A Monoclonal Antibody Quantitative Measurement And Immunohistochemical Localisation Of Carcinoembryonic Antigen In Ovarian Neoplasia

A Askalani, H Meabid, Saad El Sadek M El Sadek, Nabil M El Tabbakh, M Osman

Citation

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Abstract

Objective: To evaluate the significance of including CEA in serum and tissues in the management protocol of patients with ovarian malignancies.

Patients And Methods: The study included 68 patients which was divided into three groups. Group [A] included 21 patients with malignant ovarian tumors. Group **included three patients with boderline ovarian tumors. Group [C] included eight patients with benign ovarian tumors, Group [D] included 36 women without any apparent gynaecologic disorder (control group). Serum level of CEA was measured in all patients in group A, B and C prior to treatment and at least 12 weeks following therapy. Formalin - fixed and paraffin - embedded tissue blocks taken from 2 different sites of the studied lesions were prepared. Immunohistochemical staining for CEA was performed for the studied tissues.**

Results: All the ovarian tumors investigated in this work were of epithelial origin. All benign and borderline ovarian tumors had negative pre- and post-treatment serum CEA levels (< 5ng/ml) and were negative for CEA tissue stain.85.71% of ovarian serous cystadenocarcinomas had negative pre-treatment serum levels . All the squamous cell carcinomas and 66.67% of each of mucinous cystadenocarcinomas and undifferentiated carcinomas had positive (>=5ng/ml) pre-treatment serum levels. After treatment the different histological types of ovarian carcinimas were seronegative for CEA.The highest percentage of positive staining (100%) was found in squamous cell carcinomas [50% (+1) and 50% (+3)], followed by 66.67% of mucinous cystadenocarcinomasThe mean difference between pre-and post-treatment serum CEA was significant in mucinous cystadinocarcinoma with negative and positive tissue reaction and in negatively stained serous cystadinomacarcinoma.The percentages of cases of ovarian carcinomas with positive pre-treatment serum CEA increased with the increase of grade of malignancy from 33.33% in G1 to 55.56% in G II and 66.67% in GIII.No definite relation could be detected between the grade of malignancy and the intensity (degree) of reaction of malignant ovarian tumors to CEA immunostaining.The percentage of cases with positive pre-treatment levels of serum CEA increased progressively with the advance in clinical staging. The incidence was 37.5% in stage I, 55.56% in stage II, 66.67% in stage III, and 100% in stage IV. No definite relation could be detected between the clinical stages and the degree of reaction of ovarian carcinomas to CEA tissue stain.

Conclusion: This study indicated that immunohistochemical identification of CEA in tumor tissue and of monoclonal antibodies quantitative measurement of CEA in human serum is a useful adjunct in the management protocol of patients with ovarian malignancies. However further studies are required to fully ascertain the usefullness of this technique.

INTRODUCTION

Ovarian cancer is a serious and silent disease and has high rate of mortality among malignant gynecologic tumor. It is typically diagnosed at an advanced stage([1,2,3]). Furthermore, even when small ovarian tumors are considered to be resected , there is a recurrence in at least one-third of the patients([2,3,4,5]). Unfortunately, despite advances in surgical technique and novel chemotherapeutic agents, survival rates have not improved significantly over past 25 years([2,6]).

The oncofoetal antigens comprise one particular group of markers produced by human neoplasms, these antigens have been detected in the sera of patients with gynaecological cancer. The practical use of such markers in the diagnosis and follow-up has been limited by the low sensitivity and specificity of their tests (6)..Carcinoembryonic antigen (CEA) is one of the first known tumor markers. Since then, many more have been described, but CEA, determined alone or in combination with others, is still one of the most used. CEA is not organ specific and abnormal values may be found in a wide range of carcinomas ([7]). Epithelial ovarian cancer is known to produce CEA and the plasma CEA level is frequently elevated in patients with advanced stage and bulk of tumor.([8])

In this work we try through the study of CEA in the serum and tissues to evaluate the significance of including this tumor marker in the management protocol of patients with ovarian malignancies.

