Expression of p53 and her2/neu in serous ovarian carcinoma with different grades of differentiation (Immunohistochemical Study)

Hadeel A. Kerbel, M.B.Ch.B, FICMS(Path), Rehab H. Abdul Saheb, M.B.Ch.B, FICMS(Path.) and Dhafer S. Alabbasi, M.B.Ch.B, M.Sc(path).

التعبير المفرط لعامل النمو البشراوي Her2/neu وعامل (P53) في خبيث المبيض السطحي المصلي وعلاقتهما بدرجة التمايز النسيجي (دراسة مناعية نسيجية كيميائية)

هديل عبد الإله كربل، رحاب حميد عبد الصاحب، ظافر صاحب العباسي

الملخص

الأهداف: من اجل تقييم مغزى التعبير المفرط لعامل النمو البشراوي Her2/neu وعامل P53 في سرطان المبيض في الإناث وعلاقته مع درجة التمايز.

الطريقة: تم الحصول على ٣٠ عينة مثبتة بالفورمالين ومطمورة بالبارافين لمرضى مصابين بسرطان المبيض السطحي المصلي. كما تم الحصول على ١٠ نماذج لنسيج المبيض الطبيعي حيث استخدمت كمجوعة قياسية. استخدمت طريقة **ABC** لتحديد التعبير المناعي النسيجي لعاملي Her2/neu و P53.

النتائج والمناقشات: أظهرت ألنتائج أن التعبير النسيجي المناعي لـ **Her2/neu كان** موجبا في 8 حالات **(26.6%)** والتعبير النسيجي المناعي لـ**P53** كان موجباً في 13 حالة (43.3%) من أورام المبيض بينما لم نلاحظ أي تعبير نسيجي لـ **Her2/neu** و **P53** في نسيج المبيض الحميد**(P<0.001)**. إن زيادة شدة إظهار **p53 (3+score و 4+score)** سجلت المستويات الأعلى وإنها ترتبط بعلاقة ايجابية معنوية مع درجة التمايز مشيرة إلى إن أورام المبيض الخبيثة موجبة **p53** هي عدوانية حيويا وتتواجد أكثر تكرارا في درجة التمايز الثالثة من درجة التمايز الأولى والثانية. إن تردد إظهار **her2 /neu** وشدته لا ترتبط بعلاقة ايجابية معنوية مع درجة التمايز مشيرة إلى إن أورام المبيض الخبيثة موجبة **her2/neu** هي لا تزال موضع جدل حول أهميتها كعامل قياسي لعواقب أورام المبيض الخبيثة .إن التعبير المناعي النسيجي المتزامن للعاملين لا يشكل علاقة ايجابية معنوية مع درجة التمايز.

الاستنتاج: من النتائج أعلاه يمكن لنا أن نستنتج أن **p53** يلعب دور مهم في تولد و نشأة خبيث المبيض السطحي ويسند الدليل عن دوره في تكاثر الورم ونموه ودرجة تمايزه و ربما يسهم فهم دوره في إعاقة تكوين الورم أو في حالات متابعة فعالية العلاج المضاد للسرطان أو تقدير العواقب في حين أظهرت هذه الدراسة عن عدم وجود علاقه ايجابيه بين ظهور **her2/neu** ودرجة تمايز الورم.

Abstract

Objective: To assess the rate of immunohistochemical expression of Her2/neu and P53, and their coexistence in serous ovarian carcinoma in relation to the grade of differentiation as a prognostic parameter.

Methods: Thirty cases of serous ovarian carcinomas and their corresponding paraffin blocks from 2008-2009 were submitted in this study in the Department of Pathology, College of Medicine, Kufa University. Ten biopsies of normal ovarian tissues were considered as control group. ABC method was used to determine the expression of

Her2/neu and p53 in these cases. Statistically Chi square and regression tests were used by the help of SPSS version 10, using the P value significance at level < or 0.05 and 0.3 respectively.

