Predictive and Prognostic Relevance of p53 in Patients With Serous Epithelial Ovarian Cancer

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Abstract:

Background: Ovarian cancer is a common malignant gynecological tumor that is difficult to diagnose early, progresses rapidly, and causes high mortality. Aims of our study: to assess the relationship of P53 with other clinico-pathological parameters and the effect of P53 on patients' out-come.

Subjects and methods: This study was conducted at Clinical Oncology and Nuclear Medicine Department with Pathology Department, Faculty of Medicine, Mansoura University. The study was carried out on 50 patients with serous epithelial ovarian cancer presented to receive adjuvant treatments following a primary surgery and were followed from the first day after surgery, follow-up started on January, 2012 till February, 2016. P53 expression was assessed immunohistochemically on formalin-fixed paraffin-embedded tissues, and Secondary red Envision system. Patients were given adjuvant treatment(s) according to according to NCCN guidelines. The primary endpoint of the study was loco regional recurrence, and distant metastasis. At the end of the follow-up period, the patient clinico-pathological data and patient outcome were collected.

Results: we found that p53 negative tumors have a better OS & DFS at 3 year than with P53 positive tumors, but not reached statistically significant differences (p value = 0.98 & 0.48 respectively).

Conclusion: Our results showed no statically significant difference between p53 expression. OS & DFS, need to be evaluated in other study including large number of patients before using it as a marker for the outcome in these tumors.

Key words: Disease-free survival, ovarian cancer, p53, Prognostic markers.

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Introduction:

Ovarian cancer is one of the commonest malignant gynecological tumors, which is difficult to diagnose early, progresses rapidly, and causes high mortality. [1]. About 90% of ovarian malignancies are epithelial ones as regards the histological type and other rare histological types. Between all gynecological tumors, ovarian cancer has the highest mortality rate and the worst prognosis. **[**1].

In ovarian cancer, a number of factors are considered prognostic; these factors include patient's age, residual tumor size, histological type, grade and staging [2]. The most commonly studied putative molecular biological prognostic factors in ovarian malignancy are the tumor suppressor protein 53 (p53), the oncogenes epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER-2/neu). Results of a novel meta-analysis showed that p53, EGFR, and HER-2/neu immunostainings did not have a strong direct relationship with survival, although likely their respective pathways can affect patient prognosis [3].

p53 tumors suppressor protein, also called "the guardian of the genome" was initially recognized as an oncogenic protein, in complex with viral proteins [4]

Later, this transcription factor was considered to be important for the prevention of tumors formation, according to its ability to stimulate apoptosis [5].

Somatic mutations in p53 are found in about 50% of the cancers, positioning p53 as the most frequently mutated gene in human malignancies [6]. Commonly p53 is a 393 amino acid protein. It is comprised of three domains, defined as independently folding units, which are connected to each other by proline-rich linker regions.

The N-terminus domain is referred to as the transactivation domain (residues 1-63), and is followed by the first proline-rich region (residues 64-92).

The DNA-binding domain (residues 100-293), the largest and the most frequently mutated unit, is situated in the middle section of the protein and is flanked by proline-rich regions at each side. The second proline-rich domain (residues 294-323) links the DNA-binding domain to the tetramerization domain (residues 324-355). Finally, the C-terminus of the protein is called the basic region (residues 356-393)[7].Many promising researches have suggested that p53-dependent apoptosis is the major function needed for tumors suppression in vivo. Many years ago it was revealed that a reduction in p53-induced apoptosis correlated with the development of aggressive tumors emergence. Another recent research has emphasized on the significance of p53's ability to stimulate apoptosis in preserving a tumor-free state, a marked acceleration of tumors onset developed when p53 function was suppressed [8].