PATIENTS AND METHODS

The present study was conducted on patients treated at National Cancer Institute, Cairo university and the department of Obstetrics and Gynaecology, Al Hussain Hospital, Al Azhar university. The study included 68 patients. 21 patients with ovarian malignancies, 3 patient with boderline ovarian tumor, 8 patients with benign ovarian tumors and 36 patients without any apparent gynaecologic disorder as control group. The patient were divided into the following groups

Group [A] : Included 21 patients with malignant ovarian tumors, including mucinous cystadenocarcinoma (9 cases) ; serous cystadenocarcinoma (7 cases), unidifferentiated carcinoma (3 cases) and squamous cell carcinoma(2 cases).

Group **: Included three patients with boderline ovarian tumors, two of them were boderline mucinous tumor and the third was of the serous type.**

Group [C]Included Eight patients with benign ovarian tumors, four were mucinous cystadenomas and the other four were serous cystadenomas.

Group [D] : Included 36 women without any apparent gynaecologic disorder. They were age matched with the tumor patients - serum samples were taken from them were subjected to CEA measurement and considered as control. All the cases in group A,B and C, were subjected to the following :

1. Careful history, clinical examination and investigations

2. Clinical staging for malignant lesions in group A according to the International Federation of Gynaecology and Obstetrics (FIGO) staging systems (9). Staging of ovarian cancer showed 8 patients with stage I, 9 patients with stage II, 3 patients with stage III and one patient with stage IV.

3. Serum samples were collected from the patients in groupa A, B and C prior to treatment and at least 12 weeks following surgery or completion of radio or chemotherapy. All the patients were clinically free at the time of the post-treatment sample proved by a second look laparoscopy.

4. serum CEA was assayed in all the serum samples using a monoclonal antibody based immunoassay commercially available kit from Abbott Laboratories (North Chicago, Illinois, USA,) which provides a quantitative measurement of CEA in human serum. It is a solid phase enzyme-linked immunosorbent assay based on sandwich principle. A positive result for CEA in serum was taken as 5 ng/ml or more.

5. Surgical specimens from ovarian tumors-taken from two different sites-were fixed in 10% formalin and embedded in paraffin. Formalin fixed, paraffin - embedded tissue blocks with hematoxylin-eosin stained slides had been prepared for all cases without special processing for determination of histological type, the degree of tumor differentiation and for selection of blocks for study. Serial sections not more than 5 um thick were deparaffinized in xylene and dehydrated in a series of graded concentrations of alcohol. The

slides were incubated in methanol with 0.3% hydrogen peroxide to eliminate endogenous peroxidase activity.After incubation with polyclonal rabbit primary antibody for 60 minutes and with polyclonal enzyme for another 60 minutes at room temperature,the specimens were stained by the DAB (diaminobenzidine) working colour reagent and incubated for 5-10 minutes and counterstained with haematoxylin for for 30-60 seconds.

Three grades of malignancy were recognized histologically in the current study:

- Grade 1: well differentiated tumors (G1).
- Grade 2: moderately differentiated tumors (G 2).
- Grade 3: poorly differentiated tumors (G 3).

According to Charpin et al (1982) ($_{10}$). - a grading system was utilized quantify the staining positivity as follows :

(0) Denoting negative reaction i.e. showing no difference from the control sections.
(+1) Means that up to 25% of the cells were positive.
(+2) Means that >25 - 50% of the cells were positive
(+3) Means that >50 - 75% of the cells were positive.
(+4) Means that >75% of the cells were positive.

The patients with malignant ovarian tumors were treated with surgery alone or combined with radiation therapy or chemotherapy depending upon primary type, histologic differentiation and stage of disease. Those with non malignant lesions were treated only surgically.

STATISTICAL ANALYSIS

Statistical analysis was carried out using an IBM - AT computer and SAS program (SAS, 1988). One way analysis of variance (procedure GLM of SAS) followed by Duncan's multiple range test were used to test the significance between the different variables studied. Paired t-test (procedure Means of SAS) was run to test the significance of the difference in serum CEA levels in relation to the variables studied in the current investigations, while student's t - test (procedure test of SAS) was employed to test the significance of change in serum CEA levels between negatively and positively stained lesions in relation to the different variables investigated. Cross tabulation and chi - square test (procedure frequency of SAS) were used to obtain and compare the percentage distribution of the studied cases according to their serum CEA levels and reactions to CEA immunostaining in relation to the studied variables. The probability level 0.05 ($p = 0.05$) was used to test the significance of the previous tests.