Results: p53 overexpression was detected in 13 cases (43.3%) while her2/neu was detected in 8 cases (26.6%) of serous ovarian cancer with 6.6% coexistence overexpression of both markers. None of the normal ovarian tissues revealed immunohistochemical expression for both p53 and Her2/neu, with significant level of expression between malignant and benign tissues ($p< 0.001$). p53 overexpression was reported more frequently in higher grades of differentiation with significant level of expression ($p<0.05$) this indicates that serous ovarian tumors with positive p53 expression are biologically bearing more aggressive behavior, while her2/neu expression show no positive correlation with the degree of differentiation, the relation about which a lot of debates are still present. Only 2 cases showed coexistent overexpression of both markers without significant difference with grade of tumor.

Conclusion: p53 expression is significantly found in high grade ovarian serous carcinoma and considered to be a good prognostic parameters, while her2/neu overexpression has no significant difference among different grades of tumor. So coexistent overexpression of both markers could not be considered as significant prognostic parameter.

Key Words: P53,Her2/neu, serous ovarian carcinoma.

Introduction

Ovarian cancer is the fifth most common cause of death in women accounting for 5% of all cancer death.(1) In Iraq it is the 6th in the list of most common cancers according to the Iraqi cancer registry 2004.(2) More than 80% of these cancers are diagnosed in advanced stages due to there asymptomatic characters with low survival rate.(3) the surface ovarian neoplasms are classified into distinct morphologic categories based on the appearance of the epithelium into tumors of serous, mucinous, endometroid, clear cell, and transitional types. Histological type of ovarian cancer is one of the major prognostic factors determining clinical outcome. Recent studies indicate that each of these histological subtypes possess distinct morphological and molecular alterations.(4)

The role of molecular and biological factors in ovarian cancer is controversial. Several oncogenes and onco-supressor genes have been implicated in epithelial ovarian carcinogenesis but their clinical significance is not clear and conflicting data have been found in various studies.(5) This cancer results from a succession of genetic alterations involving oncogenes and tumor suppressor genes which have a critical role in normal cell growth regulation according to the function of these different proteins, the incidence of mutations in their genes in carcinogenesis and as potential prognostic factors in sporadic and hereditary ovarian cancer.(5) Mutations and/or overexpression of three oncogenes, her2/neu, c-myc and K-ras, and of the tumor suppressor gene p53, have frequently been observed in sporadic ovarian cancer. In the context of high risk families, the most frequently involved genes are BRCA1 and BRCA2.(4)

Patients and Methods

Thirty female patients with serous ovarian cancer were included in this study. The present study was performed in the Department of Pathology and Forensic medicine, College of Medicine, Kufa University. The cases were collected from the major hospitals and some of the private laboratories in Najaf and Hilla governorate, in the

middle of Iraq. The blocks of corresponding formalin fixed, paraffin- embedded ovarian biopsies were retrieved from the archives and hematoxylin-eosin slides of each ovarian biopsy were reviewed and marked their grades of differentiation. All biopsies were graded according to WHO classification into three grades, malignant grade I (well differentiated), malignant Grade II (moderately differentiated) and malignant Grade III (poorly differentiated). Patient's ages ranging from 35 to 75 years, with a mean age of 50.1 years. Avidin-Biotin Complex (ABC) method was employed for immuno-histochemical detection of p53 and her2/neu. A control group of 10 samples with normal ovarian tissues were involved in this study. While positive & negative controls were processed with each run.

Qualitative assessment: Faint staining pattern, whether cytoplasmic or nuclear, that could only be detected by using higher magnification (objective 40). While
Strong staining pattern, easily seen by low magnification (objective 4).

Scoring system: The criterion for positive immunoreaction is dark brown precipitate (cytoplasmic for her2/neu and nuclear for P53). While the intensity of the staining was assessed by counting the percentage of positive cells in 100 malignant cells at objective 40 total magnification. The immunostaining was calculated as the percentage of immunoreactive cells per total number of malignant cells. Each sample was scanned for at least five fields with a high power magnification. Scoring of p53 according to Sophia K. et al., at objective 40 and as follow: [6] Score 0: Negative, none of the cells revealed positivity for the marker, Score +1: Weak or mild staining, (5-10%) positive of tumor cells,
Score +2: Moderate staining, less than 25% of tumor cells are stained positive, Score +3: Strong staining, (25-50%) of tumor cells are stained positive, Score +4: Highly strong staining, over 50% of tumor cells are stained positive.

