P53 expression in ovarian tumors: (an immunohistochemical study)

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ABSTRACT

Objectives: The aim of this study, first, to evaluate the frequency of immunohistochemical expression of p53 in different types of ovarian tumors, second, to correlate p53 expression with age of patients, histological type and grade of tumors, and third, to compare the results with those of others.

Methods: This study was performed on 60 primary ovarian tumors. Samples were obtained in a prospective and retrospective fashion (cross sectional study). The samples were collected from Al-Jumhuri Teaching Hospital, Al-Salaam General Hospital, Al-Khansaa Teaching Hospital, and some private Laboratories, during a period of eight months, from June 2009 through January 2010. P53 expression was assessed immunohistochemically.

Results: The patients' age ranged from 13 to 80 years, with a mean of 43.88 year. Most of them were in the fifth decade (28.3%). Data concerning the age were missing in five patients. P53 immunoreactivity was observed in 48.1% of the malignant tumors. It was positive in 13 of 20 cases of serous adenocarcinoma, in all of the 5 cases of mucinous adenocarcinoma, in 3 of 12 granulosa cell tumors, in 2 of 4 endometrioid carcinomas, in 1 of 3 clear cell carcinoma, in 1 of 2 dysgerminomas, and in the one case of Sertoli-Leydig cell tumor. It was negative in malignant thecoma, malignant teratoma and in all borderline and benign tumors.

Statistically 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%).

Conclusions: P53 was expressed in 48.1% of malignant ovarian tumors, 80.8% were epithelial tumors, 15.4% were sex cord-stromal tumors, and 3.8% were germ cell tumors, and it was negative in benign and borderline tumors.

Keywords: Ovarian tumors, p53 expression, immunohistochemistry

الخلاصة

الأهداف: تم إجراء هذه الدراسة لتقييم حالة الظهور المناعي لبروتين p53 في أورام المبيض في مدينة الموصل والربط بينها وبين مختلف الصفات المرضية السريرية للورم ومقارنة هذه النتائج مع نتائج لدراسات أخرى.

الحالات والطرق: إن هذه الدراسة مستقبلية ورجعية تم من خلالها جمع ٦٠ حالة من أورام المبيض (٤٩ حالة من سرطان المبيض، ٥ حالات من أورام المبيض المتوسطة، بالإضافة إلى ٦ حالات من أورام المبيض الحميدة).

تم جمع هذه الحالات من مستشفى الجمهوري، السلام العام، الخنساء التعليمي، وبعض المختبرات الخاصة خلال فترة ٨ أشهر امتدت من شهر حزيران ٢٠٠٩ إلى شهر كانون الثاني ٢٠١٠. لقد تم التحري عن بروتين p53 بطريقة مناعية-نسيجية-كيميائية.

النتائج والاستنتاجات: لقد تراوحت أعمار المرضى بين ١٣ و ٨٠ سنة. وتم تقسيم الحالات السرطانية نسيجيا إلى ٣٢ حالة لسرطان المبيض السطحي الظهاري و ٣ حالات لسرطان الخلايا الجنسية (التناسلية) و١٤ حالة للسرطان المتعلق بالسدى والجنس و ٥ حالات من أورام المبيض المتوسطة. بالإضافة إلى ٦ حالات من أورام المبيض الحميدة. وقد ظهر بروتين p53 في ٢٦ حالة (٤٨.١%) من حالات سرطان المبيض و أورام المبيض المتوسطة.

لم يكن هناك علاقة معنوية بين ظهور بروتين p53 وعمر المريضة أو مرتبة الورم أو النوع النسيجي المرضي للورم (P=0.2806، P=0.8710 ، P=0.0681 بالتعاقب). كما أظهرت النتائج عدم ظهور بروتين p53 في أورام المبيض المتوسطة والحميدة.

Ovarian cancer represents the sixth most commonly diagnosed cancer among women in the world, and causes more deaths per year than any other cancer of the female reproductive system [1-3].