RESULTS

All the ovarian tumors (benign, borderline and malignant) investigated in this work were of epithelial origin, half of the benign ovarian tumors were mucinous cystadenomas while the other half were of serous type. In the borderline group of tumors, 66.67% were borderline mucinous tumors and 33.33% were borderline serous ones.

The percentages of the different histological types of ovarian carcinomas were as follows: 42.86% mucinous cystadenocarcinomas, 33.33% serous cystadenocarcinomas, 14.29% undifferentiated carcinomas, and 9.52% squamous cell carcinomas.

All the histological types of benign and borderline ovarian tumors had negative pre- and post-treatment serum CEA levels (>.5ng/ml)

A total of 85.71% of ovarian serous cystadenocarcinomas had negative pre-treatment serum levels representing 60% of all ovarian carcinomas with negative pre-treatment serum CEA. All the squamous cell carcinomas and 66.67% of each of mucinous cystadenocarcinomas and undifferentiated carcinomas had positive (>=5ng/ml) pre-treatment serum levels. After treatment the different histological types of ovarian carcinomas were seronegative for CEA.(Table I)

Figure 1

Table (Ia) : Serum CEA before and after treatment in the different histological types of ovarian tumors

Histological types of Tumors	No. (%)	Serum CEA Before Treatment Mean	S.D	dt	After Treatment Mean	S.D	dt	Difference Mean	S.D	dt
His - 1	4 (50 %)	1.162	0.638	b	1.177	0.625	a	0.014	0.917	b
His - 2	4 (50 %)	1.098	0.687	b	0.623	0.44	a	0.475	0.528	b
His - 3	2 (66.67%)	2.665	0.588	b	0.98	0.06	a	1.684	0.528	b
His - 4	1 (33.33 %)	0.457	-	b	0.74	-	a	0.283	-	b
His - 5	9 (42.86 %)	9.081	7.79	ab	0.773	0.614	a	9.028	7.526	ab
His - 6	7 (33.33 %)	3.21	3.146	b	0.626	0.307	a	2.584	3.21	b
His - 7	2 (9.52 %)	14.753	9.158	a	1.305	0.318	a	13.448	9.477	a
His - 8	3 (14.29 %)	4.511	1.245	b	0.629	0.682	a	3.882	1.924	ab

Figure 2

Table (Ib) : Serum CEA before and after treatment in different types of ovarian tumors. The different histological types of benign and borderline tumors were negative for CEA tissue stain.

Type of Tumor	No.	Serum CEA Before Treatment Mean	S.D	dt	After Treatment Mean	S.D	dt
Benign	8	1.13	0.615	b	0.9	0.582	a
Borderline	3	1.929	1.341	b	0.9	0.145	a
Malignant	21	7.32	6.854	a	0.754	0.519	a
Control group	25	1.024	0.865	b	-	-	-

Figure 3

Serum CEA Difference Mean	S.D	dt	S.E	T	P
0.23	0.763	a	0.27	0.853	N.S
1.028	1.195	a	0.69	1.49	N.S
6.566	6.686	a	1.459	4.5	0.0002
-	-	-	-	-	-

SD : Standard deviation

dt : Duncan's multiple range t-test

SE : Standard error

T : Paired t-test

N.S : Not significant

The negatively stained malignant ovarian tumors included all undifferentiated carcinomas, 85.71% of serous cystadenocarcinomas, and 33.33% of mucinous cystadenocarcinomas. On the other hand, the highest percentage of positive staining (100%) was found in squamous cell carcinomas [50% (+1) and 50% (+3)], followed by 66.67% of mucinous cystadenocarcinomas distributed as 55.56% in (+1) and 11.11% in (+3). The lowest percentage of positive reaction (14.29%) for CEA was found in serous cystadenocarcinomas, all the cases belonged to (+1) degree of reaction (Table II).