Scoring of her2/neu at objective 40 and as follow[7]: Score 0 (negative), no membrane staining observed, Score +1 (negative), faint partial membrane staining in >10% of cancer cells with rare or absent circumferential staining, Score +2 (positive), weak circumferential membrane staining in >10% of cancer cells but the membrane staining ring is thin, Score +3 (positive), intense circumferential membrane staining in >10% of cancer cells and the membrane staining ring is thick.

Statistical analysis: Statistical analysis of all results were preceded by the help of SPSS version 15 software statistical package using P value at level of significance less than 0.05, and correlation test (R at a significant level of 0.3).

Results:

In all sections of normal control samples, none of the examined samples revealed positive cytoplasmic immunostaining for her2/neu or nuclear immunostaining for p53. The overexpression was detected in malignant samples only, accounting to 13 cases (43.3%) of them with nuclear immunostaining for p53 and 8 cases (26.6%) with cytoplasmic immunostaining for her2/neu with significant differences when these percentages compared with control group ($P<0.0001$) (Table.1). However, only 2 cases (6.6%) of malignant samples revealed coexistent overexpression of both p53 and her2/neu immunostaining, both are well differentiated , and no significant difference among other grades was noticed ($P>0.05$).

Assessment of histopathological **grade** of differentiation revealed that 3 cases (10%) are well differentiated, 10 cases (33.3%) moderately differentiated, and 17 cases (56.6%) are poorly differentiated.

Among them, her2/neu showed none of grade I, 3 cases (10%) of grade II, and 5 cases (16.6%) of grade III over expression without statistically significant difference between different grades but we can see in general that higher scores were recorded in higher grades (Table.2). While positive nuclear immunostaining for p53 was reported in 1 case (3.3%) of grade I, 4 cases (13.3%) of grade II, and 8 cases (26.6%) of grade III with a significant difference ($p<0.05$) (Table.3). It looks that P53 immunoreactivity is significantly increasing as the grade of tumor increased.

Regarding **intensity** of her2/neu immunostaining, 100% of grade I revealed score 0/+1 (negative). Grade II revealed 70% score 0/+1(negative), 20% score +2 (equivocal), and 10% score +3(positive).

Grade III revealed 70.5% score 0/+1, 17.6% score +2, and 11.7% score +3 with significant difference only for grade III , it looks that the intensity of immunostaining is significantly increasing with the grade of tumor (Tabel.2) fig.(2).

Regarding **intensity** of p53 immunostaining, 6.6% of grade I revealed score 0, and 3.3% of score +2 while nil score +1, +3, and +4. Grade II revealed 20% score 0, 3.3% for each score +1, +2, +3, and +4. Grade III revealed 30% score 0, 16.6% sore +1, 6.6% score +2, 3.3% score +3, and nil score +4, with significant difference only for grade III ($P<0.05$) (Tabel.3) fig.(1). Statistical analysis revealed **no** significant correlation between her2/neu and p53 overexpression in the examined cases.

For the intensity of her2/neu and p53 immunostaining, the correlation between the intensity and the grade of the tumor revealed positive correlation especially significant in grade III ($R=0.9449$), though there is no statically significance in the samples with coexistent her2/neu and p53 overexpression ($P=0.824$).

