ORIGINAL ARTICLE

EXPRESSION OF P53 IN OVARIAN EPITHELIAL TUMOURS AND ITS CORRELATION WITH HISTOPATHOLOGICAL PARAMETERS

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Background: Ovarian cancers are the leading cause of death among gynaecologic neoplasms. The most common form of ovarian tumours is surface epithelial tumours divided as benign, borderline and malignant. Of particular interest are borderline tumours, because the pathologist may rely on some what vague morphologic criteria. The aim of this study was to evaluate the correlation of tumour suppressor protein P53 with macroscopic and microscopic criteria of ovarian surface epithelial tumours and distinction of borderline from malignant tumours. **Methods:** We studied 109 ovarian neoplasms including 74 benign, 8 borderline and 27 malignant ovarian epithelial tumours during March 2006–March 2011 in Urmia University of Medical Sciences. Immuno-histochemical staining for P53 performed on paraffin blocks and quantified with 12- point weighted score proposed by W.Y chan. **Results:** Mean P53 weighted scores in benign, borderline and malignant tumours were 0.20±0.63, 0.76±0.89 and 3.79±4.20, respectively. There was significant difference between malignant and borderline tumours(p=0.002) and between malignant and benign ones (p=0.000).None of 11 immuno-reactive benign and 4 borderline tumours showed P53 expression in > 50% of tumour cells, but 11 out of 15 immuno-reactive malignant tumours (73.3%) expressed p53 in >50% of tumour cells. P53 score significantly increases with mitotic count (p=0.000) and solidification of the tumour (p=0.001). There was no significant correlation with size (p=0.277), papillary structures (p=0.062) and grade (p=0.578). **Conclusion:** According to our results, P53 staining can be used as a helpful method in distinction of borderline from malignant ovarian epithelial tumours, especially in the manner that expression in >50% of cells favouring malignancy.

Keywords: Ovary, Epithelial tumours, P53

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INTRODUCTION

Ovarian carcinoma is the sixth most common cancer in females and the third most common gynaecologic cancer, represents the leading cause of mortality among gynaecologic malignancies.[1–3] The epithelial tumours are the most common type of ovarian neoplasms divided as benign, borderline and malignant and morphologically categorized as serous, mucinous, endometrioid, clear cell and Brenner tumours.[4–6] Among them, borderline tumours are very interesting because there is no consensus about the histologic criteria for diagnosis of these tumours.[7] The clinical course of these neoplasms are unpredictable, too. Recurrence is not uncommon, and metastasis of these tumours and even death have been reported occasionally.[8]

As the clinical course and optimal management of different epithelial tumours differs, it seems necessary to use reliable factors as complementary to clinical parameters for a better management of the patients.[9] Some researches focused on molecular markers.[9] One of these markers is P53 which is a tumour suppressor gene found in approximately 50% of human malignancies.[10] The purpose of this study was to determine the relation of this gene with subtypes of ovarian epithelial neoplasms and to investigate its association with various histopathological parameters as a prognostic factor.

MATERIAL AND METHODS

In this retrospective study, histopathology slides of 109 consecutive ovarian epithelial neoplasms during March 2006 to March 2011 retrieved from archive of pathology department of Urmia, Imam Khomeini hospital. The histological type was confirmed by reviewing Haematoxylin and Eosin (H&E) stained slides. Tumour grading was done according the Shimuzu-Silverberg scoring system.[11]

The most representative sections for immunohistochemistry were selected. Four micrometre thick sections from each tumour block were obtained. After codifying, deparaffinization in xylene, and hydration in graded alcohol, washing in phosphate buffer saline (PBS) was done. Then the samples were placed in 10 mol/L citrate buffer (PH=6) and boiled in microwave for epitope retrieval. After that, sections incubated in 3% H2O2 for 10 minutes for quenching the endogenous peroxidase activity. Incubation with mouse anti-

human P53 monoclonal antibody (clon: DO-7, Dako Denmark) in a humidity chamber at 37º C was done for 30 minutes. After washing in PBS for 5 minutes at room temperature, envision and chromogen were added and washing with distilled water was done after 10 minutes. Contrast staining was obtained with haematoxylin. Sections from colorectal adenocarcinoma were applied as positive control. In the negative control the primary antibody was omitted.

Immuno-reactivity for P53 were quantified with a 12 point weighted score as Chan WY *et al* used in their study.[12] According to this method, first the percentage of positive cells in each section was scored with a 5 point scale: 0 for <5%, 1 for 5–25%, 2 for 25–50%, 3 for 50–75% and 4 for more than 75%. Then, the intensity of positivity was scored with a 3- point scale: 1 for weak, 2 for medium, and 3 for intense. Finally, the weighted score was obtained by multiplying the percentage score by the intensity score.

All slides were studied without any knowledge of clinical information or their H&E staining results. Statistical analyses were performed in the SPSS statistical software program, version 18. ANOVA test, *t*-test, and Pearson correlation test were used to evaluate the differences in P53 expression among various tumours. A *p*-value of less than 0.05 was accepted as significant.

