Original Research Article

2 years study on p53 expression on serous and mucinous tumors of ovary

T. T. Sreeja[1*], S. Chandrasekhar[2], S. Lokesh Rao Magar[2], K. Durga[3]

[1]Senior Resident, [2]Associate Professor
Kakatiya Medical College,Warangal, India
[3]Professor, Osmania Medical College,Hyderabad, India
[*]Corresponding author email: **dr.tt.sreeja@gmail.com**

International Archives of Integrated Medicine, Vol. 3, Issue 7, July, 2016.

Available online at http://iaimjournal.com/
ISSN: 2394-0026 (P) **ISSN: 2394-0034 (O)**
Received on: 17-06-2016 **Accepted on:** 03-07-2016
Source of support: Nil **Conflict of interest:** None declared.
How to cite this article: T. T. Sreeja, S. Chandrasekhar, S. Lokesh Rao Magar, K. Durga. 2 years study on p53 expression on serous and mucinous tumors of ovary. IAIM, 2016; 3(7): 150-158.

Abstract

Background: Epithelial ovarian carcinoma is worldwide the sixth most common female cancer. This malignancy carries the highest mortality among all gynecological cancers.

Aim and objectives: To perform and interpret p53 immunostaining on the diagnosed serous and mucinous malignant surface epithelial ovarian tumors and to correlate expression of p53 with histological type of malignancy.

Materials and methods: 2 year prospective study was done i.e. from October 2011 to September 2013 on "p53 interpretation on surface epithelial ovarian tumors" in MGM Hospital, Warangal. All the ovarian surface epithelial tumor specimens, received in the pathology department during this period were considered.

Results: A total of 120 cases were studied, out of which benign tumors were the most common (64.2%), followed by malignancy (25.8 %) and 12 cases (10%) of borderline malignancy. Most of the benign tumors were unilateral, the cases which showed bilateral involvement were mostly malignant. The maximum number of cases in the present study was seen in the age group of 31-60 years. The youngest patient was 16 years old and the oldest was 68 years old. Serous cyst adenoma was the most common neoplasm found and accounted for 53 cases (44.2%) , followed by mucinous cystadenoma, which accounted for 21 cases (17.5%). 9 cases of serous cystadenofibroma (7.5%), 3 borderline serous tumors (2.5%) and 11 serous cystadenocarcinoma (9.1%) were found in the present study. Out of the 44 mucinous tumors, 21 were benign (17.5%), 3 were of borderline malignancy (2.5%) and 20 were malignant (16.7%). One case of mucinous cystadenocarcinoma was found to be associated with

adenocarcinoma of ascending colon. One case of benign Brenner tumour was also found in the present study.

Conclusion: The rate of p53 abnormalities varies with histologic type, grade and stage of the tumor. P53 expression was more in malignant serous tumors as compared to the malignant mucinous tumors.

Key words

Epithelial ovarian tumors, p53 expression, Malignant cystadenocarcinomas of ovary.

Introduction

Epithelial ovarian carcinoma is worldwide the sixth most common female cancer [1]. This malignancy carries the highest mortality among all gynecological cancers [2, 3]. Identification of new biological prognostic markers would be of great importance to select patients with a possibly favorable or poor clinical outcome and might help to improve treatment planning [1].

Regulators of apoptosis, especially p53 and Bcl-2, and steroid hormone receptors, estrogen and progesterone, have been studied as potential prognostic factors of epithelial ovarian cancer [1].

P53 is a tumor suppressor gene located on the short arm of chromosome 17. Mutation of p53 is believed to result in uncontrolled cell proliferation [1].Certain cancers, such as epithelial ovarian carcinoma, appear to display variation in the occurrence and range of p53 abnormalities according to certain disease characteristics such as histologic subtype and stage [4].

Aim of the study

- To perform and interpret p53 immunostaining on all diagnosed malignant serous and mucinous ovarian tumors.
- To correlate expression of p53 with histological type of malignancy.

