Microtubule associated protein tau as a marker of response to taxene based chemotherapy in primary epithelial ovarian cancer

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Abstract: Aim: Taxene – carboplatin is the first line in the treatment of primary epithelial ovarian cancer but only two third of women will respond, the majority will recur. One third of the patients will be resistance to treatment. Our aim to assess the microtubule associated protein and other prognostic in primary epithelial ovarian cancer. **Methods:** Fifty nine patients with stages Ic-IV epithelial ovarian cancer who underwent surgery either radical, optimal or suboptimal surgery are included in this study. These patients received combination chemotherapy taxol 135 mgm/m^2, carboplatin area under curve 5.This prospective study was carried out in oncology department, Tanta university hospital from June 2008 to June 2009. Their formalin fixed paraffin embedded tissue specimens are subjected to immunohistochemistry for microtubule associated protein (Tau). Correlation of Tau protein with progression free survival, overall survival and overall response were done. Primary endpoint was overall response with Tau; secondary end points were overall survival and progression free survival. **Results:** Thirty four patients out of 59 (57.6%) presented with advanced stage ovarian cancer. tau negative and positive was detected in 28 (47.5) and 31(52.5%) patients respectively. Tau negative was associated with high overall response than tau positive (11, 6 patients respectively, p=0.005). 3-year PFS in Tau-negative and Tau-positive groups were 60.1% and 38.2%, respectively (p = 0.01). In univariate analysis low expression of protein Tau, early stage of diagnosis, tumor residual less than or equal to 1 cm and serous tumor were associated with better PFS. In multivariate analysis no factor was statistically significant. As regard overall survival, 3 year overall survival was 79.9, 49.9% in Tau negative and positive respectively (0.026). In univariate analysis Tau positive, residual tumor more than 1 cm and resistance to first line chemotherapy were each correlated with worse OS (p <0,05). In multivariate analysis tau positive and resistance to first line chemotherapy significantly were associated with worse prognosis. **Conclusion:** Microtubule associated protein (Tau) is a good prognostic and predictive factor of response to taxene in epithelial ovarian cancer.
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1. Introduction

Ovarian cancer is the 5th leading cause of death worldwide gynecological malignancy [1]. Cisplatin, taxol has been the backbone of treatment of ovarian cancer [2-3]. The importance of both platinum salts and taxanes was highlighted in a 2006 multiple-treatment meta-analysis that included 60 trials in women (n = 15,609) with EOC which showed that a platinum-taxane combination improved survival when compared with cisplatin single and cisplatin combined with non taxene based chemotherapy [4]. Despite This about quarter of patients experience resistance and of responding patients some will recur.

Paclitaxel (Taxol®) binding to a pocket in β tubulin on the microtubule's inner surface, which counteracts the effects of GTP hydrolysis occurring on the other side of monomer [5,6].

Tau proteins belong to the family of microtubule-associated proteins. They are mainly expressed in neurons where they play an important role in the assembly of tubulin monomers into microtubules to constitute the neuronal microtubules network. Microtubules are involved in maintaining the cell shape and serve as tracks for axonal transport [7].

Tau protein (50–64 kD), a product of gene located in chromosome 17 (17q21) shows the ability of combining to beta-tubulin. It may bind to the exterior as well as to the interior microtubules surface, in the same binding site as paclitaxel, and consequently compete with this drug [6-8]. of Tau protein leads to increase of polymerization and at the same time reduces cells' flexibility [9].

Our aim of study is to test the correlation between tau microtubule associated protein in epithelial ovarian patients treated with standard surgery (radical, optimal or suboptimal debulking) followed by carboplatin taxol as regard response, overall survival and progression free survival.

Patients and methods

Fifty nine patients assigned to receive the carbo- taxol regimen (paclitaxel at a dose of 175 mg/m^2 as a 3-hour infusion followed by carboplatin area under curve 5) in the period from june 2008 to June 2009 in Oncology department, Tanta university.

Inclusion criteria included the FIGO stage (Ic-to IV), the amount of residual disease ' ≤1 cm, or >1 cm), the WHO performance status (0–1, 2,), and the tumor grade (well differentiated, moderately well differentiated, poorly differentiated). Patients were chosen with sufficient formalin embedded sufficient tissue for immunohistochemistry for microtubule associated protein.

