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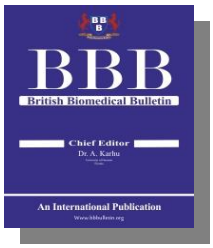

Original

Serum Levels of L-amino Acid Oxidase in Ovarian Cancer Patients

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ABSTRACT

Objective: The objective of this study was to determine the activity of L-amino acid oxidase in ovarian cancer patients.
Material and Methods: In total, 50 women with ovarian cancer enrolled in this study. Serum samples were collected from ovarian cancer patients (n=50) before and after treatment and 50 healthy women were included in this study. In total 150 samples were analysed for the serum levels of Carbohydrate antigen 125 (CA-125) and L-amino acid oxidase (LAAO) activity.
Results: In controls (n=50) the activity of L-amino acid oxidase were found to be 27.56±7.61 U/ml and the levels of CA-125 were found to be 14.62±10.11 U/L. In ovarian cancer patients, the pre treatment serum mean activity of LAAO was found to be increased significantly in serous (p<0.001), endometrioid (p<0.001), transitional (p<0.05), primary peritoneal (p<0.05), and in recurrent carcinoma patients (p<0.001). Post treatment activity of LAAO was found to be decreased compared to pre treatment levels in all histopathologic types of ovarian cancer patients except in those patients that cancer recurred. The pre treatment serum LAAO activity were found to be significantly increased (p<0.001) in patients with stage I & II disease and as well with stage III & IV disease.
Conclusion: Taking potentiality of serum biomarkers into consideration, serum level of LAAO along with CA 125 could be a potential biomarkers in future predictor of cancer recurrence.

Introduction

L-amino acid oxidases (LAAOs, EC 1.4.3.2) are homodimeric glycoproteins, that noncovalently bound flavin adenine dinucleotide (FAD) as a cofactor and catalyze the oxidative deamination of L-amino acids to concomitant reduction of FAD. The Re-oxidation of FAD by molecular O_2 leads to the production of α-keto acids, ammonia and hydrogen peroxide[1]. LAAOs are widely distributed in mollusks, bacterial, avian, plant species, fungal[1], arthropods[2], snake venoms[3], mammals such as rats, horses, dogs and pandas. Mammalian LAAOs were found to belong to three separate classes: (A) LAAO1, (B) LAAO2, (C) Human interleukin 4 induced protein 1 (IL4I1). The LAAO activity of human IL4I1 (hIL4I1) is optimal with phenylalanine as substrate and is highly active at pH 5 to 7.4[4]. hIL4I1 is expressed in myeloid cells, macrophages, dendritic cells, fallopian tube, sertoli cells of testis and brain cells including Purkinje cells, hippocampus and mitral cells in the olfactory bulb[5-8]. This enzyme is yet not proved biomarker for any clinical condition.

Ovarian cancer accounts for over 204,499 new cancer diagnoses annually and over 140,000 deaths worldwide. Over 22,280 new ovarian cancer cases were diagnosed and over 15,500 deaths due to it was estimated in India alone in 2012[9-14]. Current diagnosis of ovarian cancer relies on pelvic exam, transvaginal ultrasonography, and exploratory or diagnostic laparoscopy when evaluating a pelvic mass. CA-125 is commonly used to assess the degree and spread of tumor. Commonly accepted definitions of disease recurrence based on serum CA-125 levels alone specify a doubling of this tumor marker level, either from the upper limit of normal (35U/mL) in patients with normalization of this marker after primary treatment or from the nadir levels in patients with elevated serum marker value that never normalizes after primary treatment[15,16].

The aim of this cross sectional study was to observe the levels of serum LAAO activity in ovarian cancer patients and controls. The levels of LAAO activity were compared with CA-125 levels in patients before and after treatment and in those in whom the cancer recurred.

Materials and Methods

Ethical clearance (IEC150-2012) from the Institutional Ethical committee was obtained to conduct this study. A copy of the Information Sheet & Consent form given to participants and sufficient time is given to consider the research before consent. This study was conducted on patients attending the Department of Oncology, Kasturba Medical College, Manipal University, Manipal. Statistical Package for Social Sciences, version 13.0 for Windows (SPSS Inc., Chicago, IL) was used for statistical analysis. Plain blood samples were collected 1 week before and 3-5 months after initial treatment which included surgery and/or chemotherapy from patients (*n*=50) between the age group 20-70 diagnosed for ovarian cancer by ultrasonography and histopathologic studies. Samples were included irrespective of histopathologic findings and staging. Age and sex matched apparently healthy females were taken as controls (*n*=50). Patients with any infections and diabetes were excluded from the study. 4ml of Samples were collected in red vacutainer, centrifuged; serum was separated and stored at 4-8°C. Samples were analyzed for L-amino acid oxidase along with CA-125.

