Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq

Immunohistochemical Expression of CD44v6 and P53 Status in Borderline and Malignant Ovarian Surface Epithelial Tumors. A Clinico-Pathologic Study.

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Abstract

Background Ovarian epithelial cancer is a leading cause of death among gynecological malignancies due mainly to advanced stage at presentation.

Objectives To investigate the expression of CD44v6 and p53 in borderline tumors and malignant ovarian surface epithelial tumors, correlation with clinic-pathological parameters.

Methods A cross sectional study included a total of (101) formalin-fixed paraffin-embedded ovarian tissue blocks; of which (19) cases were borderline tumors and (82) cases were overt ovarian carcinomas. Sections from each block were immunohistochemically stained for CD44v6 and p53.

Results The expression of CD44v6 was higher in ovarian carcinomas (57.3%) than borderline tumors (26.3%) and it was significantly correlated with FIGO stage and histological grade of ovarian carcinomas. p53 was overexpressed significantly in invasive carcinoma compared to borderline tumors, and it was significantly associated with higher grade and FIGO stage of invasive carcinomas.

Conclusions CD44v6 and p53 expressions were correlated with less differentiated, advanced-stage tumor and these markers may be important molecular markers for poor prognosis.

Key words Borderline tumor, ovarian carcinoma, p53, CD44v6.

List of abbreviations: FIGO = International Federation of Gynecology and Obstetrics, IHC = immunohistochemical, SI = staining index.

Introduction

Ovarian cancer represents one of the most common malignant conditions in adult women [(1)]. In Iraq it rank 6th among top 10 cancers affecting female according to the consecutive reports of the Iraqi cancer board in 2008 and 2011 [(2)].

Surface epithelial tumors represent about 90% of primary ovarian cancers [(3)] they may be solid, cystic or mixture of both may be benign, borderline or malignant [(4)]. These tumors represent 15% of all epithelial ovarian cancers, with the serous and mucinous types making up the vast majority of cases [(5)]. Over 75% of ovarian cancer patients had already developed metastases when they were first diagnosed since it often results in poor prognosis [(6)].

Adhesion processes are involved in all levels of the metastatic cascade. Most of the cell adhesion molecules including CD44 family have played a great role in various stages of tumor progression and metastasis [(7)].

CD44v6 is an important isoform of CD44 family. It is a transmembrane glycoprotein widely distributed among different tissues and is a receptor of the extracellular matrix component hyaluronic acid [(8)].

It has been established that CD44v6 plays role in tumor development and progression in a variety of human cancers including ovarian cancer [(9)].

The p53 tumor suppressor gene mutation is the most common genetic aberration in human malignancy, including ovarian carcinomas [10]. In contrast to borderline tumors where p53overexpression is a rare event, p53 is the most commonly identified somatic genetic alteration in invasive ovarian carcinomas [11]. Cancers with p53 mutation demonstrated a trend toward more aggressive tumor behavior such as poor cellular differentiation and distant metastasis [12].

The aim of this study is to evaluate the immunohistochemical expression of CD44v6 and p53 in borderline and malignant ovarian surface epithelial tumors and to correlate these expressions with clinicopathological parameters (FIGO stage and grade).

Methods

This cross sectional study was approved by Institute Review Board of the College of Medicine, Al-Nahrain University. The collection of the samples last for the period from Mar. 2014 to Feb. 2015. A total of one hundred and one formalin fixed paraffin embedded ovarian tissue of which sixty cases were of serous carcinoma, ten cases were of endometrioid carcinoma, eight cases were of mucinous carcinoma, two were of clear cell carcinoma and two cases were malignant Brenner.

Moreover, nineteen cases of borderline tumor, of which fourteen cases with serous differentiation and five cases of mucinous type were retrieved from the histopathology archive of the Teaching Laboratories in the Medical City, Al-Yarmok Teaching Hospital and Al-Imamain Al-Kadhimyin Medical City for the period from Jan. 2011 to Dec. 2014.

All the clinic-pathological parameters such as (age; histopathological type of ovarian carcinoma and borderline tumors as well as grade and FIGO (International Federation of Gynecology and Obstetrics) pathological stage of ovarian carcinomas were obtained from patients' admission case sheets and pathology reports. Any sample lacking the clinic-pathological information was excluded from this study.

For each case, one representative (4µ) section was stained with Hematoxylin and Eosin and the histopathological diagnosis was revised, while two (4µ) sections were placed on positively charged slides and stained immune-histochemically using three steps- indirect streptavidin method for monoclonal mouse antibodies including anti CD44v6 antibody, clone (VFF-7) and anti-p53 antibody, clone (BP53-12), both manufactured by Abcam.

