ROLE OF HEAT STABLE FRACTION OF ALKALINE PHOSPHATASE AS AN ADJUNCT TO CA 125 IN MONITORING PATIENTS OF EPITHELIAL OVARIAN CARCINOMA

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ABSTRACT

Heat stable fraction (HSF) of alkaline phosphatase (ALP) was evaluated as an adjunct to CA 125 as a tumour marker for epithelial ovarian cancer in a follow-up study. In our study group 63.4% of patients had elevated HSF levels (≥ 10 U/L) and 93.3% had elevated CA 125 levels (>35 U/mL). The sensitivity of CA 125 and HSF was 93.3% and 63.3% respectively. The decline in the activity of HSF, over the pre-op levels was highly significant after the first ($p=0.001$) chemotherapy cycle and significant after the second and third cycles ($p<0.029$). Thereafter, HSF activity was almost undetectable. The decrease in CA 125 levels over the pre-op levels was significant after the first, second, third ($p < 0.001$) and fourth ($p<0.034$) chemotherapy cycle. HSF can be used alone or as an adjunct to CA 125 in screening and monitoring patients of ovarian carcinoma especially in remote areas where sophisticated facilities are not available and in patients in which CA 125 levels are not raised preoperatively.

KEY WORDS

CA 125, Heat stable fraction of ALP, Ovarian cancer, PLAP, Tumour marker.

INTRODUCTION

Ovarian carcinoma remains near the top in the mortality statistics of all gynaecological cancers (1) and is the fifth leading cause of cancer deaths in women today. Epithelial cell carcinoma being the commonest of all ovarian malignancies (80-90%). The mortality relates, at least in part, to the fact that two-thirds of the patients present at FIGO stage III and IV due to the insidious onset of the disease. Majority of patients with early / potentially curable disease voice no complaints.

To circumvent the shortcomings of diagnostic imaging techniques like CT Scan (2), ultrasound and the drawbacks of repeated surgical procedures, tumor markers are used for management of epithelial ovarian carcinoma. Cancer Antigen 125 (CA125) has been described as a useful marker and its role in monitoring therapy is indisputable as its levels correlate with

disease progression and regression in 80-90% of cases (3).

The lack of total specificity of CA 125 requires the development of HSF as a useful adjunct to the use of CA125 in the management of ovarian carcinoma (4). Non-tumoral clinical conditions such as peritonitis, pancreatitis and chronic liver disease that are associated with high serum levels of CA 125 in the range observed for ovarian and other tumours do not show similar elevation of Placental alkaline phosphatase (PLAP) (4). Levels of PLAP like enzymes (Regan type or neoplastic forms) remain elevated in ovarian carcinoma and are heat stable at 65°C, constituting the heat stable fraction of alkaline phosphatase (HSF).

In view of high mortality in women due to epithelial ovarian carcinoma and lack of high specificity of imaging techniques, this study was undertaken to evaluate the role of placental alkaline phosphatase alone as a tumor marker or as an adjunct to the available marker CA 125.

MATERIALS AND METHODS

The prospective study was conducted jointly in the Departments of Biochemistry, and Obstetrics and Gynecology, Lady Hardinge Medical College and Smt. Sucheta Kriplani Hospital, New Delhi, India.

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32 subjects were selected after informed consent, from the Gynecological wards of Smt. Sucheta Kriplani Hospital. The study was approved by the institutional ethical committee. The study subjects were divided into two groups:

Study group: 16 women with histologically diagnosed epithelial ovarian carcinoma.

Control group: 16 healthy women matched in age and parity to women in study group.

Patients in the study group were monitored for a period of six months. Serum samples were collected during pre-op, post-op, and post chemotherapy (after each cycle/monthly). Single sample was taken from the control group.

Inclusion criteria: Only confirmed cases of epithelial ovarian carcinoma were followed up in the study.