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Mutations of TP53 are the most frequently documented abnormalities in human cancer [9]. It is well known that more than 50% of a diverse group of cancers harbor mutant p53 proteins. The majority (90-95%) of these p53 mutations occur in the DNA binding domain of the protein and these alterations lead to functional inactivation of p53. When the function of p53 is lost, cells become vulnerable and can accumulate more DNA damage, such as mutations, gene amplification and chromosomal rearrangements. From a clinical point of view, functionally inactivated p53 results in resistance to chemo- and radiotherapy due to loss of apoptotic competence. [10]. P53 mutations are usually accompanied with a significantly shorter overall survival in comparison with the wild-type p53 sequence [11]

Predictive value of p53: Cell culture experiments revealed that the sensitivity of tumors cells to different chemotherapeutic agents reliant on the successful induction of apoptosis mediated by a functional p53 protein. Thus, loss of p53 can improve resistance to chemotherapy [12]

Subjects and methods:

Patients for the study: This prospective study was performed at Clinical Oncology and Nuclear medicine department, Mansoura university hospital and Surgical Oncology Unit, Oncology Centre - Mansoura University (OCMU) during the period between January 2012 and Feb 2014. The study was approved by the Research Ethical Committee and an informed consent was obtained from each patient before enrollment in the study. Fifty patients with serous epithelial ovarian cancer were enrolled in this study. Complete history and physical examination including performance status was assessed as regards the Eastern Cooperative Oncology Group (ECOG) scale. All cases underwent total hysterectomy, bilateral salpingo-oophorectomy, and omentectomy or debulking surgery (excision of as much gross tumors as can safely be performed). Samples were collected from 50 cases with primary serous epithelial ovarian malignancy. Expression of p53 protein by immunohistochemical staining was studied and compared in relation to patient's age, tumor stage, tumor grade and residual disease. Hematoxylene and eosin stained slides from formalin-fixed paraffin-embedded biopsy blocks were examined. All patients received adjuvant combination chemotherapy (Paclitaxel plus Carboplatin regimen). Paclitaxel was administered by intravenous infusion at a dose of 175 mg/m^2 over 3 hours and Carboplatin was given by intravenous infusion over 30 minutes & dose was calculated at area under the curve 6. Chemotherapy cycle was repeated every 3 weeks and continued for total of 6 cycles.

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Patients follow-up: Patients were followed from the first day after surgery as the start time of follow-up which was in January, 2012 for the 1st patient till the end of the study in the 1st February, 2016 (unless death has occurred earlier).Follow-up visits were scheduled every three months in the initial two years after adjuvant chemotherapy and every six months thereafter.

During the active treatment, patients were placed under close observation to ensure correctness and precision of treatment delivery according to the pre-determined protocols and schedules. After treatment was completed, patients were followed up by history and physical examination and metastatic work up as scheduled.

The primary endpoint of this study was to evaluate the prognostic significant of P53 gene in stage I, II & III serous epithelial ovarian cancer as well as traditional prognostic factors. The secondary end point was to evaluate correlation of P53 gene expression with age, tumor grade, tumor stage & residual disease. Overall survival was calculated from the initial day of study management until death from any cause or last follow-up.

Disease-free survival (**DFS)** is defined as the period of time from diagnosis till appearance of any relapse or distant metastasis.

Statistical analysis: The SPSS 21 version 21 (Armonk, USA) statistics program was used for statistical analysis. Patient's characteristics were presented by descriptive statistics (median, range and frequency). Correlation between p53 expression and clinic-pathological parameters was done using Spearman's correlation. The Spearman's rank-order correlation is used to determine the strength and direction of a linear relationship between two non-normally distributed continuous variables and/or ordinal variables. The Kaplan-Meier method (Kaplan & Meier 1958) was used to calculate Overall Survival & disease-free survival curves, and the log-rank test was used to determine differences in survival. The Cox proportional hazards model was used to calculate hazard ratios and 95% confidence intervals for different parameters in univariate and multivariate analyses. Results were considered statistically significant when P value was < 0.05 at 95% confidence interval.