Ovarian cancers represent one fourth of the malignancies of the female genital tract[2,3].They account for 4% of the total cancers in women in the United States[2,3], ranked behind malignant neoplasms of the lung, breast, colon and uterus[3]. In Iraq, ovarian cancer ranks sixth among the most common ten cancers involving females[4], representing about 3.2% of all female cancers, and second to cervical carcinoma in the female genital tract [5]. Since the p53 tumor suppressor gene has been found to be mutated in more than 50% of human cancers, it has attracted the interest of numerous researchers[6]. P53 is mutated and consequently overexpressed in 50% to 60% of advanced ovarian cancers[7]. P53 is rarely mutated in borderline tumors, and when this mutation occurs, it is associated with a poor prognosis[7]. The pattern of p53 abnormalities is most consistent with spontaneous mutation rather than the activity of chemical carcinogens[7].

The aim of this study is to evaluate the frequency of p53 expression in different types of ovarian tumors, to correlate p53 expression with age of patients, histological type and grade of tumors, and to compare the results with those of others.

PATIENTS AND METHODS

Selection of Cases

This study is based on samples prospectively and retrospectively collected from 60 patients with primary ovarian tumors, of which: 32 cases were malignant epithelial tumors, 14 malignant sex cord-stromal cell tumors, 3 malignant germ cell tumors, 5 borderline tumors, and 6 cases of benign tumors. Samples were collected from Al-Jumhuri Teaching Hospital, Al-Salaam General Hospital, Al-Khansaa Teaching Hospital and some private Laboratories. Expression of p53 protein by immunohistochemical staining was studied and compared in relation to patient's age, histopathological type and grade of tumors.

Hematoxylene and eosin stained slides from formalin-fixed paraffin-embedded biopsy blocks were examined. P53 expression was assessed immunohistochemically on formalin-fixed paraffin-embedded tissues, using p53 (DAKO, mouse monoclonal antibody p53-clone DO-7), and Secondary red Envision system.

Immunohistochemical staining interpretation

A semi-quantitative histochemical score was used to record results of p53 nuclear staining[8]. More than 1000 tumor cells, in multiple high power fields, had been counted for assessing the percentage. Also the average staining intensity was considered. The slides were checked more than once to exclude subjectivity.

P53 scoring guidelines "semi-quantitative score"[9]:

I- The percentage was used to score a slide semi quantitatively in one of five categories:

Proportion score (PS)	Proportional score observation
0	<5% positive cells.
1	5% - 25% positive cells.
2	26–50% positive cells.
3	51%-75% positive cells.
4	76%-100% positive cells.

II- Based on the intensity of positive reaction in the majority of tumor cells, the intensity of staining is graded and scored as:

Intensity score (IS)	Intensity score observation
1	Weak staining (+)
2	Moderate staining (++)
3	Strong staining (+++)

Positive and negative control slides were included in each run of staining. Positive control slides were prepared from a case known to be positive for p53. While negative control slides were prepared from the same tissue block incubated with Tris Buffered Saline (TBS) instead of the primary antibody.

Statistical analysis

The relationship between p53 expression and the clinicopathologic variables was analyzed by the Fisher Freeman Holton's test. The results were considered statistically significant if the p value was < 0.05.

RESULTS

For a period of 8 months (from June 2009 through January 2010), immunohistochemical study to assess the expression of p53 protein in 60 specimens from patients with primary ovarian tumors was performed.

The patients' age was in the range of 13 to 80 years, with a mean of 43.88 year. Most of them were in the fifth decade (31.5%), (**Table 1**). However, data concerning the age were missing in 5 patients.

Ovarian tumors were grouped according to their biologic behavior as: malignant tumors 49 cases (81.7%), borderline tumors 5 cases (8.3%), and benign tumors 6 cases (10%). All of the tumors that expressed P53 were from malignant type (**Figure 1**). P53 was expressed in 80.8% in carcinomas, 15.4% in sexcord- stromal tumors, and 3.8% in germ cell tumors.