CA-125 was analysed by electro chemiluminescent immuno assay (ECLIA) in autoanalyser Cobas 6000.

L-amino acid oxidase assay: A reaction mixture containing Tris buffer (80

micro-moles), catalase (60 units), and L-amino acid oxidase (2-30 units) in a final volume of 0.7mL was placed in a test tube. The reaction was started by the addition of 0.1mL of 0.04M L-phenylalanine and the test tube was immediately placed in a water bath at 37^0C. After 15minutes the reaction mixture was transferred to a conical centrifuge tube and centrifuged. A 0.5mL aliquot of the supernatant was transferred to a test tube containing borate-arsenate solution and mixed well. The solution was allowed to stand at least 30 minutes at room temperature, then the absorbance at 300 nm was measured using a blank in which enzyme was omitted. The readings remain constant for several hours. One unit of activity was the amount of enzyme required to give an absorbance of 0.030 at 300nm under the above conditions[17]. Paired t-test and Pearson's correlation were used in statistical analysis.

Result

Fifty patients with ovarian cancer were examined in this study, ranging between the ages 20-70 with mean age of 45±11.7. Demographic details of the patients are given in the Table 1. The stage of cancer, different histopathologic types, patient history including menstrual cycle status, chemotherapy, weight loss and other conditions such as diabetes mellitus, hypertension and anemia are shown in Table 1.

A total of 150 samples were analyzed for serum LAAO and CA-125 levels including 50 controls and 50 patients (both pre and post treatment samples were collected).

In controls (n=50) the levels of L-amino acid oxidase were found to be 27.56±7.61 U/mL and the levels of CA-125 were found to be 14.62±10.11 U/L. Serum LAAO levels were found to be significantly (p <0.001) increased to 2.8 fold more than the levels in controls prior to treatment in ovarian cancer patients (n=50) are shown in table 2.

Post treatment LAAO levels significantly (p< 0.001) decreased but still remained 1.7 fold higher than the control group. CA-125 levels were increased significantly (p< 0.05) in patients within 13 fold when compared to control prior to the treatment. The mean CA-125 levels were found to be decreased but remained 2.8 folds higher than controls after treatment which was statistically not significant (p>0.05). The Pearson correlation between the LAAO levels and CA-125 levels before (r=0.165) and after (r=-0.235) treatment were also found to be statistically not significant.

The pretreatment levels of LAAO were almost similar in all histopathologic types of ovarian cancer. However, CA-125 levels varied in different histopathologic types of cancer with the highest being seen in serous type (49-1350 U/L) and lowest being seen in primary peritoneal type (7-25 U/L).

Post treatment levels of serum CA-125 decreased uniformly in all histopathologic types of ovarian cancer. But the post-treatment serum LAAO levels were reduced differently in different histopathologic types of ovarian cancer with the highest being seen in recurrent carcinoma patients.

In recurrent ovarian cancer patients (n=6) the serum levels of LAAO were significantly increased (p<0.001) 3.2 fold than controls before treatment. Post treatment LAAO levels (72-93 U/mL) continued to remain as much as pre treatment (70-96U/mL) levels. These were found to be statistically significant (p<0.001) when compared to controls. The levels of serum CA-125 were found to be increased significantly (p<0.05) 8.3 folds more in recurrent cancer than controls before treatment and levels decreased within

control range which was statistically insignificant (p=0.126) after treatment. The Pearson correlation between both pre (r=0.032) and post-treatment (r=-0.414) serum levels of CA-125 and LAAO in these patients were found to be statistically not significant, indicating the LAAO continues to be evaluated despite treatment in patients in whom recurrence may be a high probability.

Hence, the role of serum levels of LAAO as a recurrent ovarian cancer marker assumes importance.