Interpretation of the results of immune-histochemical staining

1. CD44v6: Brown membranous &/or cyto-plasmic staining pattern of epithelial cells even if staining was focal in tumor cell is considered positive. Positive control is tonsil. Technical negative control was obtained by omission of primary antibody.

2. p53 protein: Brown nuclear staining is considered positive. Positive control is the lymphoid tissue in non Hodgkin lymphoma. Technical negative control was obtained by omission of primary antibody.

The results of immunohistochemical expression of the above molecular markers were analyzed in a semi-quantitative fashion as follow:

CD 44v6: was scored semi-quantitative by assessing both staining intensity as absent (= 0); weak/moderate (= 1), intense (= 2) and percentage of stained cells (staining ratio) in relation to the total number cells as follows: 0 = no staining; 1 = staining of 1–20% of cells; 2 = staining of 21-50% of cells; and 3 = staining of 51-100% of cells with final staining index range from 0, 2-5 [13].

p53: The interpretation of the p53 staining was based on the percentage of tumor cell nuclei staining and the staining intensity. The percentage of stained cells (staining ratio) was used to score a slide semiquantitatively in one of four categories: (*a*) 1+, 5-25% staining;(*b*) 2+, 26-50% staining; (*c*) 3+, 51-75% staining; and (*d*) 4+, 76-100% staining. Sections with less than 5% tumor nuclei staining were considered

negative. Intensity was graded from weak (1+) to strong (3+). The staining index was calculated for each case as the product of staining intensity and staining ratio (staining index = staining intensity + staining ratio), with final staining index range from 0, 2-7 [14].

Statistical Analysis

Statistical analysis was performed with SPSS V. 16 (statistical package for social sciences) and also Excel 2007 programs. Continuous variables were expressed as mean±SEM (standard error of the mean), while categorical variables were expressed as numbers and percentages. Statistical relations between two categorical variables were tested using Chi-square or Fisher exact tests. Relations between categorical and continuous variables were tested using unpaired t-test and ANOVA. Values were considered statistically significant when p-value < 0.05.

Results

The clinicopathological parameters of ovarian borderline tumors and carcinoma cases included in the present study (Table 1).

Table 1. Clinicopathological parameters of borderline tumors and invasive ovarian carcinoma cases

Parameters		No. (%)
Histopathological diagnosis	borderline tumors	19
	ovarian carcinoma	82
Age (years) Mean range ±SEM	borderline tumors	38.05 ± 2.61 (18-62)
	ovarian carcinoma	57.89 ± 1.13 (30-85)
Histopathological types of ovarian carcinoma	Serous Mucinous Endometrioid Clear cell carcinoma Malignant Brenner	60 (73.2%) 8 (9.8%) 10 (12.2%) 2 (2.4%) 2 (2.4%)
Histopathological types of borderline tumors	Serous Mucinous	14 (74%) 5 (26%)
Grade of ovarian carcinoma	Well-differentiated Moderately-differentiated Poorly-differentiated	30 (37%) 34 (41%) 18 (22%)
FIGO stage of ovarian carcinoma	I II III IV	27 (29%) 18 (53%) 32 (39%) 5 (6.1%)

FIGO = International Federation of Gynecology and Obstetrics

CD44v6 immunohistochemical expression

Of 82 cases ovarian carcinomas, CD44v6 expression was recognized in 47 (57.3%) cases compared to 5 (26.3%) cases of borderline tumors (Fig. 1) this data failed to achieve statistical significance (p > 0.05) (Table 2). Expression of CD44v6 was statistically correlated with histological grade (p = 0.004) (Fig. 2) and FIGO stage of ovarian carcinoma (p < 0.001), but not correlated with age and histological type in both carcinoma and borderline. The correlation found between CD44v6 expression in ovarian carcinoma and clinicopathological parameters are shown in (Table 2).