P53 was positive in 13 of 20 cases of serous adenocarcinoma (**Figure 2**), in all of the 5 cases of mucinous adenocarcinoma (**Figure 3**), in 3 of 12 granulosa cell tumors (**Figure 4**), in 2 of 4 endometrioid carcinomas (**Figure 5**), in 1 of 3 clear cell carcinoma (**Figure 6**), in 1 of 2 dysgerminomas (**Figure 7**), and in the one case of Sertoli-Leydig cell tumor (**Figure 8**). It was negative in malignant thecoma, malignant teratoma, in all borderline (3 serous cyst tumors and 2 mucinous cyst tumors), and in the 6 cases of benign tumors (2 serous cystadenoma, 2 mature teratoma, 1 mucinous cystadenoma, and 1 thecoma), (**Table 2**).

Most of the cases of malignant epithelial tumors were categorized as grade III (50%). Grade II and grade I formed (28.1%) and (21.9%) respectively, (**Figure 9**).

P53 expression

The p53 expression was found in 48.1% of the malignant and borderline ovarian tumor cases.

P53 expression and patient's age

The p53 expression had no significant correlation to age, 5 samples were excluded because of unknown age, (**Table 1**).

P53 expression and histological type of the tumors

The p53 expression had no significant correlation to histological type of the tumors, (**Table 2**).

Table 1. P53 status in malignant ovarian tumors in relation to patients' age.

Age (year)	Total		+ve		-ve		p-value
	No.	%	No.	%	No.	%	
13-20	3	5.6%	3	11.5%	0	0%	
21-30	5	9.3%	3	11.5%	2	7.1%	0.2806 (NS)
31-40	6	11.1%	2	7.7%	4	14.4%	
41-50	17	31.5%	6	23.1%	11	39.3%	
51-60	15	27.7%	8	30.9%	7	25%	
61-70	1	1.8%	1	3.8%	0	0%	
71-80	2	3.7%	0	0%	2	7.1%	
Unknown	5	9.3%	3	11.5%	2	7.1%	
Total	54	100%	26	48.1%	28	51.9%	

Table 2. P53 expression and histological type of malignant and borderline ovarian tumors.

Histological type of tumor	Total		P53 +ve		P53 -ve		p-value
	No.	%	No.	%	No.	%	
Serous	20	37%	13	50%	7	25%	
Mucinous	5	9.3%	5	19.3%	0	0%	0.0681 (NS)
Endometroid	4	7.5%	2	7.7%	2	7.1%	
Clear cell	3	5.6%	1	3.8%	2	7.1%	
Granulosa cell	12	22.2%	3	11.5%	9	32.2%	
Malignant thecoma	1	1.8%	0	0%	1	3.6%	
Sertoli-Leydig cell	1	1.8%	1	3.8%	0	0%	
Malignant teratoma	1	1.8%	0	0%	1	3.6%	
Dysgerminoma	2	3.7%	1	3.8%	1	3.6%	
Borderline serous cyst tumor	3	5.6%	0	0%	3	10.7%	
Borderline mucinous cyst tumor	2	3.7%	0	0%	2	7.1%	
Total	54	100%	26	48.1%	28	51.9%	

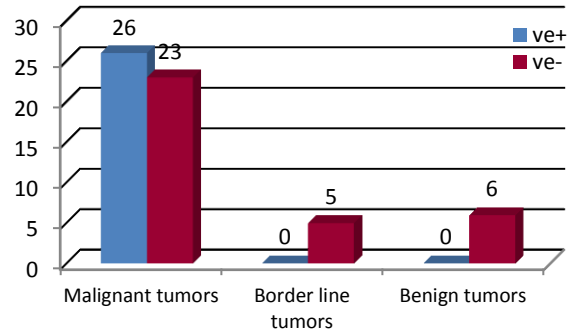

Figure 1. P53 status in ovarian tumors.