In serous type of ovarian cancer patients (n=29) serum LAAO a levels were significantly (p value <0.001) increased three fold more than control levels prior to treatment and after treatment levels decreased significantly (p value <0.001) but remained higher than normal. CA-125 levels in serous type of ovarian cancer patients significantly (p < 0.05) increased 15 folds higher than the control levels before treatment and after treatment the CA-125 levels fell (p>0.05) but remained higher (more than double) the levels in control.

In endometrioid type of ovarian cancer patients (n=8) the serum LAAO levels were found to increased significantly (p<0.001) more than double the levels in control before treatment and levels fell significantly (p<0.05) after treatment but remained higher than levels in control. CA-125 levels in endometriod type of ovarian cancer patients were remained within the control levels before (p>0.05) and after treatment (p>0.05).

In transitional type of ovarian cancer patients (n=2) the levels of LAAO were found to be increased significantly (p<0.05) more than double the levels in controls before treatment and levels came back to control levels significantly (p<0.05) after treatment. The moderately increased (less than one fold) CA-125 levels in transitional cell ovarian cancer patients control levels before treatment were found to be insignificant (p>0.05). CA-125 levels fell within control range after treatment but found to be statistically insignificant (p>0.05).

In squamous ovarian cancer patients (n=2) the increased (double the control) levels of serum LAAO were found to be insignificant (p>0.05) before treatment and the fall in levels after treatment were also found to be insignificant (p>0.05). The levels of CA-125 in squamous type ovarian cancer patients were found to be increased (not significant, p>0.05) than the control before treatment and CA-125 levels fell within the control range but found to be statistically insignificant (p>0.05) after treatment. Comparsion of LAAO and CA 125 with respect to histopathological types of cancer are shown in table 3.

In case of primary peritoneal ovarian cancer patients (n=3) the serum levels of LAAO were found to be significantly increased (p<0.05) double than the levels in control and the fall in LAAO levels (remained higher than control) after treatment were found to be statistically insignificant (p>0.05). The serum levels of CA-125 in primary peritoneal cancer patients remained within the range of control before (p>0.05) and after (p>0.05) treatment.

Pretreatment serum levels of LAAO in Stage I and II were found to be increased significantly (p<0.001) 2.26 fold more than controls. However, 1.8 fold increase in serum CA-125 than controls in stage I & II were found to be statistically not significant (p>0.05). Pearson correlation between pretreatment levels of serum LAAO and CA-125 in stage I &II was also found to be significant (r=0.662). Pretreatment serum levels of LAAO in stage III and IV increased significantly (p<0.001) 3.06 fold more than controls. Also the pretreatment serum levels of CA-125 in stage III and IV

were found to be significantly (p<0.001) increased 13.7 fold more than controls. Pearson correlation between pretreatment serum LAAO and serum CA-125 levels in stage III&IV was found to be not significant (r=0.234). Pretreatment serum levels of LAAO between Stage I & II and Stage III & IV were found to be statistically significant (p<0.001). However, pretreatment serum CA-125 levels between stage I & II and stage III & IV were statistically not significant (p>0.05). Hence, sensitivity of serum LAAO in early diagnosis of ovarian cancer is greater than the serum CA-125. Serum levels of LAAO and CA125 varied with the staging of the disease as shown in Table 4.

Post treatment serum LAAO levels in stage I and II fell significantly (p<0.05) back to control levels. Post treatment CA-125 levels also fell significantly (p<0.05) to control levels in Stage I and II disease. Pearson correlation between post treatment serum levels of LAAO and CA-125 in stage I and II was found to be statistically not significant (r=-0.262). In Stage III & IV serum levels of LAAO decreased significantly (p>0.05) but remained 1.2 folds higher than controls. However, in Stage III and IV disease the CA-125 levels decreased compared to pre treatment but were 10 fold higher than control levels (p>0.05). Pearson correlation between post treatment serum levels of LAAO and CA-125 in stage III and IV disease was found to be negative and statistically significant (r=-0.350). Post treatment serum levels of LAAO between Stage I & II and Stage III & IV were found to be statistically significant (p<0.001). Post treatment CA-125 levels between Stage I & II disease and Stage III & IV disease were found to be statistically significant (p<0.05). Hence, serum LAAO levels also have prognostic importance.

Discussion

CA-125 is the widely accepted serum biomarker of ovarian cancer. Unfortunately, its level drastically increases only in the serous epithelial ovarian cancer patients[18].