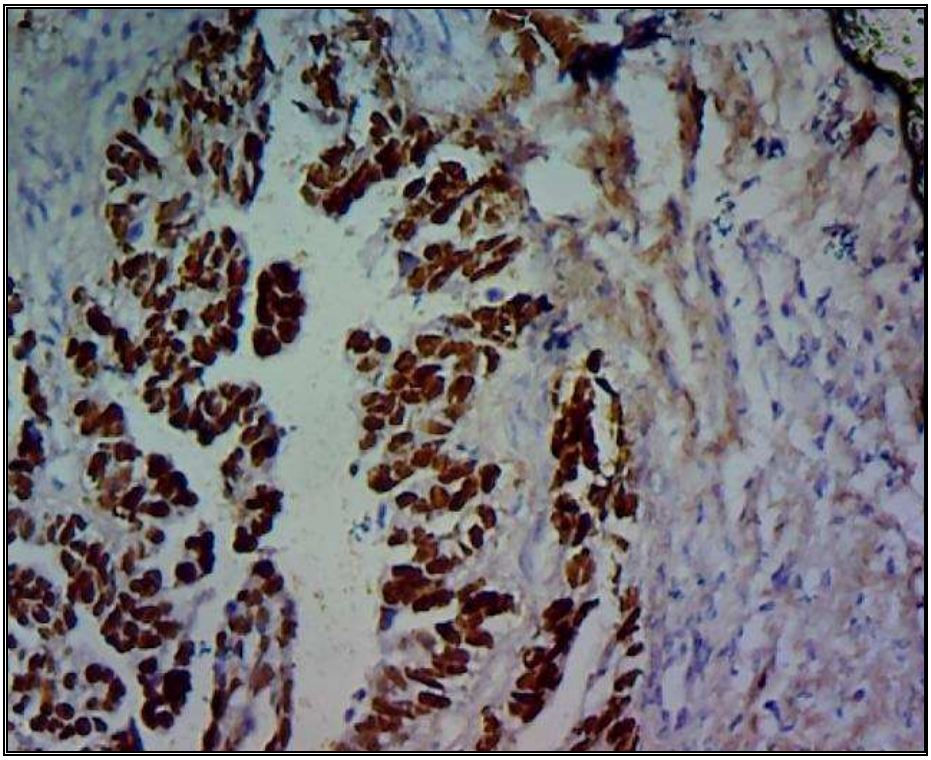

Fig.1. serous ovarian cancer (grade II) showing strong diffuse score +4 nuclear immunostaining for P53(20X)

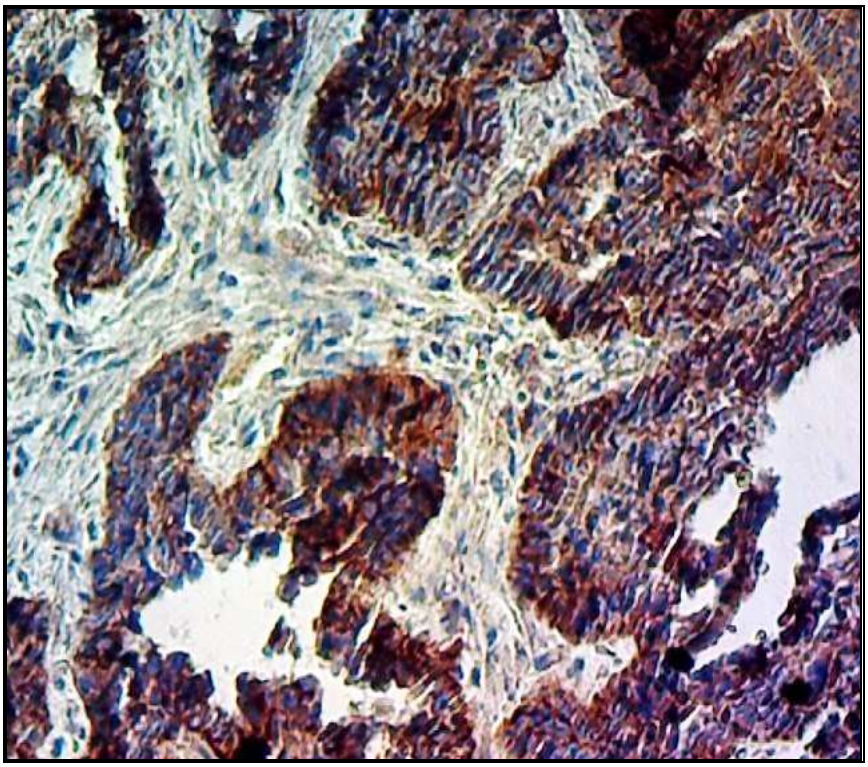

Fig. 2. Serous ovarian cancer, moderatly differentiated(grade II) showing strong diffuse score + 3 immunostaining for Her 2/ neu(10X).