Figure 4

Table (IIb) : Serum CEA in each histological type of ovarian malignancy according to reaction to CEA tissue stain

Histological Type	Reaction of Tissue to CEA stain	No.	Serum CEA Before treatment Mean	S.D	S.E	T	df	P
His - 5	- ve	3	3.126	0.807	0.466	2.2195	7	0.0619
	+ ve	6	13.138	7.531	3.075			
His - 6	- ve	6	2.04	0.614	0.251	.	.	.
	+ ve	1	10.23	-	-			
His - 7	- ve	0	-	-	-	.	.	.
	+ ve	2	14.753	9.158	6.476			
His - 8	- ve	3	4.511	1.245	0.719	.	.	.
	+ ve	0	-	-	-			

Figure 5

Aftertreatment Mean	S.D	S.E	T	df	P
0.571	0.144	0.066	0.674	7	0.5221
0.874	0.749	0.306			
0.655	0.326	0.133	.	.	.
0.457	-	-			
-	-	-	-	-	-
1.305	0.318	0.225			
0.629	0.682	0.394	.	.	.
-	-	-			

Figure 6

Difference							
Mean	S.D	S.E	T	df	P	t	P
3.555	0.919	0.53	2.233	7	0.0607	4.817	0.0405
12.264	7.251	2.96				4.143	0.009
1.385	0.551	0.225	-	-	-	6.155	0.0016
9.773	-	-				-	-
-	-	-	-	-	-	-	-
13.448	9.477	6.701				2.007	0.2943
3.882	1.924	1.111	-	-	-	3.495	0.073
						-	-

As shown in table (II), mucinous cystadenocarcinomas were the only group of malignant ovarian tumors with a number of positively and negatively stained cases suitable for comparison of serum levels. However, all the serum levels of CEA pre-, post-, and difference for this histological type of ovarian malignancy showed no significant difference between the positively and negatively stained cases. The mean difference between pre-and post-treatment serum CEA was significant in mucinous cystadenocarcinoma with negative and positive tissue reaction and in negatively stained serous cystadinomacarcinoma.

Figure 7

Table (IIIa) : Serum CEA in different degree of tissue stain of ovarian carcinomas

Degree of Tissue stain		No.	Serum CEA					
			Before Treatment			After Treatment		
		(%)	Mean	S.D	dt	Mean	S.D	dt
- VE	0	12	2.929	1.302	c	0.627	0.370	a
		(57.14)						
	+ 1	7	9.999	2.737	b	0.843	0.729	a
+ VE		(33.33)						
	+ 3	2	24.286	4.323	a	1.207	0.179	a
		(9.52)						

Figure 8

Difference					
Mean	S.D	dt	S.E	T	P
2.302	1.456	c	0.420	5.475	0.0002
9.157	2.677	b	1.012	9.048	0.0001
23.080	4.144	a	2.93	7.876	0.0804

dt : Duncan's multiple range t- test

T : Paired t-test

Means with the same letters are not significantly different at P = 0.05

S.D : Standard deviation

S.E : Standard error

Figure 10

Grade of Malignancy	Reaction of Tissue to CEA stain	No. (%)	Serum CEA					
			Before treatment					
			Mean	S.D	S.E	T	df	P
G I	- ve	4 (19.05)	2.72	0.941	0.47	2.903	4	0.044
	+ ve	2 (9.52)	9.855	5.438	3.845			
G II	- ve	4 (19.05)	1.971	0.716	0.358	2.832	7	0.0254
	+ ve	5 (23.80)	13.48	7.993	3.574			
G III	- ve	4 (19.05)	4.096	1.311	0.656	3.316	4	0.0295
	+ ve	2 (9.52)	15.73	7.78	5.499			

Figure 11

Table (IV) : Serum CEA before and after treatment and the difference between them in different clinical stages of ovarian carcinoma and according to the degree of reaction to CEA immunostaining

Clinical stage	Reaction of Tissue to CEA stain	No. (%)	Serum CEA					
			Before treatment					
			Mean	S.D	S.E	T	df	P
I	- ve	5 (23.81)	2.676	0.885	0.396	4.413	6	0.0045
	+ ve	3 (14.29)	8.513	2.876	1.661			
II	- ve	4 (19.05)	2.059	0.721	0.361	3.731	7	0.0074
	+ ve	5 (23.81)	16.56	7.639	3.416			
III	- ve	3 (14.29)	4.51	1.245	0.719	-	-	
	+ ve	1 (4.76)	10.23	-	-			

The percentages of cases of ovarian carcinomas with positive pre-treatment serum CEA increased with the increase of grade of malignancy from 33.33% in G1 to 55.56% in G II and 66.67% in G III. After treatment the serum CEA levels of all the cases were within the negative range (< 5 ng/ml).

The mean serum CEA before and after treatment and the mean difference in serum levels showed no significant change on comparing the three histological grades of malignancy.