Results:

The study included 50 female patients.The patients' age at diagnosis ranged from 41 to 70 years with a mean age of 58.7 years ± 8.2(SD). Thirty seven out of the 50 patients (74%) had performance status II according to ECOG & 13 (26%) had PS I.

Cases were followed up for 36 months. At the end of the follow-up period, patient data were collected and statistically analyzed. The most

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common presentation at diagnosis was Abdominal bloating in 26 patients (52%), pelvic & abdominal pain in18 patients (36%), Vaginal bleeding in 4 patients (8 %) & weight loss in 2 patients (4%). Staging was done according to FIGO staging system. Twenty nine patients (59%) had stage II disease, 19 patients (38%) had stage III diseased stage I disease was found in only 2 patients (4%). Immunohistochemical study to assess the expression of p53 protein was performed. P53 expression was negative in 26 patients (52%) and it was positive in 24 patients (48%).

Treatment failure was reported in 23 patients out of 50 patient representing 46% in the whole group. Nine out of 23 patients developed platinum sensitive treatment failure (39.1%) & 14 out 23 patients developed platinum resistance failure (60.9%).

Table (1): Patients and tumors characteristics

Patient Characteristics	Number	Percentage (%)
Age		
< 58	24	48.0
> 58	26	52.0
Age ranged from 41 to 70 Years with median age of 58.7 Years (Standard deviation ± 8.2)		
Performance Status		
PS I	13	26.0
PS II	37	74.0
Symptoms		
Pelvic & Abdominal Pain	18	36.0
Abdominal bloating	26	52.0
Vaginal Bleeding	4	8.0
Weight loss	2	4.0
FIGO Stage		
Stage I	2	4.0
Stage II	29	58.0
Stage III	19	38.0
Tumors Grade		
Grade I	9	18.0
Grade II	13	26.0
Grade III	28	56.0
Residual Disease		
< 2 Cm.	22	44.0
> 2 Cm.	20	40.0
No Data	8	16.0
P53 Expression		
Negative	26	52.0
Positive	24	48.0

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Table (2): Univariate analysis of different prognostic factors

Prognostic Factors	Three years OS	p value	Three years DFS	P Value
Age				
< 58 years	66.3	0.89	51.6	0.63
> 58 years	45.8		53.1	
Tumors Grade				
Grade I	50.8		55.6	0.28
Grade II	50.0	0.25	67.1	
Grade III	67.3		44.2	
Tumor Stage				
Stage I	100	0.20	50	0.91
Stage II	63.1		51.1	
Stage III	47.9		52.6	
Tumor Residual				
< 2 Cm.	80.5	0.036	71.5	0.042
> 2 Cm.	51.2		42.8	
No Data	53.6		25	
P53 Expression				
Negative	59.6	0.98	55.3	0.48
Positive	55.1		48.1	

Table (3): Treatment failure of all patients.

Treatment failure	No	%
Platinum sensitive	9/23	39.1
Platinum resistance	14/23	60.9
Total	23/23	100

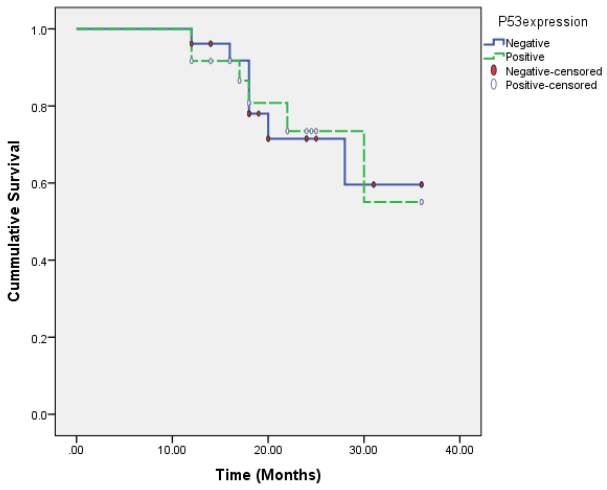

Figure (1): Overall Survival according to (P53 Expression. (p value : 0.98)