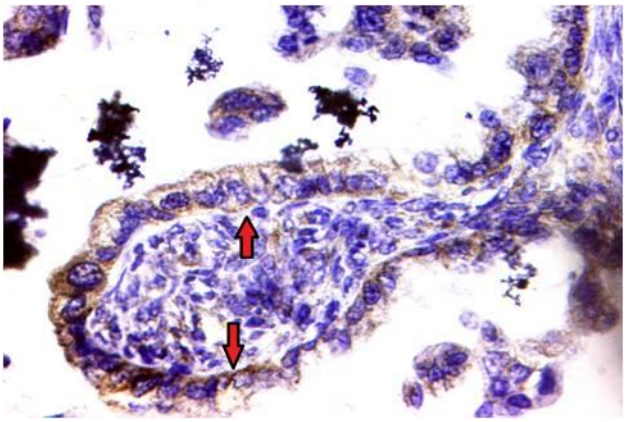

Fig. 1. Borderline serous tumor stained with anti CD44v6 showing positive brown cell membrane (arrows) and cytoplasmic immune-staining with scoring index 3, (40X).

p53 immunohistochemical expression

p53 was expressed immunohistochemically in 47 (57.3%) cases of ovarian carcinomas compared to only 1 (5.3%) case of borderline tumors(figure 4) this difference in expression was significant statistically (p = 0.004), (Table 3). The table also show significant association between IHC expression of p53 with FIGO stage and grade of ovarian carcinomas, (p = 0.01) and (p < 0.001), respectively. The current study failed to express association between IHC expression of p53 with different types of ovarian carcinoma (Fig. 3) and age in borderline tumors or ovarian carcinoma, (Table 3).

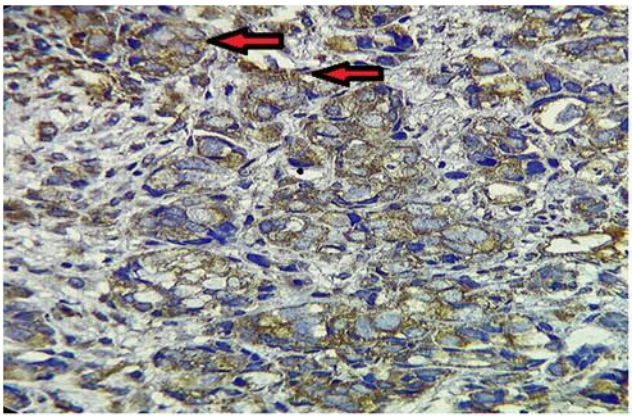

Fig. 2. Poorly differentiated serous carcinoma stained with anti CD44v6 showing positive brown cell membrane (arrows) immune-staining with scoring index 5, (40x).

Discussion

CD44v6 is involved in the production of experimental metastasis. Previous reports have indicated that the overexpression of CD44v6 was correlated with poor prognosis of human cancers [8,15]. FIGO stage and grade are the two most important prognostic factors in ovarian cancers.

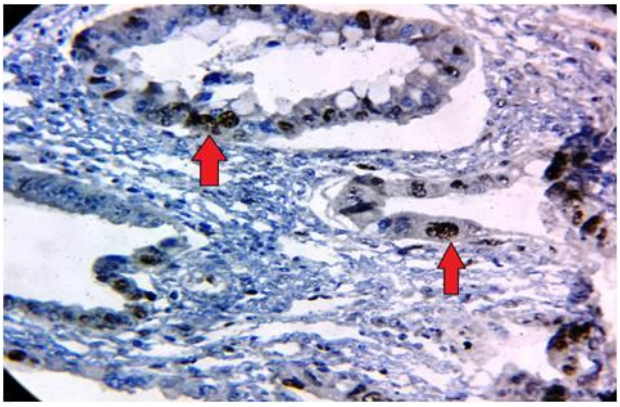

Fig. 3. Mucinous carcinoma stained with anti-p53 showing positive brown nuclear staining with scoring index of 4, (arrows), (40X).

The current study showed significant association between FIGO stages of ovarian carcinoma and CD44v6 immune expression, the expression were increased with higher stage of ovarian carcinoma. These findings were in agreements with Shi *et al* [13], Zhou *et al* [16] and Bian [17].

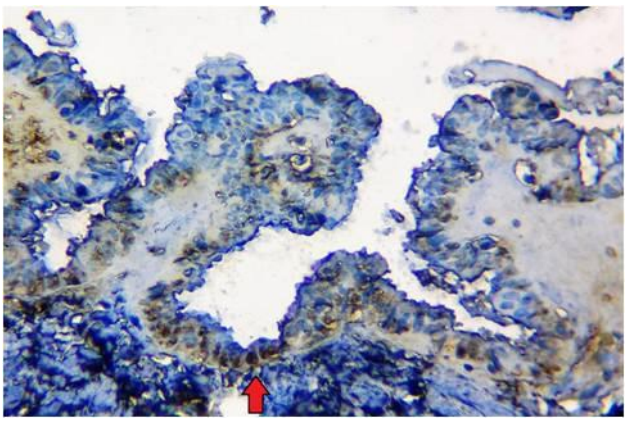

Fig. 4. Borderline serous tumor stained with anti-p53 showing positive brown nuclear staining with scoring index of 3, (arrow), (40X).