No definite relation could be detected between the grade of malignancy and the intensity (degree) of reaction of

malignant ovarian tumors to CEA immunostaining.

The mean pre-treatment serum CEA levels were significantly higher in positively stained tumor in comparison with the negative ones in each grade of malignancy.

The mean difference in serum CEA levels was significantly higher in positively stained tumors in comparison with the negative ones in each grade of malignancy (Table III).

The percentage of cases with positive pre-treatment levels of serum CEA increased progressively with the advance in clinical staging. The incidence was 37.5% in stage I, 55.56% in stage II, 66.67% in stage III, and 100% in stage IV. However, the serum levels of all the cases were negative after treatment. No definite relation could be detected between the clinical stages and the degree of reaction of ovarian carcinomas to CEA tissue stain.

The mean pre-treatment serum CEA levels were significantly higher in positively stained ovarian carcinomas in comparison with the negatively stained ones in clinical stage I and II. As for stage III the small number of positively stained tumors did not allow such a comparison.

The mean serum CEA levels after treatment showed no significant difference in relation to tissue staining of ovarian carcinomas in the different clinical stages.

The mean difference in serum CEA levels, was significantly higher in positively stained ovarian carcinomas in comparison with the negatively stained ones in clinical stages I and II. The mean difference in serum CEA levels were significant in the positively and negatively stained malignant ovarian tumors in stages I and II. The results were insignificant in stage III.

Comparison of the mean serum CEA levels before and after treatment and the mean difference in serum levels in the different clinical stages showed no significant relation.(Table IV)

Figure 12

Serum CEA													
After treatment						Difference							
Mean	S.D	S.E	T	df	P	Mean	S.D	S.E	T	df	P	t	P
0.616	0.134	0.059				2.06	1.011	0.452				4.559	0.0104
			0.287	6	0.7841				4.198	6	0.0057		
0.544	0.562	0.324				7.968	3.016	1.742				4.575	0.045
0.64	0.407	0.204				1.418	0.5	0.28				5.066	0.0148
			1.631	7	0.1469				3.565	7	0.0092		
1.244	0.639	0.286				15.31	7.672	3.431				4.464	0.0111
0.629	0.682	0.394				3.282	1.924	1.111				3.495	0.073
			-	-	-				-	-	-		
0.457	-	-				9.773	-	-				-	-

{image:12}

T : Student's t-test
t : Paired t-test

DISCUSSION

HISTOLOGICAL TYPE OF OVARIAN TUMORS

From the data of the present study, CEA is more efficient in mucinous than in serous cystadenocarcinoma, while in benign tumors showed very low sensitivety. CEA was most frequently detected in mucinous tumors(10) .Inoue et al($_{11}$) , found positive pre-treatment serum CEA in 21.42% of the cases of serous cystadenocarcinomas (cut-off level 2.5 ng/ml),while Kazuya et al($_{12}$) noted positive immunostaining in 13.8 % of serous cystadenocarcinoma and in 46.2 % of mucinous cystadenocarcinoma .Motoyama et al($_{13}$) and Phocas I(3) , using a technique of serum assay different from ours did not detect positive serum CEA levels (> 2.5 ng/ml) in any of the studied serous adenocarcinomas,and noted positive pre-treatment serum CEA (> 2.5 ng/ml) in 42.85% and 30.8% respectively of the cases of mucinous cystadenocarcinomas. While the incidence of Inoue et al.(11) , was 22.58% (using our method of serum assay but a lower cut-off level 2.5 ng/ml).

As regards undifferentiated carcinomas the incidence of positive serum levels in the current study was comparable to that of stall and Martin($_{14}$) . In patient with serous cystadenocarcinoma low level of CEA and high CA 125 was a characteristic feature in the serum, while in patients with mucinous cystadenocarcinoma, high CEA and low CA 125 was generally found in the serum.($_{15}$)

The simultaneous production of CEA probably leads to a simultaneous elevation of the serum levels of these antigens. Pure type of malignant serous tumors is not likely to have CEA.High serum levels of CEA were frequently found in patients with tumors that contained cells stained for

CEA.(13)

The benign and borderline ovarian tumors in this study were negative for CEA tissue stain. Previous investigators have evaluated the distribution of CEA in ovarian epithelial neoplasia, all of whom reported positive staining in many cases, particularly in mucinous tumors.(3, 10. 11, 12, 13, $_{16}$) .Rutanen et al. ($_{17}$), reported positive staining of serous ovarian tumors in about 12% of the cases. Negative tissue stain of benign serous ovarian tumors was found by previous investigators(10,13), but they used a different method of tissue staining. However, Tohya et al. ($_{18}$) and Neunteufel and Breitenecker ($_{19}$) , using our technique of immunostain, did not also detect positive reaction among benign serous ovarian tumors. Positive staining of benign mucinous ovarian tumors was noted by previous investigators(10,13,17) .