Materials and methods

A 2 year prospective study was done i.e. from October 2011 to September 2013 on "Interpretation of p53 in malignant serous and mucinous ovarian tumors" in MGM Hospital, Warangal. All the ovarian surface epithelial tumor specimens received in the pathology department during this period were considered.

Inclusion criteria

- All the surface epithelial ovarian tumor specimens, of females in the age groups 10-70 and above.
- Only samples with definite histopathological diagnosis were considered.

Exclusion criteria

- All the ovarian tumor specimens of females of < 10 years were excluded.
- Congenital lesions, unusual tumour types and inadequate samples were excluded.
- All other surface epithelial tumors were excluded

Specimen handling

All the ovarian tumor specimens were received in 10% formalin. The specimens were then subjected to gross examination and adequate sampling by appropriate tissue section and were sent for routine histopathological processing. After a histopathological diagnosis of the lesion was made, the paraffin blocks of the samples which had met the criteria of inclusion were collected.

Sections for histology

For cystic tumors: Up to three sections of cyst wall (particularly from areas with papillary appearance)

For solid tumors: Three sections or one section for each centimeter of tumor, whichever was greater; also one section of non-neoplastic ovary, if identifiable.

p53 immunostaining using polyclonal antibody (DAKO)

Sections underwent histological evaluation to select blocks without necrotic and hemorrhagic areas. Consecutive 3-4μm sections were taken on poly-lysine coated slides. At the next stage, sections were deparaffinised and antigen-retrieval procedure was performed by trilogy solution using microwave method. Sections were thoroughly washed with wash buffer (TRIS-buffered saline) in between every step. Endogenous peroxidase blocking was done by horse radish peroxidase. Then, monoclonal antibody against p53 protein (clone DO-7; Dako), was applied to the sections and incubated for 30 minutes at room temperature. Then, secondary antibody was added and incubated for 30 minutes. Then freshly prepared diaminobenzidine (DAB.) was added to the sections for 10 minutes and the sections were lightly counterstained with hematoxylin. Slides were then dehydrated, cleared and mounted.

Positive controls: Salivary gland tissue sections known to express p53 strongly.

Negative controls: Involved the omission of primary antibody.

Interpretation

Immunoreactivity for p53 was evaluated semi quantitatively according to the percentage of positive tumor nuclei, scored as follows:

- None (< 5%),
- Weak (+, 5–25%),
- Moderate (++, 25 – 75%),
- Intense (+++, >75%).

All tumors showing p53 immunoreactivity (at least +) were considered to be positive (**Figure – 1 to 8**).

Results

In our study on "p53 expression in serous and mucinoustumours of ovary", we have evaluated 120 cases aged between 10 years and 70 years age group from October 2011 to September 2013 in MGM hospital, Warangal.

The histomorphological types as per the main heads of the WHO classification were as per **Table - 1**. Age distribution was as per **Table - 2**. Most of the cases were seen in the age group of 31- 40 years. The lowest age in which the tumour found was a 16 year old girl who presented with pain abdomen. The tumour size was 9x8x1 cm; this was diagnosed as serous cystadenoma, which was the most common tumour in our study. The oldest lady aged 68 years, presented with abdominal discomfort. It was of 6x5x3 cm. Histopathological examination revealed a diagnosis of mucinous cystadenocarcinoma.

The number of tumors which were benign/ malignant was as per **Table - 3**. Out of 120 cases, 77 tumors were benign (64.2%), 31 were malignant (25.8%) and 12 cases were of borderline type (10%).

p53 IHC was done on all the cases of diagnosed malignant surface epithelial tumors of the ovary. Out of 31 cases 11 were positive. Of these 11 positive cases, 7 cases were of serous cystadenocarcinoma and 4 cases of mucinous cystadenocarcinoma. The results of p53 immunostaining were as per **Table – 4**. The intensity of p53 immunostaining was as per **Table - 5**.

Table – 1: Histomorphological types of ovarian tumors.