Creatinine clearance was calculated using the Jelliffe formula [11] and AUC, the Calvert formula [12]. Treatment cycles were repeated every 3 weeks provided the neutrophil count was > 1.5 × 109/L, the platelet count was > 100 × 109/L

The patients received three cycles of carboplatin. Taxol, assessment of the patients using magnetic resonance imaging of abdomenoplevis, chest computed tomography, tumor marker CA125, patient with disease progression will receive another line of treatment. Patients with complete, partial, and stable disease will receive another three cycles of chemotherapy.

The evaluation of response using RECIST criteria was done after six cycles of chemotherapy according to radiological investigation done at the baseline and/or second-look surgery assessment.

Response according to RECIST Criteria [13]

- A complete response (CR) was defined as the disappearance of all clinical evidence of tumor, including normalization of CA 125 level, determined by two observations not less than 4 weeks apart.
- Partial response (PR) was defined as a 50% or greater decrease in the sum of the products of the perpendicular diameters of the measured lesions, determined by two observations not less than 4 weeks apart. No simultaneous increase in the size of any lesion or the appearance of new lesions was permitted.
- Stable disease was defined as a steady state of response less than a PR or progression less than 25% lasting at least 4 weeks. No new lesions were to appear for inclusion in this category.
- Progressive disease (PD) was defined as the unequivocal increase of at least 25% in the sum of the products of the perpendicular diameters of the measured lesions.

PFS, secondary end point, was defined as the interval between the date of randomization and the date of progression of the disease or death or start of a new therapy without evidence of progression, whichever occurred first

Immunochemistry

Formalin fixed paraffin embedded sections of fifty nine patients with ovarian cancer were examined for tau protein using immunohistochemistry. The tissues were incubated with anti-Tau polyclonal rabbit antibody that recognizes all isoforms of human Tau irrespectively of its phosphorylation status. Specimens were assessed by means of light microscope with 20 × magnification lens. Tau staining of tumor cells was scored:

- score 0 – no staining
- + 1 poor focal staining or very poor diffuse staining (less intense than normal ovarian epithelium);
- +2 average diffuse staining (similar to normal ovarian epithelium) or strong staining (more intense than normal ovarian epithelium) in less than 25% cells
- + 3 strong staining in 25% of tumors cells or more.

Tau expression was negative (0 and +1) or positive (+2 and +3).

Statistical analysis

Statistical analyses were conducted using SPSS for Windows, version 21.0 (SPSS Inc., Chicago, IL, USA). The Kaplan–Meier method was used to calculate survival. The Cox multivariate hazards models were used to calculate the hazard ratios (HR) and their 95% confidence intervals (95% CI) in the analysis of DFS and OS. P value was considered significant if less than 0.05 [14-15].

3. Results

Patient characteristics

Tau expression was evaluated in 59 ovarian cancer patients. Tau negative and positive was detected in 28 (47.5) and 31(52.5%) patients respectively. Table (1) summarizes clinical characteristics of the patients included in this study. Median age in the study group was 51 years (range 22-61). Forty seven (79.6%) patients was diagnosed at advanced FIGO stage (III-IV). 52.5% of the patients diagnosed serous type of ovarian cancer. Forty five (76.4%) patients were chemosensitive.

In thirty four patients with measurable disease Tau negative was associated with high overall response than tau positive (11(78.6%), 6 (30%) patients respectively, p=0.005) table(2).

As regard progression free survival, 3-year PFS in Tau-negative and Tau-positive groups 60.1% and 38.2%, respectively (p = 0.01). Univariate analysis revealed following clinical parameters correlated with progression free survival: FIGO staging (early), residual tumor size after debulking surgery (p =0.033) and negative tau expression level (p =0.010) are correlated with better progression free survival. On multivariate analysis no factor was statistically significant. The results are presented in Table 3 and Figure 1.

As regard overall survival, 3 overall survival was 79.9, 49.9% in Tau negative and positive respectively (0.026). In univariate analysis Tau positive (0.026), residual tumor more than 1 cm (0.001) and resistance to first line chemotherapy (0.041) were correlated with worse OS. In multivariate analysis tau positive (0.035) and resistance to first line chemotherapy(0.001) significantly associated with worse prognosis table (4) and figure (2).