In this study the highest percentage of positive staining in malignant ovarian tumors was found in squamous cell carcinomas (100%), followed by mucinous cystadenocarcinomas (66.7%), and finally serous cystadenocarcinomas (14.3%). The frequency of positive staining of mucinous ovarian carcinomas showed wide variation in the literatures.

Oishi et al (15) reported that immunostaining of CEA was strongly positive in mucinous cystadenocarcinoma associated with high serum CEA levels, tissue localization of CEA in serous cystadenocarcinomas was negative. There was a good correlation between serum CEA and immunohistochemical tissue levels of CEA in mucinous cystadenocarcinoma. (15)

Van Nagell et al ($_{20}$) , Heald et al. ($_{21}$) , Tohya et al. (18) and Neunteufel and Breitenecker (19) using polyclonal antiserum for tissue staining (same technique used in the present study) reported positive staining in 20%, 100%, 73% and 81% of their cases respectively. On the other hand charpin et al.(10) , Motoyama et al. (13) and Hammond et al. ($_{22}$) , using monoclonal antiserum in immunohisto - chemical staining found positive results in 88%. 100% and 80% of the cases in the same order. Staining intensity of CEA in serous carcinomas was lower than that in mucinous carcinomas. ($_{23}$)

A great variability was also observed in the incidence of serous ovarian carcinomas showing positive reaction for CEA tissue staining. Previous authors used polyclonal antiserum , founded that all serous ovarian carcinomas had a negative reaction (18,$_{24}$) . While Neunteufel and Breitenecker (19) and Dabbs and Geisinger ($_{25}$) , reported 21% and 52% as incidences of positivity respectively. On the other hand, charpin et al. (10) , Motoyama et al. (13) and Hummond et al.(22) , using monoclonal antiserum in their techniques found positive cases of serous ovarian carcinomas in 0%, 0% and 24% of their studied groups respectively.

As regards undifferentiated carcinomas the results of the present study were consistent with the findings of Hammond et al. (22) , (using monoclonal antiserum). and Fleuren and Nap ($_{26}$) , (using polyclonal antiserum). Dietel et al. ($_{27}$) , attributed these wide variations in results to the differences in antisera applied (mono or polyclonal), absorption procedures used for antiserum purification, and antiserum dilutions used for immunoassay (27).

Two degrees of positivity of staining were detected among ovarian carcinomas in the current study. Casper et al. ($_{28}$) , showed that positively stained serous and mucinous carcinomas were distributed only between 2 levels of positivity but their grading system for positive reaction was different from that used in the present study. Motoyama et al.(13) , noted positive staining reaction in mucinous ovarian carcinomas only not in the serous ones. They used 3 levels of positivity for grading of tissue reaction. The positively stained mucinous lesions were distributed between the 3 levels but the main bluk (82% of the cases) were in the first level. However, it should be noted that their technique of immunostaining and the system of grading of tissue reaction were different from those of our research (13). Charpin et al. (10) , using the same grading system of our study, found that positively stained mucinous ovarian carcinomas were distributed between 3 levels of positivity mainly in (+ 2) level (67% of cases), but they used monoclonal antisera for tissue staining.

A relation could be observed in the current investigation between pre-treatment serum levels of CEA and the reaction of tissues to CEA immunostaining in malignant ovarian tumors. These results are consistent with the findings of Motoyama et al. (13) , as they considered that immunohistochemical identification of a marker in tumor tissue is a pre-requisite to the use of that marker in the serum to monitor disease status. The only exception to this finding is the undifferentiated carcinomas because all of them were negative for CEA immunostaining but 66.67% of the cases had positive serum levels.

