DOI:10.21276/sjpm.2017.2.3.3

Saudi Journal of Pathology and Microbiology
Scholars Middle East Publishers
Dubai, United Arab Emirates
Website: http://scholarsmepub.com/

ISSN 2518-3362 (Print)
ISSN 2518-3370 (Online)

Original Research Article

Immunohistochemical Study of P53 and Ki 67 Expression in Surface Epithelial Tumor of the Ovary

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Abstract: Surface epithelial tumors of the ovary account for approximately two-thirds of all ovarian neoplasms and their malignant forms represent about 90% of ovarian cancers. For years, efforts to identify reliable prognostic factors have focused on molecular markers; large number of them has been investigated to date, usually by immunohistochemistry. Mutation of the p53 gene has been reported in a variety of human malignant tumors and it has been claimed to be a marker of poor prognosis. Aim of the study is to assess the immunoexpression of p53 and Ki 67 in surface epithelial tumors of the ovary and to evaluate their correlation with the clinicopathological parameters. There were 60 cases of surface epithelial tumors encountered over the period of 7 years from 2007 to 2014 in addition to10 samples of non-neoplastic ovarian tissues were included as a control. The samples were collected from Hawler Maternity Teaching Hospital. Formalin fixed, paraffin-embedded specimens were studied and sections were prepared for immunohistochemical staining for both p53 and Ki 67 markers. Ethical consent has been obtained from Hawler Medical University. Data were analyzed using the Statistical Package for Social Sciences (SPSS, version 19). High immunoexpression of p53 and Ki 67 were seen in patients with ovarian tumor aged more than 40 years. Statically highly significant correlation was found in the immunoexpression of both p53 and Ki 67 marker between benign, borderline and malignant surface epithelial tumors (P value < 0.05); while no significant correlation was found between their expression in relation to tumor grade and stage in epithelial ovarian cancer. Maximal immunoexpression of p53 and Ki-67 immunohistochemical markers was seen in epithelial ovarian cancer, this emphasizes their important carcinogenic role in surface epithelial tumor of the ovary. Their over expressions are not correlated with tumor grade and stage**.**
Keywords: P53, Ki 67 immunohistochemical markers and ovarian tumors.

INTRODUCTION

Surface epithelial tumors of the ovary account for approximately two-thirds of all ovarian neoplasms, and their malignant forms represent about 90% of ovarian cancers [1]. Epithelial ovarian cancer is the most lethal gynecological malignancy. Due to its lack of symptoms, this disease is diagnosed at an advanced stage when the cancer has already spread to secondary sites. Accordingly, reliable markers that are independent and complementary to clinical parameters are needed for a better management of these patients. For several years, efforts to identify prognostic factors have focused on molecular markers, with a large number having been investigated [2].

Surface epithelial tumors are classified based on tumor cell type (serous, mucinous, endometrioid, clear cell, transitional) and are then further subclassified as benign, borderline or malignant carcinoma [3].

Different subtypes of epithelial ovarian cancer reflect a heterogeneous group of diseases, and their determination is relevant for prognostication and treatment prediction [4,5]. A correct classification of histological subtype is important while clear cell, endometrioid and mucinous carcinomas commonly present with stage I or II disease, high-grade serous carcinoma often present in more advanced clinical stages. Serous carcinoma is the most common type of ovarian cancer, accounting for 68% of ovarian cancers in one large population-based study [6].

The distinction between borderline versus carcinoma is utmost significance for prognostic purposes [7]. Borderline tumors have a favorable prognosis, even in advanced stages [8].

Mutations in the p53 gene and subsequent gene product have been related to most cancer types [2]. Wild-type p53 protein has a very short half-life and thus

the protein level is too low to be identified immunohistochemically. In contrast, mutant p53 proteins have a longer half-life and can be easily detected by immunohistochemical methods [9]. Although P53 is the most frequently studied molecular biological parameter in epithelial ovarian cancer, but their prognostic impact is still unequivocal [10].