In this work, we estimated the levels of serum LAAO and CA-125 in controls and ovarian cancer patients. In other ovarian cancer patients such as endometrioid, primary peritoneal, serum CA-125 levels remained similar to control levels. Serum LAAO activity is increased in all histopathologic types of ovarian cancer. Post treatment serum LAAO activity decreased in all histopathologic types except in patients whom cancer recurred. Hence, serum LAAO seems to be the potential biomarker for recurrence of cancer.

Previously, it was reported that the progressive increase in three consecutive serum CA-125 levels within normal range was high predictor of tumor recurrence within the 4-24 months[19]. Here, we showed that the post treatment serum LAAO activity remained as high as pre treatment levels indicating the high predictive of tumor recurrence. Pre treatment serum activity of LAAO is also increased in all patients with stage I and II disease in contrast to CA-125 which increases more in stage III & IV, indicating its higher sensitivity in diagnosing ovarian cancer at the earliest.

In humans, IL4I1 is shown to have secreted L-phenylalanine oxidase activity. hIL4I1 also shown to have antibacterial and antiviral activity. hIL4I1 is shown to inhibit T-cell proliferation in vivo. It is a lysosomal protein which has antibacterial, antifungal, anti-protozoan, and antiviral properties. The presence of carbohydrate components on this molecule promotes the attachment to the cell surface and creation of high local concentrations of H_2O_2. Increased activity was found in primary mediastinal large B-cell lymphoma. IL4I1 is the first LAAO

described in mammalian innate immune cells[20].

Current study suggests that the L-amino acid oxidase can be used as a serum diagnostic and prognostic biomarker along with CA-125 and the levels of L-amino acid oxidase activity not falling within controls after post treatment increases the risk of recurrence in patients.

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Conflict of interest

Nil.

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Table 1. Demographic data of patients aged between 20-70 with mean of (45±11.17)

Characteristic	No. of cases	Percentage (%)
Stage of Cancer		
Stage I & II	14	28
Stage III & IV	36	72
Histopathologic types		
Serous	29	58
Endometrioid	8	16
Transitional cell	2	04
Squamous	2	04
Primary peritoneal	3	06
Recurrent carcinoma	6	12
Patient History		
Post menopausal bleeding	40	80
Pre-menopausal	10	20
Chemotherapy	26	52
Weight loss	45	90
Asthma	05	10
Diabetes mellitus	23	46
Hypertension	16	32
Anemia	01	02

Table 2. Serum LAAO and CA125 levels of controls and pre and post treatment of ovarian cancer patients. Values are expressed as mean ± SD

	Levels of L-amino acid oxidase (units/ml)	CA 125 levels (units/L)
Control (n=50)	27.56±7.61	14.62±10.11
Pre-treatment (n=50)	79.53±12.8**	210.24±56.34*
Post-treatment (n=50)	38.27±20.30**	49.15±18.33

**p<0.005-significant (compared with controls)
*p<0.05- significant (compared with controls)
CA 125 (Carbohydrate antigen 125)

Table 3. Pre- and Post-treatment levels of CA-125 and L-amino acid oxidase in different histopathologic types of ovarian cancer patients (levels are compared with controls)

Parameter	Type of ovarian cancer					
	Serous	Endometrioid	Transitional	Squamous	Primary peritoneal	Recurrent carcinoma
CA-125 (units/L)						
pre-treatment	49-1350*	20-36	48-140	48-60	7-25	125-200**
post-treament	37-471	15-30	12-23	10-30	6-25	12-26
L-amino acid oxidase (units/mL)						
pre-treatment	60-100**	50-90**	78-80*	60-82	55-80*	70-96**
post-treatment	15-50**	20-48*	26-29*	26-50	27-41	72-93**

**p<0.005- highly significant (compared with controls)
*p<0.05- significant (compared with controls)
CA 125 (Carbohydrate antigen 125)

Table 4. Pre- and Post-treatment levels of CA-125 and L-amino acid oxidase expressed as mean ± SD in different stages of ovarian cancer

Stage of Cancer	CA125 (units/liter)		L-amino acid oxidase (units/ml)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Stage I & II	43.58±20.41	29.71±13.9	60.50±17.33**	22.35±4.36*
Stage III & IV	279.96±189.7**	127.6±43.77	84.79±19.88**	45.29±20.12

**p<0.005- highly significant (compared with controls)
*p<0.05- significant (compared with controls)
CA 125 (Carbohydrate antigen 125)