.
13. Ramos-Vara JA. Technical Aspects of Immunohistochemistry. Vet Pathol. 2005; 42:405-26.
14. The Lancet Oncology: Ovarian cancer: breaking the silence. Lancet Oncol. 2012; 13(2):111. doi: 10.1016/S1470-2045(12)70052-9..
15. Levesque MA, Katsaros D, Yu H, et al. Mutant p53 protein expression is associated with poor outcome in patients with well or moderately differentiated ovarian carcinoma. Cancer. 1995; 75:1327-38.
16. Berchuck A, Kohler MF, Hopkins MP, *et al*. Overexpression of p53 is not a feature of benign and early-stage borderline epithelial ovarian tumors. Gynecol Oncol. 1994; 52:232-6.
17. Gannon JV, Greaves R, Iggo R, et al. Activating mutations in p53 produce a common conformational effect. A monoclonal antibody specific for the mutant form. Embo J. 1990; 9:1595-602.
18. Van der Zee AG, Hollema H, Suurmeijer AJ, et al. Value of P-glycoprotein, glutathione S-transferase pi, c-erbB-2, and p53 as prognostic factors in ovarian carcinomas. J Clin Oncol. 1995; 13:70-8.
19. Thigpen T, Brady MF, Omura GA. Age as a prognostic factor in ovarian carcinoma. The Gynecologic Oncology Group experience. Cancer. 1993; 71(2):606-14.
20. Kerbel HA, Abdul Saheb RH, Alabbasi DS. Expression of p53 and her2/neu in serous ovarian carcinoma with different grades of differentiation (Immunohisto-chemical Study). Kufa Med J. 2011; 14(1):98-107.
21. Hamdi EA, Saleem SH. P53 expression in ovarian tumors: (an immunohistochemical study). Ann Coll Med Mosul. 2012; 38(2):73-9.

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