

Original Article

Enzyme-Linked Immunosorbent Assay and Immunohistochemical Localisation of Carcinoembryonic Antigen in Ovarian Neoplasia

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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 divided into three groups: Group A included 21 patients with malignant ovarian tumors. Group B included three patients with borderline ovarian tumors. Group C included eight patients with benign ovarian tumors. Group D included 36 women without any apparent gynecologic disorder and acted as our control group. The serum level of CEA was measured in all patients in Groups A, B and C prior to treatment and at least 12 weeks following therapy. Formalin-fixed and paraffin-embedded tissue blocks taken from two sites of the studied lesions were prepared. Immunohistochemical staining for CEA was performed for the studied tissues.

Results: All benign and borderline ovarian tumors had negative pre- and post-treatment serum levels of CEA (< 5 ng/ml) while 52.38% of malignant ovarian tumors had positive pre-treatment serum values. After treatment, all the malignant ovarian tumors were seronegative for CEA. The mean pre-treatment serum CEA level in malignant

ovarian tumors (7.32 ng/ml) was significantly higher than that of the other groups. The mean post-treatment serum values and the mean difference in serum levels showed no significant differences between the three groups. The mean difference between pre- and post-treatment serum CEA was significant only in malignant ovarian tumors. A total of 12.5% of the benign ovarian tumors, and 42.86% of the malignant ones had a positive reaction for CEA tissue stain. The mean values of serum CEA before treatment were significantly higher in positively stained malignant ovarian tumors ($P < 0.0001$). The mean difference in the serum CEA was significantly higher in positively stained malignant ovarian tumors ($P < 0.0001$). The mean pre-treatment serum CEA and also the mean difference in serum levels showed significant progressive increase with the increase in degree of tissue stain of ovarian carcinomas.

Conclusion: This study indicates that immunohistochemical identification of CEA in the tumor tissue and monoclonal antibodies quantitative measurement of CEA in human serum is a useful adjunct in the management protocol of patients with ovarian malignancies.

KEYWORDS: carcinoembryonic antigen, ovarian tumor, serum and tissue levels

INTRODUCTION

Ovarian masses fall into two broad categories, benign and malignant. The former are a nuisance but rarely dangerous while the latter are the most lethal of the common gynecological malignancies. The surgical management of ovarian cancer is complex and often involves gastrointestinal surgery. Differentiating between benign and malignant masses is of paramount importance^[1,2]. In the US, ovarian cancer is the fifth leading cause of cancer death from gynecologic malignancies. Most patients, however, are diagnosed with advanced-stage diseases when the prognosis is poor, despite radical surgery and combined chemotherapy^[2]. More than 70% of cases are diagnosed at an advanced stage, where 5 years survival approaches only 20%^[3].

If detected in the early stages, ovarian cancer can be cured. Consequently, there is an increasing need for a reliable cost-effective method for detecting ovarian cancer early. Unfortunately though, despite advances in surgical technique and novel chemotherapeutic agents, survival rates have not improved significantly over past 25 years^[4].

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^[5]. Carcinoembryonic antigen (CEA) is one of the first known tumor markers. Since its discovery, many more have been

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described, but CEA, determined alone or in combination with others, is still one of the most widely used tumor markers. CEA is not organ specific and abnormal values may be found in a wide range of carcinomas^[6].

In this work, we investigate the 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

This study was conducted on patients treated at Egypt's National Cancer Institute, Cairo University and the Department of Obstetrics and Gynecology, Al Hussain Hospital, Al Azhar University. The study included 68 patients: 21 patients with ovarian malignancies, three with borderline ovarian tumors, eight with benign ovarian tumors and 36 patients without any apparent gynecologic disorder who acted as the control group. The patients were divided into the following groups:

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

Group B: Included three patients with borderline ovarian tumors, two of whom were borderline mucinous tumors 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 gynecologic disorder. They were age matched with the tumor patients and serum samples taken from them were subjected to CEA measurement and considered as control.

All the cases in Groups A and B, 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 Gynecology and Obstetrics (FIGO) staging systems^[7]. Staging of ovarian cancer showed eight patients with stage I, nine with stage II, three with stage III and one patient with stage IV.
3. Serum samples were collected from the patients in Groups 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 of the tumor at the time of the post-treatment sample as proven 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 the sandwich principle. A positive result for CEA in serum was taken as 5 ng/ml.
5. Surgical specimens from ovarian tumors, taken from two 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 diagnosis confirmation and for selection of blocks for study. Serial sections, not more than 5 µm 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 (Dako, Carpinteria, CA) for 60 minutes and with polyclonal enzyme (Dako, Carpinteria, CA) for another 60 minutes at room temperature. The specimens were stained by the DAB (diaminobenzidine) working color reagent and incubated for 5-10 minutes and counterstained with haematoxylin for 30-60 seconds.