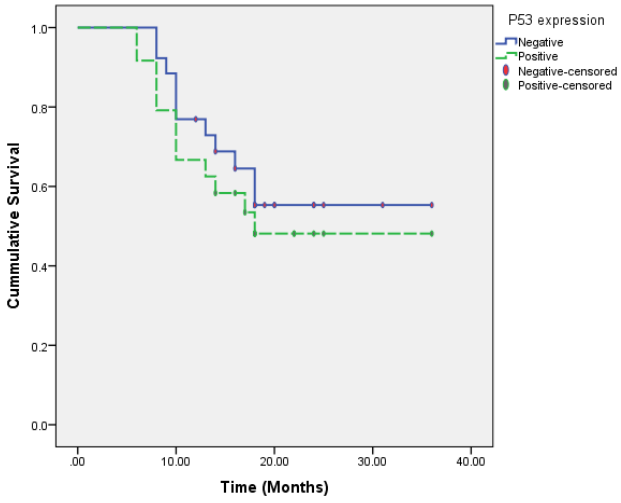

Figure (2): Disease Free Survival according to p53 expression. (p value: 0.48)

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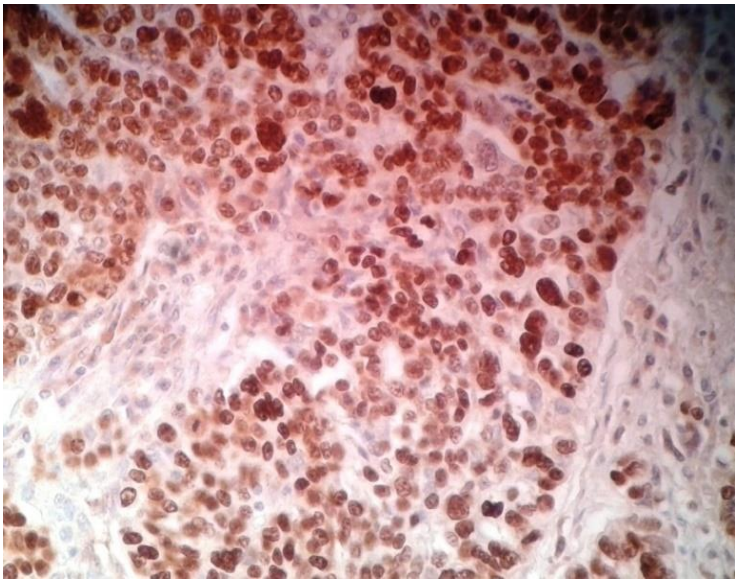

Fig [3]: Higher magnification Photomicrograph of high grade ovarian serous adenocarcinoma of ovary showing strong intensity nuclear p53 staining with expression in more than 90 % of cells (immunoperoxidase x100)