The current study also demonstrates increase in CD44v6 expression with loss of differention of malignant cells of in ovarian carcinoma. This tendency for increase CD44v6 expression along with increase grade of ovarian tumor had been demonstrated by several previous studies like Bar *et al* [18], Shi *et al* [13], Zhou *et al* [16] and Ryabtseva *et al* [19]. The positive correlation between histological grade, FIGO stage and expression of CD44v6 may indicate that this marker play an important role predicting the prognosis of patient with ovarian carcinoma. Regarding the expression of CD44v6 in relation to histological types of ovarian tumors, this study showed no statistically meaningful correlation neither among different types of invasive ovarian carcinoma, nor between the 2 subtypes of borderline tumors. This is in agreement with Shi *et al* [13] and Zhou *et al* [16].

Table 2. Association of CD44v6 immunohistochemical expression with clinicopathological parameters of borderline tumors, and ovarian carcinoma

Clinicopathological parameter		CD44v6		p value
		Positive	Negative	
Age (years) mean± SEM	Borderline tumors	42.80 ± 5.17	36.36 ± 3.01	0.245
	Ovarian carcinoma	59.30 ± 1.56	56.00 ± 1.62	0.336
Histopathological diagnosis	Borderline tumors	5 (26.3)	14 (73.7)	0.228
	Ovarian carcinoma	47 (57.3)	35 (42.7)	
Histopathological type of Borderline tumors No. (%)	Serous	4 (28.5)	10 (71.5)	0.554
	Mucinous	1 (20.0)	4 (80.0)	
Histopathological type of invasive carcinomas No. (%)	Serous	38 (63.3)	22 (36.7)	0.312
	Mucinous	4 (50.0)	4 (50.0)	
	Endometrioid	2 (20.0)	8 (80.0)	
	Clear cell Carcinoma	2 (100.0)	0 (0)	
	Malignant Brenner	1 (50)	1 (50.0)	
Grade of invasive carcinoma No. (%)	Well-differentiated	12 (40.0)	18 (60.0)	0.004
	Moderately-differentiated	19 (55.9)	15 (44.1)	
	Poorly-differentiated	16 (88.9)	2 (11.1)	
Pathological stage of invasive carcinoma No. (%)	I	4 (14.9)	23 (85.1)	<0.001
	II	14 (77.7)	4 (22.3)	
	III	24 (75.0)	8 (25.0)	
	IV	5 (100)	0	

Although there is a similarity in expression pattern between different types ovarian carcinoma, there are some differences; a possible explanation for the difference may be that ovarian carcinomas are heterogeneous entities, some derived from borderline tumors and others arising de novo.

Comparing CD44v6 expression in carcinoma versus borderline groups, the expression was higher among invasive carcinoma group (57.3%) than borderline tumor (26.3%), this results were in concordance with Zagorianakou *et al* (50 %vs. 42.9 %) [20] and Hong *et al*. (40% vs. 27%) [21]. However, this difference in expression of CD44v6 didn't achieve statistical significance either in the current study, or in the two other studies mentioned, while Bian [17] prove a significant difference among the two groups. This may attributed to larger sample of borderline tumors (32 cases) compared to (19 cases) in the current study, (15 and 14 cases) in Hong *et al* [21] and Zagorianakou *et al* [20], respectively.

Table 3. Association of p53 immunohistochemical expression with clinicopathological parameters of borderline tumors, and ovarian carcinoma

Clinicopathological parameter		p53		P value
		Positive	Negative	
Age (years) (mean± SEM)	Borderline tumors	40.0 ± 0.00	37.94 ± 2.76	0.867
	Ovarian carcinoma	57.60 ± 1.31	58.29 ± 2.02	0.767
Histopathological diagnosis	Borderline tumors	1 (5.3)	18 (94.7)	0.004
	Ovarian arcinoma	47 (57.3)	35 (42.7)	
Histopathological type of invasive carcinomas No. (%)	Serous	38 (63.3)	22 (36.7)	0.948
	Mucinous	5 (62.5)	3 (37.5)	
	Endometroid	3 (30.0)	7 (70.0)	
	Clear cell Carcinoma	1 (50.0)	1 (50.0)	
	Malignant Brenner	0	2 (100.0)	
Grade of invasive carcinoma No. (%)	Well-differentiated	10 (33.3)	20 (66.7)	<0.001
	Moderately-differentiated	24 (70.6)	10 (29.4)	
	Poorly-differentiated	13 (72.2)	5 (27.8)	
Pathological stage of invasive carcinoma No. (%)	I	6 (22.3)	21 (77.7)	0.01
	II	10 (55.5)	8 (44.5)	
	III	26 (81.25)	6 (18.75)	
	IV	5 (100)	0	