The grading system introduced by Charpin et al^[8] was utilized to quantify the staining positivity as follows :

- (0) Denoting negative reaction, i.e. no difference from the control sections.
- (+1) Up to 25% of the cells were positive
- (+2) > 25 - 50% of the cells were positive
- (+3) > 50 - 75% of the cells were positive
- (+4) 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. 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. Student's t-tests (procedure test of SAS) were 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. Sensitivity, specificity, positive predictive value and negative predictive value were calculated using 2 x 2 table.

RESULTS

All benign and borderline ovarian tumors had negative pre- and post-treatment serum levels of CEA (< 5 ng/ml) while 52.38% of the malignant ovarian tumors had positive pre-treatment serum values (> 5 ng/ml). After treatment all the malignant ovarian tumors were sero negative for CEA. The sensitivity, specificity, positive predictive value and negative predictive value of CEA were 34%, 67%, 52% and 50%, respectively.

The mean pre-treatment serum CEA in malignant ovarian tumors (7.32 ng/ml) was significantly higher than that of the other groups, whereas the mean post-treatment serum values and the mean difference in serum levels showed no significant differences between the three types of ovarian tumors (Table 1).

The mean difference between pre- and post-treatment serum CEA was significant only in malignant ovarian tumors (Table 1). When comparing the mean difference between pre- and post-treatment, serum CEA was highly significant in malignant ovarian tumors only (Table 1). On comparing this mean difference with those of benign and borderline tumors, it was found insignificant because of the large standard deviation (SD) of the malignant group (reflecting a wide range of variability) which affects the significance of results.

As shown in Table 2, 12.5% of the benign

ovarian tumors, and 42.86% of the malignant ones had a positive reaction for CEA tissue stain, while all the borderline tumors showed a negative reaction. Of all the positively stained ovarian tumors, 90% were malignant. The mean values of serum CEA before treatment were significantly higher in positively stained malignant ovarian tumors (13.174 ng/ml) in comparison with the negative ones (2.929 ng/ml). The other two groups were not valid for such a comparison as they were almost devoid of positive cases.

The mean serum CEA levels after treatment were decreased in all types of ovarian tumors. This decline was most marked in malignant ovarian tumors but with no significant differences between these cases according to their stain reaction. The mean difference in serum CEA was significantly higher in positively stained malignant ovarian tumors in comparison with the negative ones.

The mean difference between pre- and post-treatment serum CEA was highly significant in the positively and negatively stained malignant tumors (Table 2).

All the positively stained ovarian tumors (12.5% of this group) belonged to (+1) degree of reaction. Positive staining of malignant ovarian tumors was detected in 42.86% of the studied cases, most of them (33.33%) had a (+1) degree of reaction and only 9.52% showed a (+3) level of positivity.

A total of 83.33% of the negatively stained ovarian carcinomas had negative pre-treatment serum CEA levels and 16.67% showed positive serum values. All positively stained ovarian carcinomas had positive pre-treatment levels of serum CEA. The post-treatment serum CEA was negative for all the studied cases of ovarian carcinomas.

The mean difference between pre- and post-treatment serum CEA was highly significant in negatively stained tumors (0) and in positively stained tumors with (+1) degree of reaction.

The mean pre-treatment serum CEA and also the mean difference in serum levels showed

Table 1
Serum CEA before and after treatment in different types of ovarian tumours

Type of Tumor	No.	Serum CEA											
		Before Treatment			After Treatment			Mean		Difference		T	P
Mean	SD	dt	Mean	SD	dt	Mean	SD	dt	S.E				
Benign	8	1.13	0.615	b	0.9	0.582	a	0.23	0.763	a	0.27	0.853	N.S
Borderline	3	1.929	1.341	b	0.9	0.145	a	1.028	1.195	a	0.69	1.49	N.S
Malignant	21	7.32	6.854	a	0.754	0.519	a	6.566	6.686	a	1.459	4.5	0.0002
Control Group	25	1.024	0.865	b	-	-	-	-	-	-	-	-	-

ovarian carcinomas investigated by Inoue et al^[9], and in 33% of cases with frankly invasive epithelial ovarian carcinoma investigated by Roman et al^[11].