Uncontrolled proliferation is a driver for cancer and is one of the hallmarks of this disease [11]. Among proliferation markers, Ki67 is the most studied marker in cancer research. It is a nuclear protein expressed in proliferating cells during the G1, S, G2 and M cell cycle phases and is absent in quiescent cells (phase G0) [12].

The aim of the study, to assess the expression of p53 and Ki 67 proteins in surface ovarian tumor in an attempt to investigate their involvement in ovarian carcinogenesis and to find out whether a correlation exists between overexpression of these markers and histopathological parameters.

MATERIAL AND METHODS

The data has been recruited retrospectively from the medical record of Hawler Maternity Teaching Hospital for the period from 2007 to June 2014, a total of 60 formalin fixed, paraffin-embedded surface epithelial tumors of ovary specimens were studied , in addition 10 samples of non-neoplastic ovarian tissues were included as a control . Sections were prepared for IHC staining for both p53 and Ki 67 markers.

For all cases 4µm histological sections were deparaffinized in xylene and rehydrated in descending dilutions of ethanol and were mounted on positively charged glass slides for immunohistochemical staining and subjected to antigen retrieval. Only nuclear p53 and Ki 67 expressions were accepted as specific reactions.

Immunohistochemical Interpretation:

Evaluation of paraffin IHC was performed by three reviewers. The distribution of p53 immunoreactivity in surface epithelial tumors of ovary were quantitatively assessed as –ve (less than 10% are negative cells) and +ve (equal or more than 10% are positive cells) and the positive cases were graded as + (10-30%) ; ++ (30-50%) ; and +++ (more than 50%) are positive cells [13].

The distribution of Ki-67 immunoreactivity in surface epithelial tumors of ovary were quantitatively assessed as -ve (less than 1% are negative cells) and +ve (equal or more than 1% are positive cells) and the positive cases were graded as + (1-30%) ; ++ (30-50 %); and +++ (more than 50%) are positive cells [14].

Positive cells determined by counting 1000 cells in at least 10 HPF were measured for each case Luminita *et al* [15].

Non-surface ovarian epithelial tumor has been excluded from the study. The institutional ethical committee approved the study.

STATISTICAL ANALYSIS

Data were analyzed using the Statistical Package for Social Sciences (SPSS, version 19). Chi square test of association was used to compare between proportions in the study. P value of ≤ 0.05 was considered as statistically significant.

RESULTS

The mean age was (51) for malignant tumors, (46) for borderline tumors and (32) for benign tumors. The immunostaining of both p53 and Ki 67 showed more frequent expression in patients with ovarian tumor aged more than 40 year as shown (figure 1).

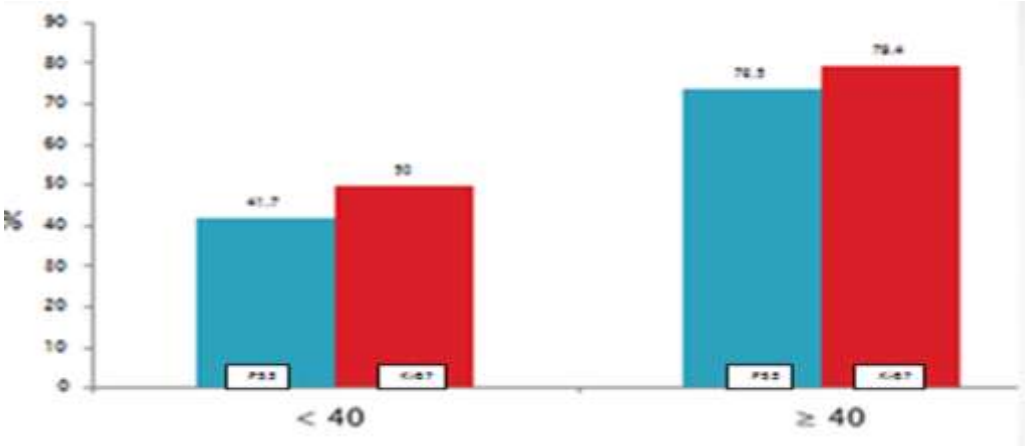

Fig-1: showing correlation of P53 and Ki67 in relation to the age

The frequency of p53 overexpression in all surface epithelial tumors was (51%).