Table (1) Characteristics of 59 patients with epithelial ovarian tumor:-

Characteristics	Number	Frequency
Age	22-61 median(51)	Mean ± SD 46.96±10.92
Performance status		
• 0-1	48	81.4%
• 2	11	18.6%
FIGO		
• I	5	8.5%
• II	7	11.9%
• III	36	61%
• IV	11	18.6%
Surgery		
• Radical	13	22
• Optimal debulking	23	39
• Suboptimal debulking	23	39
Pathology		
• Serous	31	52.5%
• Mucinous	9	15.3%
• Endemetroid	9	15.3%
• Others	10	16.9%
Grade		
• well differentiated	8	13.6%
• moderately differentiated	20	33.9%
• poorly differentiated	31	52.5%
Chemotherapy		
• chemosensitive	45	76.3%
• chemo resistant	14	23.7%
Residual disease		
• ≤ 1 cm	36	61%
• > 1cm	23	39%
Tau		
• positive	31	52.5%
• negative	28	47.5%

Table (2) Correlation of response with microtubule associated protein in 34 patients with measurable disease

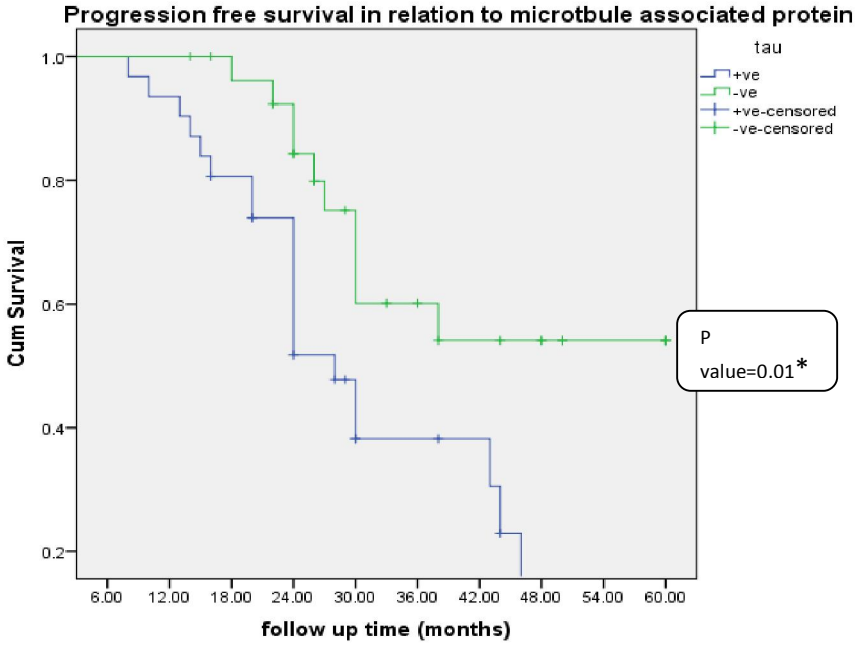
Response	Tau negative=14	Tau positive n=20	*P* value
Complete response	10(71.4%)	5(25%)	0.04
Partial response	1(7.1%)	1(5%)	
Stable disease	1(7.1%)	8(40%)	
Disease progression	2(14.3%)	6(30%)	
Overall response	11(78,6%)	6(30%)	0.005
Non responsive	3(21.4%)	14(70%)	

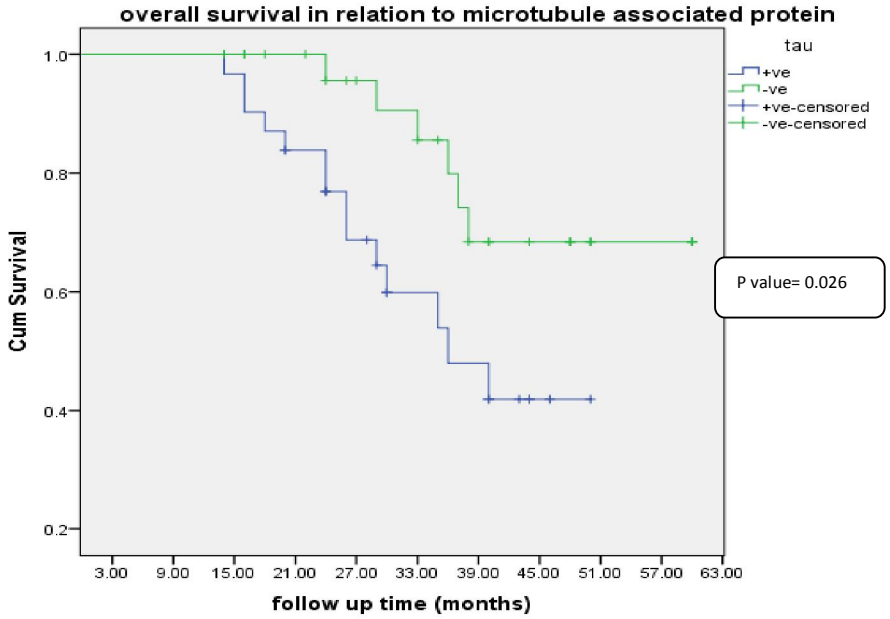
Table (3) Univariate and multivariate analysis of progression free survival