Exclusion criteria: Women with any other malignancy besides ovarian were excluded from the study. Also excluded were pregnant women, women with endometriosis, pelvic inflammatory disease, pancreatitis, hepatitis, cirrhosis, lymphoma, pericarditis, peritonitis and renal failure.

Stage of the disease was classified in accordance with International Federation of Gynecologists and Obstetricians criteria (5). The histological grading was performed in accordance with the WHO classification (6). Total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy and in some appendectomy were the standard surgical procedures used for treatment.

Postoperatively all patients received combination chemotherapy including cis-platinum (50 mg/m²), cyclophosphamide (500 mg/m²) and /or doxorubicin (50 mg/m²). One patient received a regimen that included Taxol.

Venous blood samples were taken from each patient at predefined stage under sterile conditions using disposable syringe and needle. Fresh sera were used for routine investigations and for measurement of HSF by thermal inactivation method described by Fishman *et al.* (7). Rest of the sera was stored at -20°C till CA 125 was batch analyzed using Quorum Diagnostics kit by EIA. Serum total ALP was estimated using enzymatic rate method described by Hausamen *et al.* (8) on Synchron CX5 Clinical Chemistry auto analyzer (Beckman) using Randox kit. All the data was analyzed on SPSS (statistical package and subjected to Students) 't' test (independent and paired) for statistical evaluation.

RESULTS AND DISCUSSION

In our study the mean age in the study group was 45.3±12.9 years with 43.75% patients belonging to

peri/ post menopausal age group (Refer Table 1). According to Young *et al.* (9), highest incidence is found in postmenopausal women.

Table 1. Important findings in study group

Study group	
Mean age (years)	45.3±12.9
Mean parity	2.3±1.4
No. of patients with family history of cancer	2
Bilateral occurrence (%)	81.3%
Ascites	Present in 50%
Patients in stage III and IV	87.5%

The mean parity in the study group was 2.3±1.4 with 12.5% patients being nulliparous (Refer Table 1). There was no grand multipara. This relates well with the fact that a higher incidence of epithelial ovarian tumors is seen in women with a lower mean number of pregnancies and in women with infertility (10, 11). In our study, 12.5% patients gave a family history of cancer though not of ovarian cancer.

As seen in our study, ovarian cancer patients usually don't present with any menstrual symptoms unlike other gynecological malignancies. This also contributes to the delay in diagnosis.

Ovarian malignancy is known to occur bilaterally in majority of patients (12). 81.3% of patients showed the same in our study. Ascites was seen in 50% of patients in the study group.

In the study, 68.75% patients belonged to stage III, followed by 18.75% patients in stage IV and 12.5% in stage II (Refer Table 1). No patient was diagnosed as stage I. According to Silveston *et al.* (12), 57% patients belonged to stage III and IV at the time of diagnosis. This may be because of the fact that ovarian carcinoma lacks any specific diagnostic symptoms. This could also be due to the tendency of uneducated Indian women of procrastination and for social reasons, in seeking medical advice especially for non-specific symptoms.

The mean pre-op level of total ALP in our study group was 261.5±96.7 U/L with significant difference (p=0.001) from the control group (Refer Table 2). The increase in total ALP could be because of relative increase in HSF in the study group.

In our study group, the mean pre-op level of HSF was 14±11.4 U/L which showed significant difference

Table 2. Mean pre-operative levels of ALP, HSF and CA 125

	Mean pre-op serum level in U/ml	
	Study group	Control group
Total ALP	261.5±96.7	164.1±43.2
HSF	14.0±11.4	2.8±2.1
CA 125	1066.6±1081.8	9.3±4.0

($p=0.001$) from pre-op level in control group. 63.4% of patients in the study group had elevated HSF levels (≥ 10 U/L) and none of the controls had increased HSF levels (<10 U/L). Nozana *et al.* (13) reported high level in 60% of patients of serous cystadenocarcinoma of ovary and also reported that none of the healthy women studied were PLAP positive. Elevated levels of HSF have been reported by a number of other investigators in 30-60% of ovarian carcinoma (14, 4).