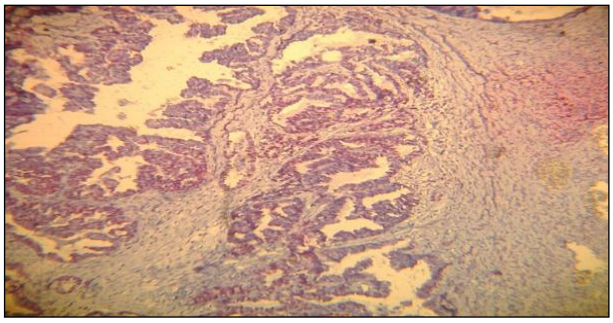

Figure 2. Papillary serous cystadenocarcinoma, IHC staining positive for p53 protein, strong expression (x 100).

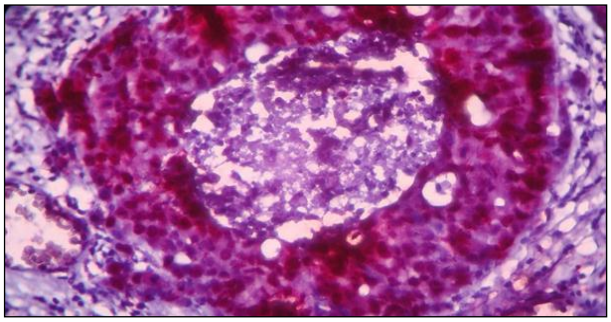

Figure 3. Mucinous cystadenocarcinoma of ovary, IHC staining positive for p53 protein, strong expression (x 400).

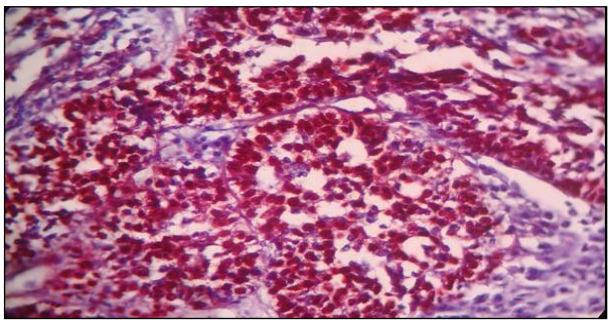

Figure 4. Granulosa cell tumor, IHC staining positive for p53 protein, strong intensity (x 400).

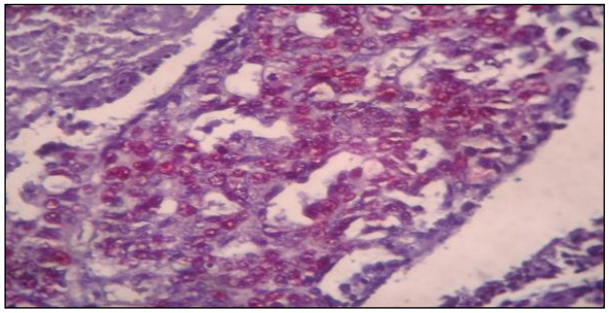

Figure 5. Endometrioid carcinoma grade II, IHC staining positive for p53 protein, moderate intensity(x 400).

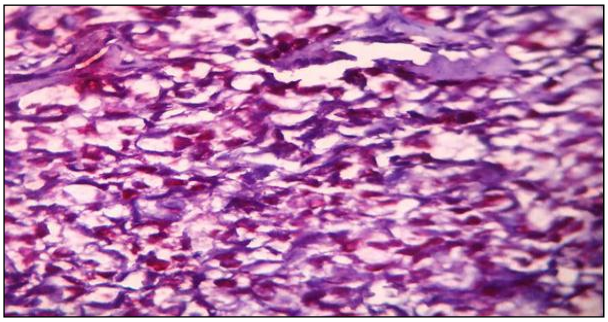

Figure 6. Clear cell carcinoma of ovary, IHC staining positive for p53 protein (x 400).