Histological types	No.	%
Serous tumors	76	63.3%
Mucinous tumors	44	36.7%
Total	120	100%

Table - 2: Age distribution.

Age group (Years)	Number	%
11-20	3	2.5%
21-30	14	11.7 %
31-40	46	38.3 %
41-50	36	30.0 %
51-60	18	15.0 %
61-70	3	2.5 %

Table - 3: Benign/ Malignant lesions.

Type of lesion	No.	%
Benign	77	64.2%
Borderline	12	10.0%
Malignant	31	25.8%

Figure – 1: Gross photograph of serous cystadenocarcinoma.

Figure – 2: Gross photograph of mucinous cystadenocarcinoma.

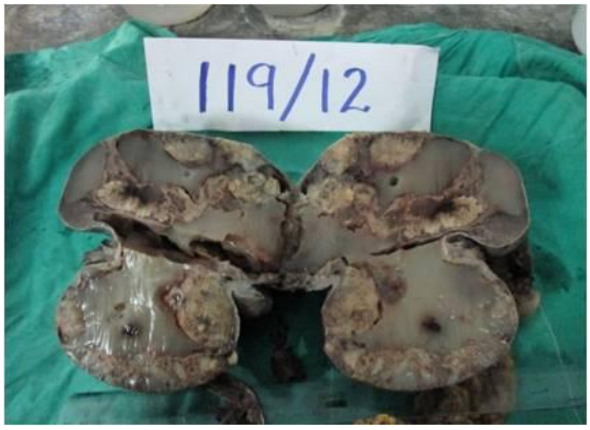


Figure – 3: Photomicrograph of serous cystadenocarcinoma.

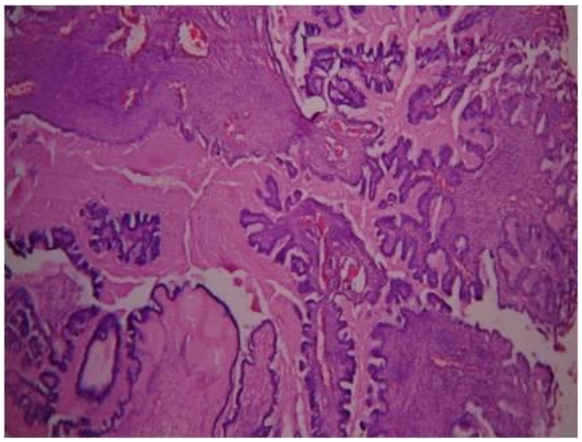


Figure – 4: Immunostaining of serous cystadenocarcinoma (grade 1).

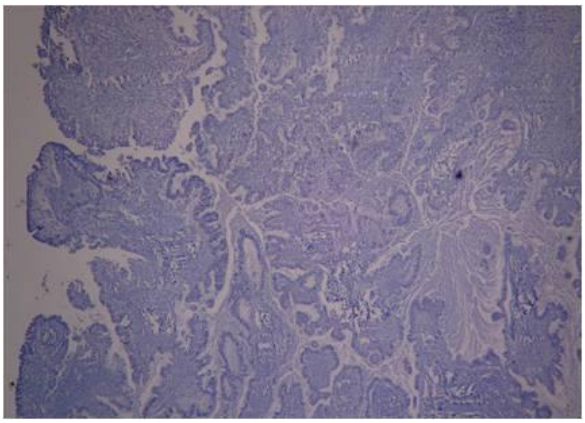


Figure – 5: Photomicrograph of serous cystadenocarcinoma.