There was a significant correlation between both these IHC markers overexpression with the biological tumor behavior as p53 are differently expressed with maximal value (90%) in malignant surface epithelial tumors compared with borderline tumors (20%) and benign tumor (35%) while the frequency of Ki 67 expression was (100),(60),(10) respectively (figure 2).

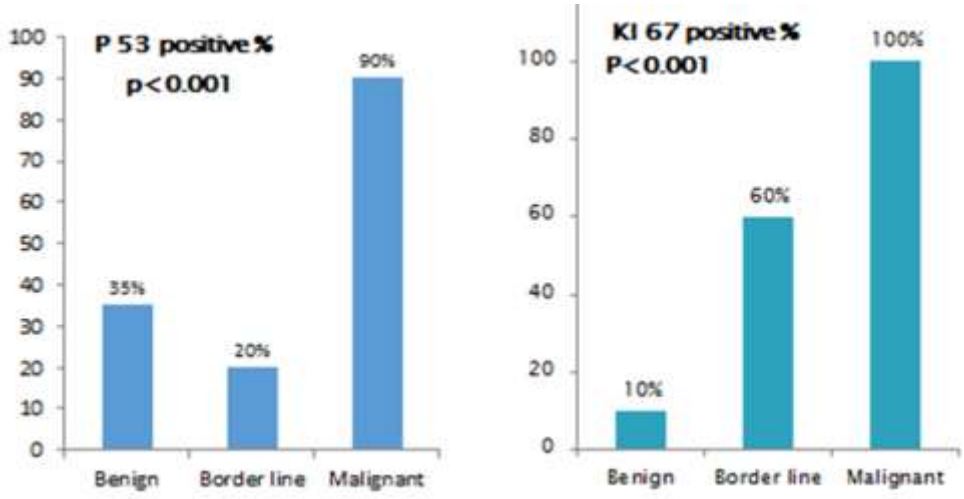

Fig-2: Correlation of P53 and Ki67 expression with biological behavior of surface epithelial tumors of the ovary

The distribution of positive p53 immunoreactivity among various histological malignant surface epithelial tumors of ovary was studied. There are differences in the rate of p53 abnormalities ,the highest frequency was recorded with highly statistical significant difference among mucinous cyst adenocarcinoma (100%) and endometrioid carcinoma (100%), followed by serous cyst adenocarcinoma (88.9%), while no p53 expression among clear cell carcinoma were recorded (table 1& 2).

Table 1: Distribution of p53 immunoreactivity in various histological subtypes of surface epithelial tumors of ovary

Histological types of ovarian tumors	Benign Positive P 53	Borderline Positive P 53	Malignant positive P 53	p-value
Serous tumors	2(33.3%)	2(33.3%)	8(88.9%)	0.056*
Mucinous tumors	0(0%)	0(0%)	9(100%)	0.000***
Endometrioid tumors	3(50%)	0(0%)	10(100%)	0.036**
Brunner tumors	2(100%)	0(0%)	0(0%)	
Clear cell ca.	0(0%)	0(0%)	0(0%)	

Table 2: Expression of P53 according to various histological types of malignant surface epithelial tumors of ovary.

Malignant histological types of tumors	P53 Positive	P53 Negative	Total
Serous cyst adenocarcinoma	8 (88.9%)	1 (11.1 %)	9 (100%)
Mucinous cyst adenocarcinoma	9 (100 %)	0 (0 %)	9 (100%)
Endometrioid carcinoma	10 (100%)	0 (0 %)	10 (100%)
Clear Cell Carcinoma	0 (0 %)	2 (100 %)	2 (100%)
Total	27 (90 %)	3 (100 %)	30 (100%)

P<0.01

In the present study, ki-67 was expressed (54.3%) of all surface epithelial tumors of the ovary including (57.1%) of serous tumors, (63.2%) of mucinous tumors, (68.8%) of endometrioid tumors, (50%) of Brenner tumors, (100%) of clear cell carcinoma and (0%) of control cases (non neoplastic ovarian tissue) as shown in (table 2).