The p53 tumor suppressor gene mutation is the most common genetic aberration in human malignancy, including ovarian carcinomas [10]. p53 was detected in only one case of borderline tumor (5.3%), compared to 47(57.3%) out of 82 case of carcinoma. These data were similar to those of Anreder *et al* [22] where only 2 (10.5%) of 19 borderline tumors showed reactivity to p53 compared to 30(62.5%) of 48 carcinomas.

Kupryjanczyk *et al* [23] demonstrated immune-reactivity with anti-p53 in 14% of the borderline tumors while Zagorianakou *et al* [20] found very low expression (less than 10%) or even absent in all 14 cases of ovarian borderline tumors compared to 23 (47.9%) of 48 carcinoma cases show greater than 10% expression of p53. These statistically significant results can be interpreted to justify use of the p53 status as an immunohistochemical marker to help differentiate invasive from borderline ovarian neoplasms. Although p53 mutations have been detected in all histological types of ovarian carcinoma, the relationship between p53 protein expression and the histopathological subtype in ovarian carcinomas is still controversial.

In studies of Milner *et al* [24] and Gottlieb and Berek [25] both show that the highest incidence of p53 positivity were reported in serous carcinoma and The lowest rate of p53 expression was found in endometrioid carcinoma. These observations were in agreement with the results of the current study which also showed that serous carcinoma expressed p53more frequently than other types of invasive carcinoma, furthermore the only positive single case of borderline tumor that express p53 was of serous differention, however statistical comparison did not reach significance in this field. This statistical non-significant correlation between histological types of invasive ovarian carcinoma and p53 IHC expression was found by Hamdi and Saleem [26], Skirnisdottir *et al* [27] and Levesque *et al* [28] as well. This controversy of findings in this field could be due to random case selection and different types of antibodies used in different studies.

Focusing on association of p53 IHC expression with grade of ovarian carcinoma many authors found a significant association between grade and p53 expression [29-31].

The current study also demonstrates this association, i.e., increase in IHC expression of p53 was associated with increase grade of ovarian carcinoma which was highly significant from statistical point of view. The study show that 13 (72.2%) out of 18 cases with poorly differentiated tumors are positively expressed the marker compare to 10 (33.3%) out of 30 cases of well differentiated tumors. Apparently the most important determinant of clinical outcome of ovarian carcinoma is the clinicopathologic stage at the initial time of diagnosis.

Shelling *et al* [32] found that the prevalence of TP53 gene alterations appears to raise with increasing stage and it occur more often in stage III and IV ovarian cancers when compared to stage I and II, i.e., in 58% versus 37% in stage III/IV and in stage I/II, respectively.

In the current study we are in agreement with these finding as there were a significant association between p53 immune expression and stage of invasive ovarian carcinoma. The data of the current study show that while expression of p53 did not exceed 25% in stage I we found the expression hit 100% in stage IV, this raise in immune expression of P53 with increase in FIGO stage was supported by findings of many previous studies [23,33,34]. The current study show no significant correlation between p53 IHC expression and age of the patients and this is consistent with many previous studies [26,31,35].

We concluded that CD44v6 expression was higher with higher grade and FIGO stage of ovarian carcinoma, ovarian carcinoma with p53 mutation demonstrated a trend toward more aggressive tumor behavior such as poor cellular differentiation and distant metastasis and CD44v6 and p53 may be important molecular markers for poor prognosis.

Acknowledgment

Special thanks to the staff members of pathology department in Collage of Medicine, Al-Nahrain University for their valuable help and advice.

Author contributions

All authors coordinated study subject recruitment, implementation and progress of this study, and helped with data interpretation and manuscript organization and editing.

Conflict of Interest

The authors have no conflicts of interest

Funding

This research was funded by the College of Medicine, Al-Nahrain University.

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Received 4th Oct. 2015: Accepted 4th Jan. 2016.