Variant	Univariate	Multivariate	
		P value	HR-95%CI
FIGO • Early (I+II) • Late (III-IV)	0.013	0.116	2.086(0.834-5.222)
Tumor residual • < 1cm • > 1m	0.033	0.127	1.838(0.841-4.017)
Pathology • Serous • others	0.007	0.111	0.451(0.169-1.201)
Tau • -ve • +ve	0.010	0.315	0.618(0.242-1.580)

Table (4) Univariate and multivariate analysis of overall survival

Characteristics	Univariate	Multivariate	
		P value	HR-95%CI
Residual • ≤ 1cm • > 1cm	0.001	0.002	4.800(1.812-12.715)
Chemotherapy • Chemosensitive • Chemoresistant	0.041	0.384	1.985(0.424-9.300)
Tau • -ve • +ve	0.026	.035	0.345(0.129-0.629)





4. Discussion

Despite that platinum combined with paclitaxel is the gold standard in epithelial ovarian cancer. About two third of patients respond to this regimen and of the responders some will recur[2]. There are no predictive factors of chemotherapy response in ovarian cancer. Tau protein bind to the exterior as well as to the interior microtubules surface, in the same binding site as paclitaxel, and consequently compete with this drug[8]. We study Tau protein as a predictive factor in patients with epithelial ovarian cancer treated with carboplatin – taxol.

Tau negative and positive were detected in 28 (47.5) and 31(52.5%) patients respectively. These results are similar to that reported by other authors [8,16]

In thirty four patients with measurable disease Tau negative was associated with high overall response than tau positive (11(78.6%), 6 (30%) patients respectively, p=0.005) and this was constant with other authors reported that Tau negative patients are associated with high overall response than Tau positive patients[16-17]. The remaining of non responders in Tau negative may be due to tubulin mutation or Overexpression of multidrug resistance gene [18].

As regard progression free survival, 3-year PFS in Tau-negative and Tau-positive groups were 60.1% and 38.2%, respectively (p = 0.01). Univariate analysis revealed following clinical parameters correlated with progression free survival: FIGO staging (early), residual tumor size after debulking surgery (p=0.033) and negative tau expression level (p =0.010) are correlated with better progression free survival. On multivariate analysis no factor was statistically significant. As regard overall survival, 3 overall survival was 79.9%, 49.9% in Tau negative and positive respectively (0.026). In univariate analysis Tau positive (0.026), residual tumor more than 1 cm (0.001) and resistance to first line chemotherapy (0.041) were correlated with worse OS. In multivariate analysis tau positive (0.035) and resistance to first line chemotherapy (0.001).

These finding was different from that reported Steffensen *et al.,*[19] who reported hat Tau protein was not associated with progression free survival or overall survival or progression free survival.

Smoter *et al.,*[20], 74 patients with epithelial ovarian cancer treated with cisplatin, paclitaxel are tested for Tau protein status. Low expression of protein Tau was associated with better OS, whereas an advanced stage at diagnosis, suboptimal surgery, serous histological type and resistance to first line chemotherapy were each correlated with worse OS (p <0,05). This was constant with our study. In multivariate analysis only resistance to first line chemotherapy remained significant (HR 22.59; 95% CI, 8.71-58.55; p <0.0001) but our study in multivariate analysis, resistance to chemotherapy and Tau protein positive patients were correlated with bad OS.

Conclusion:

Better response to paclitaxel is correlated with negative status of Tau protein in primary epithelial ovarian cancer associated with increased PFS, overall survival. A large number of epithelial ovarian cancer patients are needed to assess the Tau protein as prognostic and predictive factor in ovarian cancer patients receiving taxol based chemotherapy.

Conflict of interest:

No conflict of interest to declare.

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