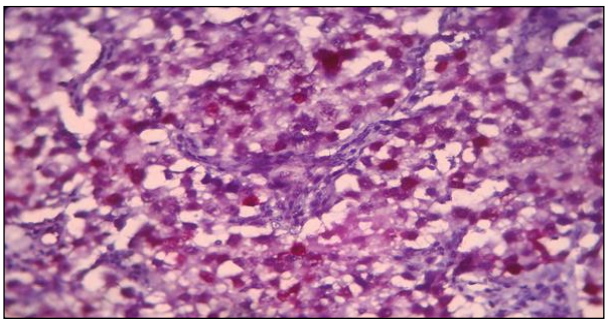

Figure 7. Dysgerminoma, IHC staining positive for p53 protein, scaterred positivity (x 400).

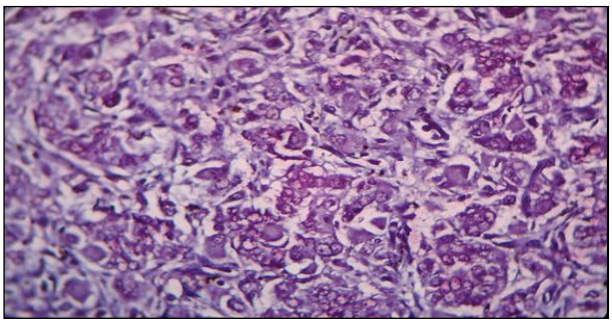

Figure 8. Sertoli-Leydig cell, IHC staining positive for p53 protein weak intensity (x 400).

P53 expression and grade of the carcinomas

P53 expression has no significant correlation to the grade of the tumors p-value= 0.8710 (NS), (**Figure 9**).

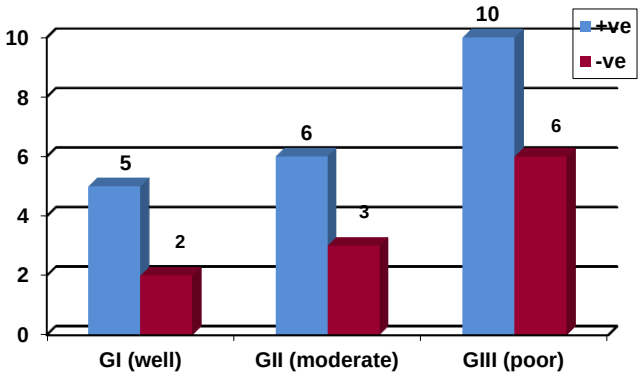

Figure 9. P53 expression & grade of ovarian carcinomas (P=0.8710, NS).

DISCUSSION

In this study, p53 was expressed in 48.1% of the malignant ovarian tumors. Other similar studies have shown variable ratios (25.6%-61%) (**Table 3**). The reasons for this variation are unknown[10]. However possible sources for this variation may be attributed to[10]:

a. The properties of different antibodies.
b. The scoring methods applied for p53 immunoreactivity.
c. The enzyme and microwave treatments of the tissue during the staining process.
d. The tissue fixation procedure.

Table 3. Frequency of p53 expression in ovarian tumors in different studies.

Study	Year	Site	No. of Cases	% of P53 +ve Cases
Current study	2010	Mosul	54	48.1%
Abubaker J. et al[11].	2009	Saudi Arabia	154	32.5%
Kupryjanczyk J. et al[12]	2008	Poland	452	58.8%
Shao HL. et al[13].	2007	China	79	28.3%
Graeff P. et al[14].	2006	UK	476	52.1%
Dogan E. et al[15].	2005	Turkey	82	54%
Nielsen JS. et al [16].	2004	Denmark	868	53%
Havrilesky L. et al[17].	2003	USA	125	44%
Chan WY. et al[18].	2000	China	46	54%
Ferrandina G. et al [19].	1999	Italy	168	50%
Anttila MA. et al[20].	1998	Finland	316	25.6%
Eltabbakh GH. et al[21].	1997	NewYork	221	48.4%
Herod J J. et al[22].	1996	UK	70	61%
Klemi PJ. et al[23].	1995	Finland	136	46.3%
Marks JR. et al[24].	1991	North carolina	107	50%

P53 expression in relation to age

P53 expression was mainly found in the 6th decade of life (30.9%). This may be related to the accumulation of somatic mutations[25]. It is known that loss of heterozygosity on chromosome 17 increases with age[25]. Li-Fraumeni patients with p53 mutations develop tumors earlier and with a higher frequency when adjusted for age[26]. The promoter of MDM2 (Murine Double Minut2) and p53 interaction partner contains a functional estrogen receptor signal in the DNA[27]. Therefore, the effect of the p53 on risk of cancer in women could depend on menopausal status[27].