P53 and Ki 67 immunostaining in different histological types of surface epithelial tumors presented in (figure 3 &4).

Table 3: Distribution of ki-67 immunoreactivity in various histological subtypes of surface epithelial tumors of ovary

Histological types of ovarian tumors	Benign Positive Ki-67	Borderline Positive Ki-67	Malignant Positive Ki-67	p-value
Serous tumors	0(0%)	3(50%)	9(100%)	0.000***
Mucinous tumors	0(0%)	3(75%)	9(100%)	0.000***
Endometrioid tumors	1(16.7%)	0(0%)	10(100%)	0.000***
Brunner tumors	1(50%)	0(0%)	0(0%)	
Clear cell ca.	0(0%)	0(0%)	2(100%)	

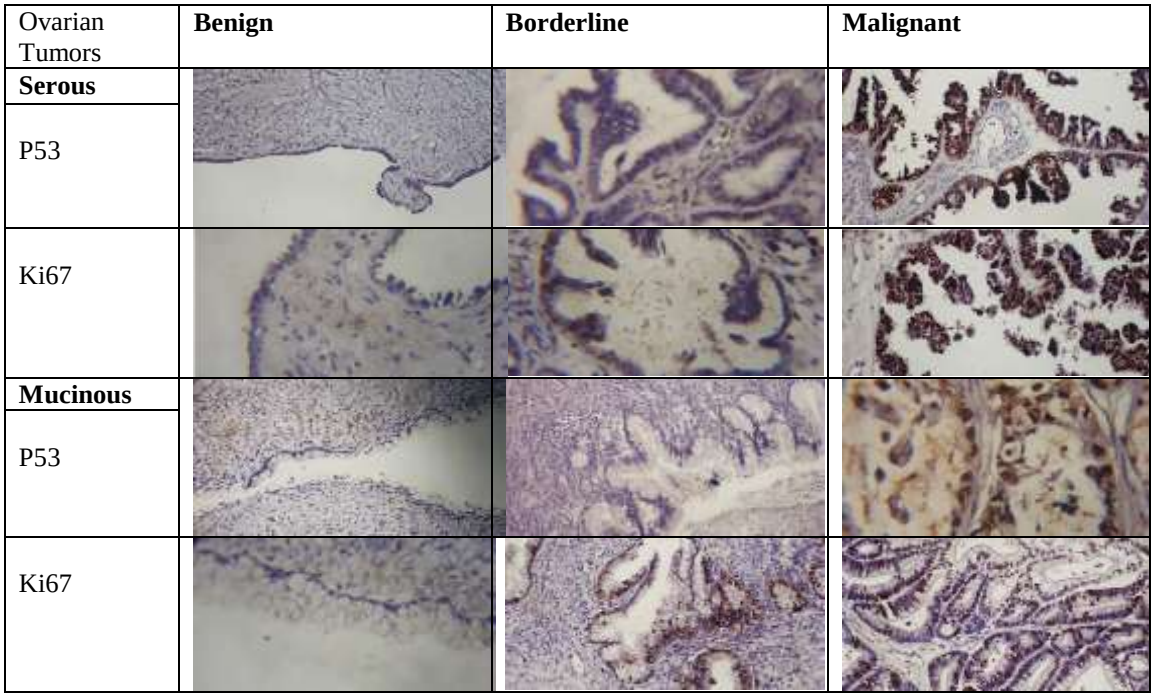

Fig-3: showing p53 and Ki67 IHC staining of Serous and Mucinous Tumors of ovary

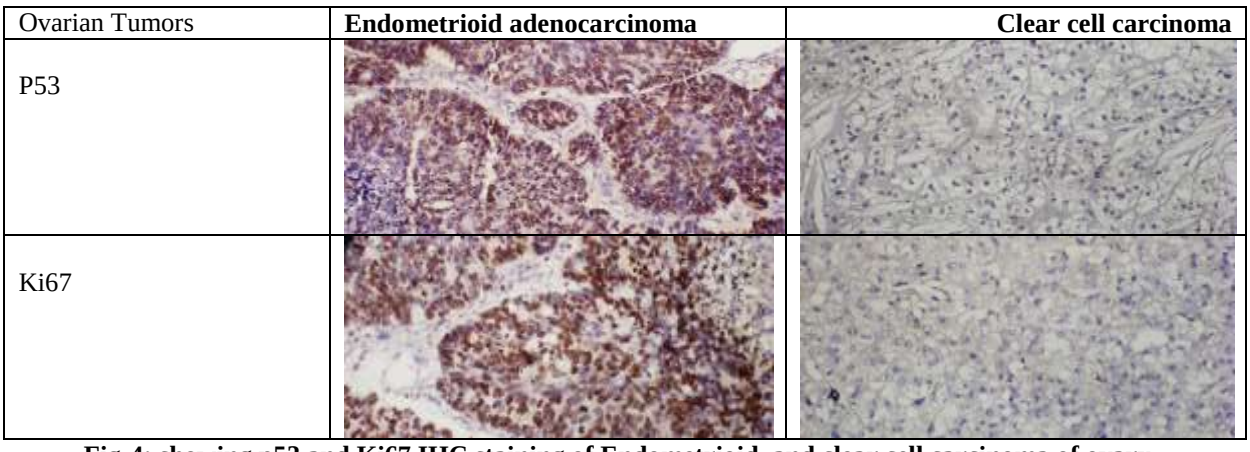

Fig-4: showing p53 and Ki67 IHC staining of Endometrioid and clear cell carcinoma of ovary

There was a linear increase between these two immunohistochemical markers as with high p53 overexpression, there was a tendency toward a higher expression of Ki 67, their co-expression was seen in 80% of cases (table 3).

Table 4: The correlation between p53 and Ki67 immunoexpression in all cases of surface epithelial tumors of ovary

P53	Ki-67 Negative		Ki-67 Positive		Total	
	N	%	N	%	N	%
P53 Negative	17	56.7	13	43.3	30	100
P53 Positive	8	20	32	80	40	100
Total	25	35.7	45	64.3	70	100

The expression of p53 and Ki67 IHC markers were assessed for all cases of epithelial ovarian cancer in relation to tumor grade and stage which showed no statistical significant differences. Ki 67 scoring analysis was high across all grades, although there was relative more frequent overimmunoexpression in grade II, III in comparison with grade I.

DISCUSSION

The mean age in our studied samples was higher for malignant cases, this can be explained by well-known fact that the frequency of cancer increases with age, which can be explained by the accumulation of somatic mutation associated with emergence of malignant neoplasm [16].

Comparable results were done by Luminita *et al* [15] who showed the average age for malignant tumors was 59 years and for borderline tumors was 43 years.

In the present study we found increasing age more than 40 years associated with increase expression of both p53 and Ki 67, this has been supported by other studies on the base of loss of heterozygosity on chromosome 17 increases with age [17].

The promoter of MDM2 (Murine Double Minut2) and p53 interaction partner contains a functional estrogen receptor signal in the DNA, therefore, the effect of the p53 on risk of cancer in women could depend on menopausal status [18].

The estimated frequency of p53 positive immunoreactivity in the all ovarian surface epithelial tumor samples analyzed in the present study was (51%), and in carcinoma was 90%, the comparative analysis with other studies shows variable results. Ranging from 14-75 % [19].