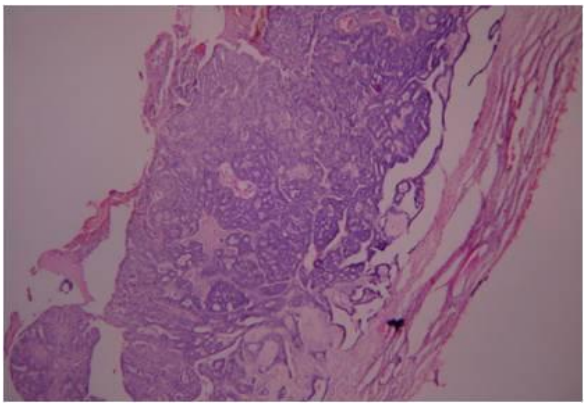
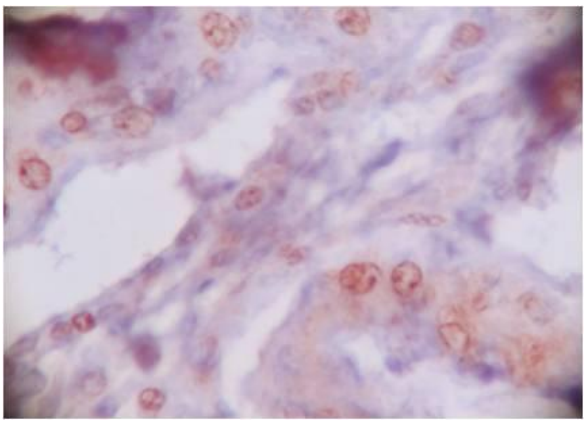


Figure – 6: Immunostaining of serous cystadenocarcinoma (grade 2).



Discussion

The present study was in concordance with both studies as per **Table - 6**, where most of the cases were seen between 30-50 years of life [5, 6]. Comparative analysis of benign/malignant

lesions with other studies [5, 6] was as per **Table – 7**.

Figure – 7: Photomicrograph of mucinous cystadenocarcinoma.

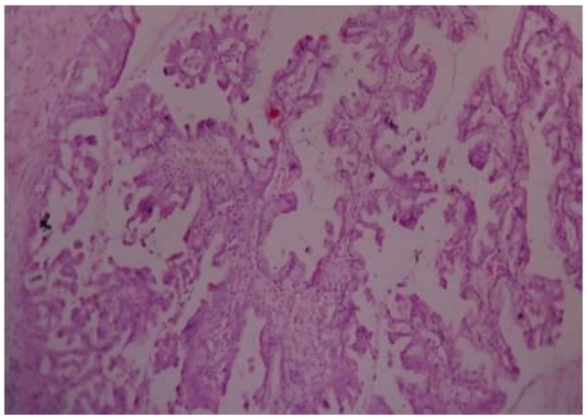
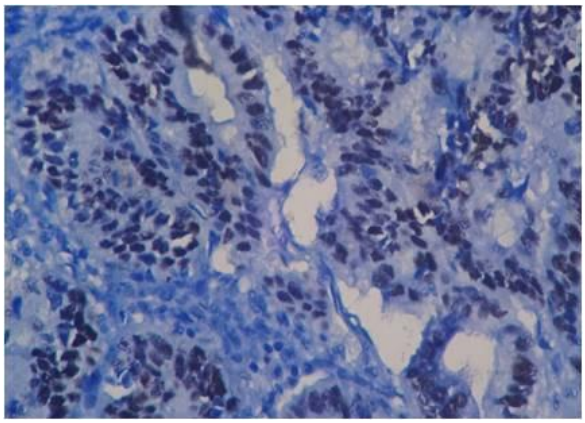


Figure – 8: Immunostaining of mucinous cystadenocarcinoma (grade 4).



Distribution of ovarian tumors on the basis of microscopic diagnosis

Histologically, a total 120 patients who were diagnosed with surface epithelial ovarian tumors were studied. The tumors were classified according to the WHO classification. Serous tumors (62%) were the most common, followed by mucinous tumors (36.3%). 0.8% of the cases were other surface epithelial tumors. The comparative analysis of study with other authors [5-7] showed the similar results as per **Table - 8**. In the present study, serous tumors were the commonest of all the surface epithelial ovarian neoplasms.

The present study revealed statistically significant correlation with other studies [8-13] as per **Table - 9**. A little over 50% of human tumors contain mutations in the gene. Homozygous loss of p53 occurs in virtually every type of cancer [14]. p53 induced apoptosis of cells with irreversible DNA damage is the ultimate protective mechanism against neoplastic transformation [15].