Table 1: Detection rate among control samples and malignant samples for both her2 and p53 immunostaining

Control			***Total***	***Malignant***	
	+ve	***-v***		***+ve***	***-ve***
Her2/neu	0	10	40	8(26.6%)	22(73.4%)
P53	0	10	40	13(43.3%)	17(56.7%) **(P<0.05)**

Table 2:A scored detection rate of her2 immunostaining among different grades of differentiation

Her2/neu	***+ve***	***-ve***	***Total***		***Score 0/1+***	***Score 2+***	***Score 3+***
well	0	3(10%)	3(10%)		3(100%)	0	0
Moderate	3(10%)	7(23.3%)	10(33.3%)		7(70%)	2(20%)	1(10%)
poor	5(16.6%)	12(40%)	17(56.6%)		12(70.5%)	3(17.6%)	2(11.7%)
total	8(26.6%)	22(73.3%)	30		22	5	3 (**P>0.05**)

Table 3: A scored detection rate of p53 immunostaining among different grades of differentiation

P53	***+ve***	***-ve***	***Total***	***0***	***1+***	***2+***	***3+***	***4+***
well	1(3.3%)	2(6.6%)	3	2(6.6%)	0	1(3.3%)	0	0
Moderate	4(13.3%)	6(20%)	10	6(20%)	1(3.3%)	1(3.3%)	1(3.3%)	1(3.3%)
poor	8(26.6%)	9(30%)	17	9(30%)	5(16.6%)	2(6.6%)	1(3.3%)	0
Total	13(43.3%)	17(56.6%)	30	17(56.6%)	6	4	2	1

P(<0.05)

Discussion:

The present study is designed to correlate the p53 and her2/neu over-expression to the grade of differentiation and to assess whether such relation could be used as a prognostic factor for the early diagnosis of the malignancy. Moreover, we attempted to assess whether there is any increase in the frequency of gene expression in comparison to studies done abroad due to other factors specially that our samples were taken from area suffered as a war zone by this study which has been conducted in the middle of Iraq in an area in which many preliminary studies indicated to the increase in cancer incidence to more than three folds from the older Iraqi cancer registries.[(2)]

Her2/neu and ovarian cancer: Her2/neu oncogene which belongs to epidermal growth factor receptor family has been implicated in malignant transformation and may have a

driving force in the carcinogenesis of several human cancers including ovarian cancer.[9] Several reports have examined the prognostic significance of her2/neu expression in epithelial ovarian cancer. The role of her2/neu immunohistochemistry in ovarian cancers is not that clear yet with contradicting results and conflicting data.[5] Thus, the prognostic influence of her2/neu is still a matter of debate since the percentage of her2/neu positive patients varies considerably among different individual studies dealing with different samples. Although expression of her2/neu oncogene may be of some prognostic importance in advanced ovarian cancer its role in early stage disease has not been established.[10]

Increased expression of her2/neu oncogene has been reported to occur in ovarian tumors and possibly to correlate with its biologic behavior and prognosis.[10] In addition, many studies concluded that her2/neu overexpression has been associated with advanced stages, poorly differentiated tumors, resistance to chemotherapy and shortening survival. However, other studies had the ability to prove a positive association of her2/neu overexpression with an increased risk of progression and death among women with early stage ovarian carcinoma.[11]

Garcia-Velasco A et al (2008) [12] revealed only 5% detection rate of her2/neu overexpression in 72 malignant samples of ovarian tissues without significant difference.

D pils et al (2007) [5] revealed that 35(27.6%) out of 127 cancer tissues of patients with malignant tumors were found to be overexpressed her2/neu gene product including tissues with high intensity of immunostaining.

Elena Verri et al (2005)[13] revealed 27.3% positive immunostaining for her2/neu, among which 13.4% were weakly stained (score +1), and 13.9% were intensely positive (score +2 to +3), without any significant relationship between her2/neu detection rate and intensity of immunostaining. While other study showed 18% overexpression in 79 samples of stage I or II ovarian neoplasms[14].