Statistically, there was no significant correlation between p53 overexpression and age of the patients. This is consistent with the results of other studies[12,14,19,20,22,23,26], with the exception of a single one with a reported significant correlation[21].

P53 expression in relation to histological type of tumors

Our study showed p53 expression in carcinomas mainly, this was reported by others who found that surface epithelial tumors, especially serous cystadenocarcinomas, and non Hodgkin lymphomas of the ovary showed high expression of p53 compared to the benign and borderline tumors[28]. On the otherhand, the expression of p53 in tumors arising from germ cells and sexcord-stromal cells were observed to be very low[28]. P53 deficiency alone is not sufficient for ovarian epithelial tumorigenesis[29]. Thus, other genetic lesions are likely to be required to develop ovarian cancer[29]. However, the leading role of alterations in p53 gene in the development of ovarian carcinoma was further substantiated by results of a study, which revealed a striking association between the number of life time ovulatory cycles and overexpression of mutant p53 protein in ovarian carcinoma tissue[30]. Abendstein B, et al. suggested that p53 mutations occur spontaneously during the repeated injuries of the ovarian surface caused by ovulations[30]. This repeated repair requires high rates of DNA synthesis with increased likelihood of mutations especially in the ovarian surface epithelium from which epithelial ovarian carcinomas originate[30].

Statistically, there was no significant correlation between p53 expression and histological type of tumors. Review of literature showed conflicting results; some with no significant relation-

ship[13,16,18,25] and others with significant relationship[12,20,22,23].

P53 expression in relation to grade of tumors

It has been well established that TP53 mutations are frequent in both hereditary and sporadic high-grade serous carcinoma[29,31-34]. Boyd and co-workers[35] demonstrated TP53 mutations in microcarcinomas, and Werness et al,[36] demonstrated loss of heterozygosity at BRCA1 and TP53 and expression of p53 in one microscopic ovarian surface carcinoma-in situ[36].

These data demonstrating increasing atypia and accumulating genetic alterations in surface epithelial inclusion glands, ovarian "carcinoma in situ", microcarcinomas, and typical high-grade serous carcinoma, suggest that high-grade serous and perhaps endometrioid ovarian adenocarcinomas arise from these structures[35]. Additionally, the data from a small number of cases indicate that TP53 mutation occurs in preinvasive epithelium with loss of BRCA1 or BRCA2 function in the majority of tumors, findings compatible with the high degree of genetic instability of these tumors[35].

In the present study P53 expression was mainly found in poorly differentiated tumors (47.6%). However, the expression of p53 in relation to grade was not significant statistically and this is also shown in three other studies[11,22,37] but it was significant in eight studies[12,14,16,18-21,23,26] which reported significant correlation with higher grades while Havrilesky et al.[17] reported significant correlation with lower grade, which is attributed to the fact that most of their cases were in lower grade.

The degree of p53 expression increased with the increasing grade of ovarian tumors, as seen by the presence of strong p53 expression in grade III [21]. Apparently, cancers with p53 mutation demonstrated a trend toward more aggressive tumor behavior such as distant metastasis and poor cellular differentiation[38]. A better understanding of the factors and mechanisms determining the aggressive behavior of some epithelial ovarian carcinoma is critical in developing new treatment [11].

CONCLUSIONS

1. P53 expression was found in 48.1% of malignant ovarian tumors, and this result is within the range observed by others.
2. P53 was expressed in 80.8% in carcinomas, 15.4% in sexcord-stromal tumors, and 3.8% in germ cell tumors.
3. Age of the patients, grade, and histological type of the tumors had no significant correlation with p53 expression.
4. All of the benign and the borderline tumors were negative for p53.

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