RESULTS

The age of the patients ranged from 13 to 78 years (mean±SD: 44.8±17.2). Sixty seven patients were below the 50 years of age and 42 cases above the 50. Of the 109 ovarian epithelial tumours, 74 were benign, 8 borderline and 27 malignant. According the Shimuzu- Silverberg grading, 5 cases (18.51%) were grade-I, 13 cases (48.14%) grade-II and 9 cases (33.33%) grade-III. Papillary structures (larger than 1 cm in diameter) were present in 28 tumours and 81 cases were non-papillary. Eighty eight specimens were cystic, 11 specimens were solid and 10 cases were solid/cystic. Tumour sizes ranged from 2.3 to 30 cm (mean±SD:12.7±6.5).

Distribution of various histologic subtypes of ovarian epithelial neoplasms are seen in table-1 and age distribution and macroscopic features of the tumours according their nature are seen in table-2.

No mitotic figures found in benign ovarian tumours. Small number of mitoses are seen in borderline tumours, but malignant tumours showed significantly higher mitotic count than benign and borderline ones (Table-3).

Large numbers of malignant tumours showed P53 immunoreactivity in comparison with benign and borderline tumours. Also, P53 weighted score is prominently higher in malignant tumours. (Table-3)

All of the 11 immuno-reactive benign tumours and all of the 4 immuno-reactive borderline tumours showed positivity in less than 50% of tumoural cells. In contrary, of the 15 immuno-reactive malignant tumours, 11 cases showed immunoreactivity in more than 50% of tumoural cells. (Table-3)

Regarding the mitotic count and its correlation with P53 weighted score, we observed a positive linear correlation between them (r=0.551, *p*=0.000, Pearson correlation).

Among malignant tumours, P53 expression did not observed in clear cell carcinoma, malignant Brenner tumour and transitional cell carcinoma. Four of 13 serous carcinomas and one of 5 mucinous carcinomas were also negative in p53 staining.

In consideration of tumour nature, P53 weighted score was significantly different among benign, borderline and malignant tumours (*p*=0.000, ANOVA). The one by one comparison of these tumour groups showed significant difference between benign and malignant tumours (*p*=0.000) and between borderline and malignant ones (*p*=0.002) but there is no significant difference between benign and borderline tumours (*p*=1.000).

In addition, mean p53 weighted score did not differ between the different histologic subtypes of benign and also borderline tumours (*p*>0.05 ANOVA, Bonferroni).Among the most common types of malignant epithelial tumours, there were also, no difference between malignant serous tumours with their mucinous counterpart (*p*=0.4).

Tumours with cystic growth pattern showed significantly lower p53 weighted score than solid tumours (*p*=0.001) or solid/cystic tumours (*p*=0.000). (Table-4) We did not find any correlation between mean p53 weighted score and patient age, tumour size, histologic grade and presence or absence of papillary structures. (Table-5)

Table-1: Distribution of ovarian epithelial tumours according to the histologic subtypes

Histologic Type	Number (Percent)
Benign Serous	34 (31.2%)
Benign Mucinous	29 (26.6%)
Benign Mixed Seromucinous	9 (8.3%)
Benign Brenner	2 (1.8%)
Borderline Serous	5 (4.6%)
Borderline Mucinous	3 (2.8%)
Serous Carcinoma	13 (11.9%)
Mucinous Carcinoma	5 (4.6%)
Endometroid Carcinoma	3 (2.8%)
Adenocarcinoma ,undifferentiated	2 (1.8%)
Other Types of Carcinoma	4 (3.7%)
Total	**109**

Table-2: Age distribution and macroscopic features of ovarian epithelial tumours

Tumour nature	Age		Tumour Size			Macroscopic Pattern			Papilla	
	<50 years	≥50 years	<10 cm	10–20 cm	>20 cm	Cystic	Solid	Mixed	Presence	Absence
Benign	52	22	29	29	16	71	2	1	11	63
Borderline	6	2	2	5	1	7	0	1	5	3
Malignant	9	18	11	16	0	10	9	8	12	15
Total	**67 (61.5%)**	**42 38.5%)**	**42 (38.5%)**	**50 (45.9%)**	**17 (15.6%)**	**88 (80.7%)**	**11 (10.1%)**	**10 (9.2%)**	**28 (25.7%)**	**81 (74.3%)**

Table-3: Mitotic count and P53 weighted score expression in benign, borderline and malignant epithelial ovarian tumours

Tumour nature	Total Number	Mitotic figures/10HPF		P53 immunoreactivity			P53 weighted score	
		Mean	SD	Negative	Positive		Mean	SD
					<50% of cells	>50% of cells		
Benign	74	0	0	63 (85.1%)	11 (14.9%)	0	0.20	0.63
Borderline	8	3.38	4.13	4 (50%)	4 (50%)	0	0.76	0.89
Malignant	27	27.67	21.32	12 (45.5%)	4(14.8%)	11(40.7%)	3.79	4.2

Table-4: Mean P53 weighted score expression in ovarian epithelial tumours according to macroscopic pattern of growth (ANOVA-Bonferroni)