The ability of p53 to control apoptosis in response to DNA damage has important practical therapeutic implications. Irradiation and chemotherapy, the two common modalities of cancer treatment, mediate their effects by inducing DNA damage and subsequent apoptosis. Tumors that retain normal p53 are more likely to respond to such therapy than tumors that carry mutated alleles of the gene. By contrast, tumors which frequently carry p53 mutations, are relatively resistant to chemotherapy and irradiation. Various therapeutic modalities aimed at increasing normal p53 activity in tumor cells that retain this type of activity or selectively killing cells with defective p53 function are being investigated [16].

Several lines of experimental evidence support the concept that p53 is involved in the cellular response to cytotoxic agents and that loss of p53 is associated with resistance to agents such as cisplatin [17]. In contrast, p53-deficient cell cultures show increased sensitivity to paclitaxel treatment or no difference. Few studies show the association of aberrant p53 staining with a poor prognosis.

The importance of p53 accumulation as a marker of adverse outcome in ovarian carcinoma has been demonstrated in several studies. Expression of p53 is associated with other unfavorable prognostic factors such as advanced FIGO stage, suboptimal cytoreduction, serous histologic subtype and increasing tumor grade. Nevertheless, its independent prognostic value remains controversial [18, 19]. Some investigators have demonstrated that p53 mutation or overexpression is a significant prognostic factor [20-22]. Other studies have been unable to confirm such results [23-25].

Table - 4: Results of p53 immunostaining.

Type of tumor	p53 +ve	p53 -ve	Total cases
Serous cystadenocarcinoma	7 (63.6%)	4 (36.4%)	11
Mucinous cystadenocarcnoma	4 (25%)	16 (75%)	20

Table - 5: Intensity of p53 immunostaining.

	Serous cystadenocarcinoma	Mucinous cystadenocarcinoma
Negative	4 (36.6%)	16 (80%)
+1	1 (9%)	2 (10%)
+2	3 (27.2%)	1 (5%)
+3	3 (27.2%)	1 (5%)

Table - 6: Comparative analysis of age incidence of surface epithelial tumours with other studies.

Age group (Years)	Kar, et al. [5]	Jha, et al. [6]	Present study
11 – 20	0%	2%	2.5 %
21 - 30	13%	11%	11.7 %
31 – 40	28%	30%	38.3 %
41 – 50	38%	25%	30.0 %
51 – 60	15%	20%	15.0 %
61 - 70	6%	12%	2.5 %
Total	100%	100%	100%

Table - 7: Comparative analysis of benign/malignant lesions with other studies.

	Kar, et al. [5]	Jha, et al. [6]	Present study
Benign	57%	79%	64.2%
Borderline	9%	0%	10.0%
Malignant	34%	21%	25.8%
Total	100%	100%	100%

Table - 8: Comparative analysis of the various histological types with other studies.

	Kar, et al. [5]	Jha, et al. [6]	Maheshwari, et al. [7]	Present study
Serous tumors	70%	68%	58 %	63.3%
Mucinous tumours	24%	32%	36%	36.7%
Total	100%	100%	100%	100%

In the present study, we have studied the expression of p53 in malignant surface epithelial ovarian tumors by immunohistochemistry using monoclonal antibody against p53 protein (clone DO-7; Dako). In the light of the literature and controversy that exists regarding the expression of p53 in various surface epithelial ovarian tumors, this study was planned to study the expression of p53 in these tumors.IHC was performed on 31 malignant surface epithelial ovarian tumors which included 11 cases of serous cystadenocarcinoma and 20 cases of

mucinous cystadenocarcinoma. Among the serous type, 7 cases (63.6%) were positive and among the mucinous type, 4 cases (25%) were positive. Positivity rates in our study are high as compared to Pde Graff, et al. [9] and J.R.Mark, et al. [11] (**Table - 9**). This may be due to heterogenesity of the lesion, inter observer variability in interpretation of slides and technical problems with antigen retrieval.