Prema P et al (2003)[15] revealed 2% detection rate for her2/neu in sample of 43 cases which scored as zero in 30 patients, +1 in 12 patients and +3 in only one patient without significant difference. While her 2/neu overexpression were noted in only 21% of cases with advanced disease.[16]

Stephen C et al (1994)[10] studied her2/neu expression in 40 patients with stage I and II epithelial ovarian cancer. Positive detections were higher in grade I and III than grade II. Moreover, 70% of the recorded positive immunostaining cases were scored as +1 and +2, 20% were score +3, while 10% were score zero.

Many other studies revealed various degrees of detection rate for her2/neu immunostaining such as **Berchuch et al 1990** (32%), **Salmon et al 1989** (26%), **Bookman et al 2003** (11%), **Dimova et al 2006** (11%), **Nielsen JS et al 2004** (35%), and **Malamou-Mitsi V 2007** (18%).[17]

P53 and ovarian cancer: The guardian of the genome, p53 is the most common tumor suppressor gene involved with human malignancies. It is a multifunctional and often altered in High grade serous carcinoma of the ovary.[18] Although the biologic and clinical roles that p53 play in cancer remain areas of intense investigation and debate, a number of studies have shown that alterations in p53 may or may not associate patient's outcome, such as response to therapy or survival. However, several studies have shown that alterations in p53 are associated with patient outcome, such as response to therapy or survival and hence p53 has been studied extensively as a prognostic indicator in ovarian carcinoma. In several studies, p53 overexpression has been shown to be significantly associated with advanced stage and revealed p53 as an independent prognostic factor along with grade of epithelial ovarian cancer.[19]

If mutation of p53 and its consequent overexpression is an early event in ovarian tumorigenesis, then p53 assessment may prove useful prognostically in the assessment of either low grade ovarian carcinomas, as a possible indicator for progression, or in early stage ovarian tumors, as a marker of tumor progression or the likelihood of recurrence[20].

Bartel F et al (2008)[21] identified a large group of patients with p53 overexpression to be associated with a significantly shortened overall survival and refractory to chemotherapy compared with patients with normal p53.
Buchynska LG et al (2007)[22] found significant correlation between p53 immunoreactivity and high grades of ovarian cancer, While other study demonstrate that the average scores for p53 immunoreactivity in normal ovaries and different types of ovarian tumors were increasing in order of benign, normal, borderline, grade I, grade II and grade III malignant samples[23].

LC Hartmann et al (1994)[24] showed that p53 immunoreactivity was present in 177(62%) cases out of 284 patients with epithelial ovarian cancer using immunohistochemical techniques in paraffin embedded specimens, revealing a significant correlation between p53 immunoreactivity and high grades.
Many other studies shown the almost the same results with very much similar conclusion that there is a strong correlation between p53 overexpression and tumor grade, stage, recurrence and survival rate. Such as: **John p et al (2000)** [25]**, Levesque MA et al (1995)** [26]**, M. Baekelandt et al (1999)** [27]**, and David M. (1999)** [28] In addition, **Michael B et al (1999)** [29] found that the detection rate of p53 immunostaining decreasing with increasing the intensity of immunostaining. However, **Hogdall et al (2008)** [30] revealed **no** significant difference in frequency of p53 tissue expression in low malignant potential ovarian tumors with increasing stage while significant increase in ovarian cancer with increasing stage.
On the other hand, there is a study showed that p53 did not correlate with stage grade and recurrence but we didn't found another published paper that supports such results [31].

Conclusion: From the above results and discussion we can see that P53 is over expressed significantly among different grades while her2/neu expressed without significant difference. Thus our study confirms many previous studies in different parts of the world regarding significance of p53 immunostaining among different grades of differentiation. On the other hand, her2 immunostaining was not that useful regarding ovarian cancers. Moreover, Coexistent overexpression of p53 and her2/neu is not significant as a prognostic parameter.

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