In our study group, the mean pre-op CA125 level was 1066.6 ± 1081.8 with 93.3% patients with level greater than 35 U/ml. None of the control samples showed elevated CA 125 levels. The difference from control group was highly significant ($p=0.001$). Bast *et al* (15) also reported CA 125 levels > 35 U/ml in 91% patients.

The mean post-op level of HSF was 8 ± 8.3 U/L in our study group with highly significant ($p=0.001$) difference between pre-op and post-op HSF levels (Refer Table 3). The sudden significant decrease seen postoperatively could be because of the bulk removal of tumor tissue thereby reducing tissue source of HSF production.

The mean post-op CA 125 level in our study group was 411.6 ± 754.2 U/L with a mean decrease over the pre-op levels being 61% and this difference was highly significant ($p=0.001$).

Table 3. Pre and post operative levels of HSF and CA125

	Mean (U/L)	p value
HSF		
Pre-op	14.3	0.001
Post-op	8.0	0.001
CA 125		
Pre-op	1066.6	0.001
Post-op	411.6	0.001

In our study the decline in the activity of HSF, over the pre-op levels was highly significant after the first ($p=0.001$) chemotherapy cycle. It also decreased significantly after the second and third cycles ($p<0.029$). Thereafter, HSF activity was almost undetectable.

In our study group the decrease in CA 125 levels over the pre-op levels was significant after the first, second, third ($p<0.001$) and fourth ($p<0.034$) chemotherapy cycle. A persistently high level after several rounds of chemotherapy is the single most important prognostic variable (16).

In our study, the sensitivity of CA 125 was 93.3% and of HSF 63.3%. Specificity of both was 100%. Positive predictive value of both was 100% and negative predictive value of CA 125 was 93.75% and HSF 78.94%.

We conclude that though the sensitivity of HSF is less than that of CA125, it is useful in patients of ovarian carcinoma where CA 125 is not elevated or is elevated due to other medical conditions. Due to ease of testing and cost effectiveness, HSF of alkaline phosphatase can be used alone or as an adjunct to CA 125 in screening and monitoring patients of ovarian carcinoma especially in remote areas where sophisticated facilities are not available.

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Actual Findings and Study Values

Patient	Age	Parity	CA125_pre	CA125_pos1	CA125_pos2	CA125_pos3	CA125_pos4	CA125_pos5	CA125_pos6	HSF_pre	HSF_pos1	HSF_pos2	HSF_pos3	HSF_pos4	HSF_pos5	HSF_pos6
1	52	2	932	301	76	31	27	46	52	999	999	999	999	999	16	19
2	42	2	9999	9999	9999	164	138	181	9999	999	999	999	11	41	9	999
3	58	3	960	500	86	27	19	9999	9999	7	2	1	3	4	999	999
4	25	2	320	234	27	20	18	20	8	1	1	1	1	1	1	1
5	35	4	462	160	89	9999	9999	9999	9999	10	7	2	999	999	999	999
6	20	0	35	84	142	126	132	128	9999	999	13	14	17	20	19	999
7	53	2	956	366	42	22	9999	9999	9999	10	2	3	1	999	999	999
8	50	2	4275	3100	2800	2327	2115	1783	1419	999	23	17	14	19	10	7
9	54	3	1400	202	162	114	76	42	36	2	1	1	1	1	1	1
10	43	3	936	380	20	14	12	9999	9999	40	30	12	8	4	999	999
11	70	4	840	86	32	30	22	23	9999	13	7	3	1	2	1	999
12	40	2	710	132	74	34	25	9999	9999	26	12	3	1	3	999	999
13	32	0	729	108	55	26	22	9999	9999	22	12	6	2	4	999	999
14	49	3	312	64	22	16	9999	9999	9999	9	7	3	4	999	999	999
15	45	3	432	210	47	26	19	23	18	999	999	999	999	999	1	2
16	56	2	2700	247	64	39	31	9999	9999	14	7	10	11	8	999	999
9999 Represents invalid entry.																

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