McDicken and Rainey ([29]), found that in the lesions with more undifferentiated cell forms CEA may be masked and difficult to be demonstrated immunohistochemically. They recommended pre-treatment of tissues with trypsin to facilitate exposure of cellular antigens. This finding may explain the apparent controversy shown before in undifferentiated carcinomas.

HISTOLOGICAL GRADE OF MALIGNANCY OF OVARIAN CARCINOMAS

The percentage of cases with positive pre-treatment serum CEA values increased with the increase in grade of malignancy as shown in this study. After treatment all ovarian carcinomas with different grades of malignancy showed negative serum CEA values (< 5 ng/ml) as shown in table (III). Similar results were observed by previous investigators(14,20,[30])

So generally we can assume that no definite relation could be proved between the histological grade of malignancy and the intensity of staining reaction in the present study. Breiteneckere et al. ([31]) and Tholander et al ([32]) using the same technique of immunostaining could not also find a relation between these 2 variables.

Positive stain of benign and borderline tumors were mostly located on the lumina border while malignancies were situated both on the lumina border and in the cytoplasm and tended to concentrate in the latter with the increase in histological grade. (23)

After treatment a marked drop in serum CEA occured in the different grades. These results are consistent with those of previous reports(14,32)

CLINICAL STAGING OF OVARIAN CARCINOMAS

The percentage of cases with positive pre-treatment levels increased progressively with the advance in clinical staging. (Table IV). These results are consistent with the findings of Motoyama et al. (13) and Magon et al. ([33]) , found an elevated pre-treatment serum CEA levels in 16.67% of the patients with stage I disease, the incidence increased to 38.89% in those with stage III, but all the cases with stage II disease were seronegative for CEA as the majority of them were of the serous type which is not usually associated with a high incidence of elevated serum CEA. Tholander et al. (32) , showed elevated serum CEA values in 53% of the patients with limited disease (stage I and Iia) and in 20% of those with extensive disease (stage IIb, III and IV), but they used a different technique of serum assay (polyclonal rabbit antiserum) and the majority of their cases (86.6%) were non mucinous carcinomas.

Massi et al. ([34]) , reported a decreased incidence of elevated serum CEA with advance of the disease stage, but most of their cases were of the serous and undifferentiated types. Kazuya et al(12) reported that CEA level was not correlated with the clinical stage suggesting that this tumor marker may be more useful in screening for early stage disease.

The positively stained malignant ovarian tumors in the different clinical stage were distributed between 2 levels of positivity (+1) and (+3). No specific relation could be found between clinical staging and degree of tissue staining in the current work. The same finding was also observed by Motoyama et al. (13) .

On comparing the mean values of pre-and post-treatment serum CEA and the mean difference between them in the different clinical stages, no statictically significant difference can be detected between them as shown in table (IV). These results are comparable to those of Tholander et al. (32) and Koh and Cauchi ([35]) . All of them observed no correlation between the absolute level of the marker and stage of the disease in positive serum cases.

Several factors were shown to affect the serum and tissue levels of CEA ,e.g histological type of tumors ,grade of malignancy ,clinical stage of disease, technique of serum assay and tissue staining and the cut-off level of CEA in serum. These factors must be put in consideration before evaluating the results of CEA assay.

In conclusion this study indicates that immuno-histochemical identification of CEA in tumor tissue and of monoclonal antibodies quantitative measurement of CEA in human serum is a useful adjunct in the management protocol of patients with ovarian malignancies. However further studies are required to fully ascertain the usefulness of this technique.

CORRESPONDENCE TO

Dr. Nabil El-Tabbakh Hadi Hospital P.O.Box : 44630 Hawally 32061 Kuwait. Telephone : 5327849 Fax : 531 4717 E-mail : neltabbakh@yahoo.com

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Author Information

Ahmed H Askalani, MD
Department of Obstetrics and Gynecology, Faculty Of Medicine, Al Azhar University

Hasan A Meabid, MD
Department of Obstetrics and Gynecology, Faculty Of Medicine, Al Azhar University

Saad El Sadek M El Sadek, MD
Department of Obstetrics and Gynecology, Faculty Of Medicine, Al Azhar University

Nabil M El Tabbakh, MD
Department of Obstetrics and Gynecology, Faculty Of Medicine, Al Azhar University

Mohamed S M Osman, MD
Department of Obstetrics and Gynecology, Faculty Of Medicine, Al Azhar University