Table 5: Comparative analysis of the results of p53 immunostaining with other studies

Author	No. of cases	Percentage of positive cases %
Pysrri *et al* [20]	141	81.6%,
Hamdi E Ab *et al* [21]	60	80.8%
Ayadi *et al* [22]	57	73.6%
Kuprjanczyk *et al* [23]	38	68%
Present study	60	90.6

The possible sources for this variation may be due to number of the sample analyzed , also may be attributed to the properties of different antibodies, the scoring methods applied for p53 immunoreactivity [24].

The correlation of P53 with various histological subtypes of surface epithelial tumors was significant. The comparative analysis of the current study with other authors showed similar results done by Hamdi [21] Nielsen *et al* [24], while Gursan *et al* [25] found most significant in serous carcinoma.

In the study of Ayadi *et al* [22], there was no significant difference in expression of P53 between serous and non-serous tumors (p=0.84).

Studying of p53 status in malignant cell has important practical therapeutic implications. Recently 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 [26].

In the current study the maximal value of immunoexpression of p53 and Ki67 marker was found in most histological type of malignant surface epithelial tumors showed significant positive p53 expression except in clear cell carcinoma may be due to limited number of cases.

The overexpression of p53 protein was a common feature in invasive epithelial ovarian cancers while was lower frequency rate of expression in benign and borderline tumors. Sylvia *et al* [27] also showed a higher p53 expression in malignant tumors. Kmet *et al* [28] found that the estimated prevalence of p53 mutation as 45%, 5%, and 1%, respectively, for invasive, borderline and benign tumors.

The rarity of p53 among benign ovarian tumors compared with increasing prevalence of these abnormalities among carcinoma might be taken as evidence that ovarian carcinogenesis follows a

multistep model [14, 16].

Korkolopoulou *et al* [12] observed that Ki 67 is overexpressed in malignant tissues compared to benign or borderline tissues. Similar results were done by Luminita *et al* [15]. Other studies with different frequency ki-67 expression Heeran *et al* [29].

The higher frequency rate of co-expression was found between these two IHC markers, this pattern of association and concordance may indicate that these tumor markers may run in parallel manner in relation to tumor behavior and similar results has been observed by Gursan *et al* [24] .

Conflicting results has been recorded regarding these IHC marker in relation to tumor grade and stage in ovarian cancer. In the present study we noticed that their expression showed no significant correlation but there was only mild degree of overexpression in high-grade tumors, this may be attributed to small sample size. Our results are comparable with other studies done in Saudia Arabia [30].

In contrast to studies obtained from Poland [31], UK [32] and Denmark [24] , which reported significant correlation with higher grades.

In an effort to detect the prognostic value of p53 in ovarian cancer, de Graeff *et al* [32] found that their prognostic value with respect to survival is still inconclusive.

Assessment of p53 status through meta-analysis study reported an association with poor prognosis in most but not in all studies [31]. Also the prognostic value of Ki-67 index in ovarian cancer have posted controversial results [34, 35].

In reviewing the literature and controversy that exists regarding their expression in various surface epithelial ovarian tumors, this variability and apparent inconsistencies is a feature of most of the biomarkers; may be due to a number of factors which could affect the percentage of positivity which been explained at least in part by technical problems with antigen retrieval and biological factors [36]. Other explanation was the access to appropriate patients can be limited, as most studies have analyzed only a small number of samples. Also due to the inter-individual biological variability and heterogeneity of ovarian tumor tissues and interobserver variability in interpretation of slides [37, 38].

CONCLUSION

Both p53 and Ki-67 immunohistochemical markers are more frequently overexpressed in patients with surface epithelial ovarian tumors aged over 40 years.

Maximal expression was seen in malignant tumor, this emphasize their importance in the pathogenesis of epithelial ovarian cancer and suggest a relevant role in the progression to the invasive phenotype and therefore immunohistochemistry is a good screening method that can be used to predict malignant versus proliferative tumors. No significant correlation was found between p53 and ki-67 with tumor grade and stage.

RECOMMENDATIONS

Larger cohorts must be studied to clarify the role of p53 in ovarian tumorigenesis and their real prognostic impact in patients with surface epithelial ovarian cancer.

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