Table – 9: Comparative analysis of the results of p53 immunostaining with other studies.

Author	Total cases	Method of evaluation	No. +ve cases	% of +ve cases
Psyrri, et al. [8]	120	AQUA	98	81.6
Pde Graff, et al. [9]	476	IHC of TMA	248	52.1
Ayadi, et al. [10]	57	IHC	42	73.6
J.R.Marks,et al. [11]	107	IHC	54	50.4
Kuprjanczyk, et al. [12]	38	IHC, SSCP	26	68
Reles, et al. [13]	178	IHC	110	62
Present study	31	IHC	Serous – 7 Mucinous – 4 Total - 11	63.6% 25%

Among the malignant surface epithelial ovarian tumors, positivity was high in serous type compared to the mucinous tumors. Positivity rates varied with histologic type, grade and stage of the tumor. In our study, benign tumors were excluded from immunostaining with p53 because review of literature strongly shows normal expression of p53 in benign lesions of the ovary.

To clarify the effects of specific mutations, larger cohorts must be studied. Analysis by mutations at critical functional sites such as DNA binding residues or protein folding sites may be useful in this regard.

Conclusion

Benign tumors were more common than malignant tumors. Age group of these tumours ranged from 16 years to 69 years. The age group of benign tumors ranged from 16 years to 69 years. The age group of malignant tumors ranged from 25 years to 68 years. Bilateral tumors were mostly malignant. Endometrioid tumors and clear cell tumors were the rarest among the surface epithelial tumors. p53 expression was high in malignant lesions compared to benign lesions. There are differences in the prevalence of p53 abnormalities based on the histologic type. p53 over expression was higher in serous tumors compared to mucinous tumors. For invasive carcinomas, the rate of abnormalities of p53 increases with grade of the tumor. Further studies are warranted to clarify the role of p53 in ovarian tumorigenesis. Standardisation of methods used to store paraffin-embedded tumor tissue and perform IHC analysis, the use of tumor tissue obtained in clinical trials with clearly defined end points and clearly defined, stringent, inclusion criteria, may further elucidate the prognostic impact of p53 immunostaining in the future.

References

1. Muir CS, Nectoux J. International patterns of cancer. In Cancer epidemiology and prevention. 2nd edition. Edited by Schottenfeld D, Fraumeni JF. New York: Oxford University Press; 1996, p. 141-67.
2. Kristensen GB, Trope C. Epithelial ovarian carcinoma. Lancet, 1997; 349(9045): 113-117.
3. Runnebaum IB, Stickeler E. Epidemiological and molecular aspects of ovarian cancer risk. J Cancer Res Clin Oncol., 2001; 127(2): 73-79.