Macroscopic Pattern of Growth	Number of Cases	Mean P53 weighted Score	SD
Cystic	88	0.4633	1.39703
Solid	11	3.2082	4.09126
Solid/Cystic	10	4.7270	4.68716
Total	**109**	**1.1315**	**2.63684**

Table-5: Mean P53 weighted score correlation with the age, tumour size, tumour grade and papillary structures

Parameter	Number of Cases	Mean p53 weighted score	*p*-value
Age			
<50 years	67	0.74	0.052
≥50 years	42	1.75	
Size			
<10 cm	42	1.34	0.277
10–20 cm	50	1.27	
>20 cm	17	0.19	
Histologic Grade			
I	5	3.55	0.578
II	13	3.04	
III	9	4.99	
Papilla			
Present	28	1.93	0.062
Absent	81	0.85	

DISCUSSION

Ovarian carcinomas consists of 90% of malignant tumours of the ovary.[5]To definite diagnosis and better management of these tumours, different methods were used recently. P53 is a tumour suppressor gene found approximately in 51% of ovarian carcinomas.[10]

In this study, the mean P53 weighted score was 0.2±0.63, 0.76±0.89, and 3.79±4.2 in benign, borderline and malignant ovarian epithelial tumours, respectively. In comparison of benign and malignant tumours and also of borderline and malignant tumours the difference was significant (p=0.000 and p=0.002, respectively), but there is no significant difference between benign and borderline tumours (P=1). Similar to our results, Gursan *et al* found significant difference in the expression of P53 between malignant and benign ovarian epithelial tumours (p<0.005).[7] In the study of Kadkhodayan *et al* no benign ovarian epithelial tumour was immune-reactive for p53 but 1 case from 24 borderline tumours and 16 cases from 24 malignant ones were immune-reactive (p<0.001).[5]

In our study, none of 11 immuno-reactive benign tumours and none of 4 immuno-reactive borderline ones showed more than 50% of positive immune-reactive cells but in 40.7% of malignant tumours more than 50% of tumoural cells showed positivity. Similarly, Leonne M. *et al* found more than 50% of immune-reactive tumoural cells in 29% of malignant tumours and only in 5% of intermediate group.[1] In our study, in comparison of different histologic subtypes, the mean P53 weighted score was not significantly different in each groups of benign, borderline or malignant tumours (p>0.05, ANOVA). Also, in the study of Ayadi *et al*, there was no significant difference in expression of P53 between serous and non-serous tumours (p=0.84).[13] But, Ozer et al in their study about mucinous and serous tumours found significant difference only between borderline serous and borderline mucinous tumours (p<0.05). The serous tumours showed higher immunoreactivity in comparison with their mucinous counterpart.[14]

In our study, the mean p53 weighted score was 3.53±3.95, 3.04±4.2 and 4.99±4.53 in carcinomas

grade-I, II and III, respectively. The difference was not significant (p=0.578). Arik et al in Turkey and Ayadi *et al* in Tunisia found similar results (p=0.169 and p=0.061, respectively).[11,13] But, in the study of Ozer *et al* in Turkey, grade-II and III carcinomas showed significantly higher expression of P53 than that of grade I carcinomas (p<0.05).[14] Hui-Rong Shi, also found that the immunoreactivity for P53 significantly rises with the grade of malignant tumours (p=0.015).[3] In our study, there was a linear increase and significant difference between the mean p53 weighted score and mitotic count (r=0.551, p=0.000), but Arik *et al* found no such relation.[11] In a similar study, Gursan *et al* found positive relation between P53 expression and Ki-67 immunoreactivity as a proliferation marker (p<0.05).[7]

We found no relation between the age groups (more than 50 and less than 50 years) and P53 immunoreactivity (p=0.052). Similarly, Hui- Rong Shi *et al* and Kadkhodayan et al found no such relation, too (p=0.46 and p=0.39, respectively).[3,5] In evaluation of macroscopic appearance, in our study, cystic tumours showed less P53 immuno-reactivity in comparison with solid and solid/cystic tumours. The difference was significant (p=0.001, p=0.000, respectively). It should be remembered that malignant tumours have more solid portions in comparison with benign and borderline tumours.

CONCLUSION

In summary, it seems that P53 can be useful in distinction of borderline tumours from malignant ones, in the manner that expression in more than 50% of cells and also higher mean P53 weighted score favouring malignancy. Also, we found that there was a linear correlation between P53 immunoreactivity and mitotic count which is a parameter in grading of ovarian carcinomas, though there was no significant difference among grades of carcinomas, independently. The limitation of our study were restricted numbers of the samples (109 cases) and using only one marker. It seems that extensive study of higher numbers of tumours and using different markers are necessary to achieve more reliable results. At the other hand, in our study, only one block per case representative for the tumour was selected for immunohistochemistry staining. Maybe, selection of more sections could be show different results.

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AUTHOR'S CONTRIBUTION

FA: Study the histopathology slides and writing the manuscript. AE: Study the histopathology slides as advisor. ZY: Statistical analysis. AS: Collecting the data and literature review

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