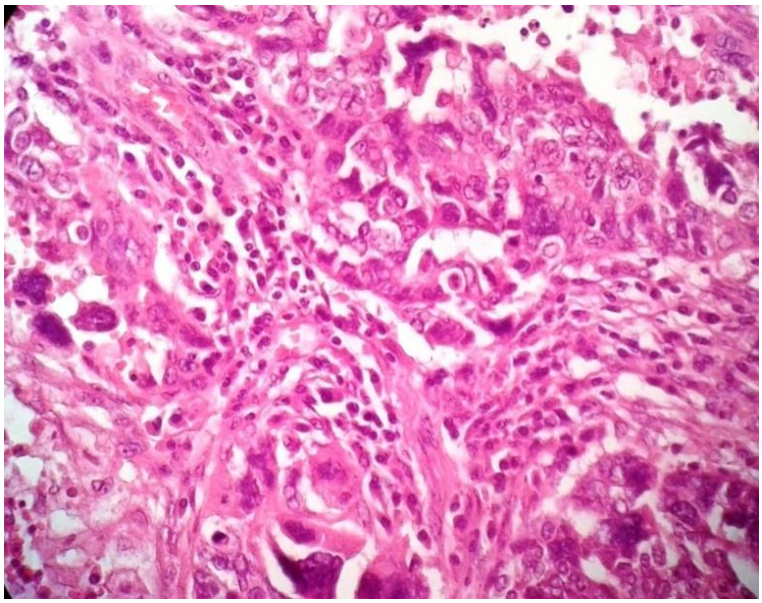

Fig [4]: Photomicrograph of high grade ovarian serous adenocarcinoma (H&E x100)

Discussion:

The aim of the current study was to investigate the prognostic value of p53 expression in tumors samples from 50 cases with serous epithelial ovarian cancer as well as different traditional prognostic factors.

P53 correlation with patient and disease characteristics

In our study we found no significant correlation between P53 expression with the age ($p > 0.05$)

The relationship between p53 expression with age has been previously explored in number of studies. In one of these [13], the authors

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failed to prove any association between p53 and age. Our results were in agreement with that reported by ***Ndukwe* and *de Graeff,***

In this study we found a statistically significant correlation between P53 expression and tumor grade (P <0.001). These results were in agreement with that reported by ***de Graeff,*** and ***Ndukwe***. who found a statistically significant association between p53 positivity and tumor grade ($p < 0.01$). [13].

Again, we did not found any significant correlation between p 53 expression and Tumor stage (P > 0.05). These results coincide with that reported by ***de Graeff, et al.***, who found a non-significantly different between p53 expression & tumor stage (p = 0.85 in the prospective arm, Scottish patients and p = 0.38 in retrospective arm, Dutch patients).

In our study, we did not found any significant correlation between P 53 expression and Residual disease (P > 0.05). These results coincide with that reported by ***de Graeff, et al.***, who found a non-significantly different between P53 expression & residual disease (P = 0.40).

In this study we found a statistically significant correlation between P53 expression and tumors grade (P <0.001).these results was in agree with that reported by ***de Graeff, et al.*** and ***Ndukwe, et al.***.

These two studies discussed the correlation between p53 and histological grade and both found a significant correlation between p53 expression &tumors grade.

There was no significant correlation between p53 expression with the age (p > 0.05). Also, there was not found any significant correlation between p 53expression and residual disease and tumor stage (p > 0.05 for each).

Overall and disease free survival of the whole Group

Cancer statistics often use 5-year OS rate to present a better idea of the longer-term outlook for people with cancer. In this study the OS was 94%, 64.7% & 64.7% at 1, 2 & 3 years respectively. Also the DFS was 67.9%, 51.8% & 51.8% at 1, 2 & 3 years respectively. There is a decline in OS & DFS after first years after diagnosis.

In In this study we found that P53 negative tumors have a better OS & DFS at 3 year than with p53 positive tumors, but not reached statistically significant differences (p value = 0.04 & 0.05 respectively).

p53 Expression

TP53-mutated tumors in general have an aggressive phenotype and are characterized by poor differentiation, increased invasiveness, and high metastatic potential [13]. A considerable high mutation frequency of 50%–100% is recorded in all ovarian malignancies [13].

Mutations in TP53 are infrequently occur in low-grade serous carcinomas or serous

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borderline tumors, while they are ubiquitous in high-grade serous ovarian cancer HGS-OvCa, in which TP53 mutations are recorded in up to 100% of the patients [13].

Conclusion:

Our results showed no statically significant difference between p53 expression OS & DFS. The use of p53 as a prognostic and/or predictive factor in serous epithelial ovarian cancer needs to be assessed in other study including large number of patients before using it as a marker for the outcome in these tumors.

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