4. Greenblatt MS, Bennett WP, Hollstein M, et al. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res., 1994; 54: 4855–4878.
5. Kar T, Kar A, Mohapatra PC. Intraoperative cytology of ovarian tumours. JObstet Gynecol India, 2005; 55: 345-9.
6. Jha R , Karki S . Histological pattern of ovarian tumors and their age distribution. Nepal Med Coll J., 2008; 10: 81-5.
7. Maheshwari V, Tyagi SP, Saxena K. Surface epithelial tumours of the ovary. Indian J Pathol Microbiol., 1994; 37: 75-85.
8. A. Psyrri, P. Kountourakis, Z. Yu, C. Papadimitriou, S. Markakis, R.L. Camp, Economopoulos T, Dimopoulos MA. Analysis of p53 protein expression levels on ovarian cancer tissue microarray using automated quantitative analysis elucidates prognostic patient subsets. Annals of Oncology, 2007; 18(4): 709-15.
9. P de Graeff, J Hall, APG Crijns, GH de Bock, J Paul, KA Oien. Factors influencing p53 expression in ovarian cancer as a biomarker of clinical outcome in multicentre studies. British Journal of Cancer, 2006; 95: 627 – 633.
10. Lobna Ayadi, Salma Chaabouni, Abdelmajid Khabir, Habib Amouri, Saloua Makni, Mohamed Guermazi, Mounir Frikha. Correlation Between Immunohistochemical Biomarkers Expression and Prognosis of Ovarian Carcinomas in Tunisian Patients. World J Oncol., 2010; 1(3): 118-128.
11. Marks J.R., Davidoff A.M., Kerns B.J., Humphrey P.A., Pence J. C., Dodge. Overexpression and mutation of p53 in epithelial ovarian cancer. Cancer Res., 1991; 51: 2979–2984.
12. Jolanta Kupryjanczyk, Ann D. Thor, Roberta Beauchamp, Victor merritt, Susan M. Edgerton, Debra A.bell. p53 gene mutations and protein accumulation in human ovarian cancer. Proc. Natl. Acad. Sci. USA, 1993; 90: 4961-4965.
13. Angela Reles, Wen H. Wen, Annette Schmider, Conway Gee, Ingo B. Runnebaum. Clinical Cancer Research, 2001; 7: 2984–2997.
14. Frebourg T, et al. Germ-line p53 mutations in 15 families with Li-Fraumeni syndrome. Am J Hum Genet., 1995; 56: 608.
15. Murray-Zmijewski , et al. A complex barcode underlies the heterogenous response of p53 to stress. Nature Rev Med., 2008; 9: 702.
16. Robbins and Cotran Pathologic Basis of Disease, 8th edition, Molecular basis of cancer, tumor suppressor gene p53, Elsevier.
17. Righetti SC, Della TG, Pilotti S, Menard S, Ottone F, Colnaghi MI. A comparative study of p53 gene mutations, protein accumulation, and response to cisplatin-based chemotherapy in advanced ovarian carcinoma. Cancer Res., 1996; 56: 689–693.
18. Eltabbakh G. H., Belinson J. L., Kennedy A. W., Biscotti C. V., Casey G., Tubbs R. R. p53 overexpression is not an independent prognostic factor for patients with primary ovarian epithelial cancer. Cancer (Phila.), 1997; 80: 892–898.
19. Marx D, Meden H, Ziemek T, Lenthe T, Kuhn W, Schauer A. Expression of the p53 tumour suppressor gene as a prognostic marker in platinum-treated patients with ovarian cancer. Eur J Cancer, 1998; 34(6): 845-850.
20. Hartmann L. C., Podratz K. C., Keeney G. L., Kamel N. A., Ed- monson J. H. Grill. Prognostic significance of p53 immunostaining in epithelial ovarian cancer. J. Clin. Oncol., 1994; 12: 64–69.
21. Henriksen R., Strang P., Wilander E., Backstrom T., Tribukait B., Oberg K. p53 expression in epithelial ovarian neoplasms: relationship to clinical and

pathological parameters, Ki-67 expression and flow cytometry. Gynecol. Oncol., 1994; 53: 301–306.

22. Levesque M. A., Katsros D., Yu H., Zola P., Sismondi P., Giatdina G., Diamandis E. P. Mutant p53 protein overexpression is associated with poor outcome in patients with well or moderately differentiated ovarian carcinoma. Cancer (Phila.), 1995; 75: 1327–1338.
23. Kohler M. F., Kerns B. J., Humphrey P. A., Marks J. R., Bast R. C., Berchuck A. Mutation and overexpression of p53 in early- stage epithelial ovarian cancer. Obstet. Gynecol., 1993; 81: 643–650.
24. Allan L. A., Campbell M. K., Milner B. J., Eccles D. M., Leonard R. C. F. Parkin. The significance of p53 mutation and overexpression in ovarian cancer prognosis. Int. J. Gynecol. Cancer, 1996; 6: 483–490.
25. Sheridan E, Silcocks P, Smith J, Hancock BW, Goyns MH. P53 mutation in a series of epithelial ovarian cancers from the U.K., and its prognostic significance. Eur J Cancer, 1994; 30A(11): 1701-1704.