The mean pre-treatment serum CEA was significantly higher in malignant ovarian tumors in comparison with the other groups (Table 1). This is in agreement with the findings of Inoue et al^[9].

After treatment serum CEA was found to have decreased in all tumor patients. The findings in this respect are comparable to those of previously reported studies^[13,14].

REACTION OF OVARIAN TUMORS TO CEA IMMUNOSTAIN

In the current work, 12.5% of the benign ovarian tumors were positive for CEA tissue staining (Table 2). Tohya et al^[15] showed positive staining in 25% of the cases of benign ovarian tumors but all their studied cases were of mucinous type. Neunteufel and Britenecker^[16] reported a lower incidence (16.67%) of positive staining. The technique of immunohistochemical staining used by this group of investigators was the same as ours. Using different methods of tissue stain, Motoyama et al^[17] found positive staining of benign ovarian tumours in 30.9% of the cases.

All the borderline ovarian tumors showed a negative reaction for CEA tissue staining (Table 2). This result is contradictory to the 55% positive staining of Tohya et al^[15], 11% of Dietel et al^[14], 31.25% of Neunteufel and Britenecker^[16], and 70% of Motoyama et al^[17]. The borderline lesions in the present study did not show definite CEA immunostaining.

As a whole, 90% of all the positively stained ovarian tumors were malignant (Table 2). These results are consistent with the incidence of positive staining of ovarian carcinomas (45.45%) detected by Neunteufel and Britenecker^[16] using the same tissue staining technique.

Tohya et al^[15] found positive staining in 100% of the cases of ovarian carcinomas but all the studied cases were of the mucinous type. Motoyama et al^[17] reported positive staining of ovarian carcinomas in 37.79% of the cases but they employed methods of immunostaining different from that used in the present investigation.

The mean pre-treatment serum CEA in positively stained malignant ovarian tumors (13.174 ng/ml) was highly significant in comparison with the negatively stained ones (2.929 ng/ml) as observed in Table 2. These findings are in agreement with those of Motoyama et al^[17].

Degree of positivity of staining reaction of ovarian tumors:

As shown in Table 2, 12.5% of the benign ovarian tumors showed positive staining for CEA, all of

them were in the (+1) degree of positivity. Charpin et al^[8] showed a (+2) degree of positivity (same grading system of tissue staining reaction) but they used a different method of immunostaining and their cases were of the mucinos type only.

Positive staining in malignant ovarian tumors was found in 42.86% of the studied cases, 33.33% of them had a (+1) degree of reaction, while the remaining 9.52% showed a higher level of positivity (+3), as shown in Table 2. Charpin et al^[8] found that positively stained ovarian carcinomas were distributed between three levels of positivity as follows: 13.04% in (+1), 13.04% in (+2) and 6.52% in (+3). Although they had the same system of grading of tissue staining, they used a different technique of immunohistochemical staining. Motoyama et al^[17] found different percentage distributions of positively stained ovarian carcinomas among the various grades of positive staining reaction. However, direct comparison with their results is not possible because they used a different grading system and staining techniques.

Among the negatively stained ovarian carcinomas, 83.33% had negative pre-treatment serum CEA levels (< 5 ng/ml), and 16.67% had positive levels (> 5 ng/ml). All the positively stained malignant ovarian tumors (+1 and +3 degrees) showed a positive pre-treatment serum CEA. After treatment, all the studied ovarian carcinomas were seronegative for CEA. The association between the positivity of tissue stain for CEA and the positive serum levels was also proven by Motoyama et al^[17].

Although the mean pre- and post-treatment serum CEA were below the cut-off level of the present study in negatively stained ovarian carcinomas (degree "0"), the mean difference in serum levels were significant. An important finding which should be considered here is that 16.67% of negatively stained carcinomas had positive pre-treatment serum CEA levels (> 5 ng/ml). In the (+1) degree, the result is comparable to those of Motoyama et al^[17], and is a logic outcome of the high mean pre-treatment and low mean post-treatment serum levels. As regards the (+3) level of positivity, the mean difference in serum CEA levels were insignificant because of the small number of cases in this group.

In conclusion, this study indicates 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. Further studies are, however, required to fully ascertain the usefulness of this technique.

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