Scholars Academic Journal of Biosciences (SAJB) **ISSN 2321-6883 (Online)**
Sch. Acad. J. Biosci., 2014; 2(9): 633-639 **ISSN 2347-9515 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com **DOI:** 10.36347/sajb.2014.v02i09.015

Research Article

Serum level of CA-125, Salivary Amylase and CEA in Epithelial Ovarian Cancer in North Indian Population

Dholariya Sagar[1*], Prakash C Ray[2], Alpana Saxena[3], Gauri Gandhi[4], Nita Khurana[5], Devajit Sharma[6]
[1]Assistant Professor, Department of Biochemistry, R.D. Gardi Medical College, Ujjain-456006, M.P., India
[2] Director Professor, Department of Biochemistry, Maulana Azad Medical College and Associated Hospitals, New Delhi-110002, India
[3]Director Professor and H.O.D, Department of Biochemistry, Maulana Azad Medical College and Associated Hospitals, New Delhi-110002, India
[4]Director Professor, Department of obstetrics & Gynecology, Maulana Azad Medical College and Associated Hospitals, New Delhi-110002, India
[5]Professor, Department of Pathology, Maulana Azad Medical College and Associated Hospitals, New Delhi-110002, India
[6]Assistant Professor, Department of Biochemistry, R.D. Gardi Medical College, Ujjain-456006, M.P., India

***Corresponding author**
Dr. Dholariya Sagar Jayantilal
Email: drsagar.dholariya@gmail.com

Abstract: Ovarian cancer is most lethal of all the gynecological Cancer. The early stages of ovarian cancer are asymptomatic and more than 75% of the cases are diagnosed with regional or distant metastases. In present study our aim is to estimate the pre operatively serum level of CA-125, Salivary amylase and CEA in malignant and benign Epithelial ovarian cancer and their integrate use to predict malignant ovarian cancer. 50 malignant and 50 benign cases of epithelial ovarian cancer were included in this cross sectional study. Serum salivary amylase was estimated by SBio α AMYLASE (Direct Substrate Method) kit and CA-125 & CEA by electrochemi- luminescence immunoassay method. There was significant higher concentration of serum CA-125 and salivary amylase in malignant cases compare to benign cases of epithelial ovarian cancer ($P<0.0001$). Sensitivity and specificity of combine CA-125 and salivary amylase was 88% and 84% respectively. Concentration of CA-125 and salivary amylase was higher in advanced stage of ovarian cancer ($p<0.05$). Study concludes that Integrate use of CA-125 and salivary amylase can be used as marker to predict malignant ovarian cancer.
Keywords: Epithelial Ovarian Cancer, CA-125, Salivary Amylase, CEa

INTRODUCTION

Ovarian cancer emerged as one of the most common malignancies affecting women in India. It is the sixth most common malignancy worldwide and second most common amongst all gynecological malignancies in India [1]. It is the most lethal of all Gynecologic malignancies accounting for 52% of all gynecological cancers related deaths [2]. This is because of late diagnosis due to unavailability of effective screening and diagnosis strategy. In late stage disease five year survival rate is between 20 to 25%. Chemotherapy permanent remission rate is only 10-15% in chemotherapy sensitive patients [3-4]. So there is a need of screening test to diagnosed ovarian cancer at early stage and for monitoring therapy that reduces mortality.

Epithelial Ovarian Cancer is a most common type. So many Biomolecules are released in serum during the process of ovarian carcinogenesis and that can be used as a tumor marker. Cancer Antigen-125 (CA-125) also known as Mucin 16 (MUC 16), one of the member of mucin family glycoprotein that increased in serum during ovarian cancer [5-6]. It promotes the carcinogenesis and metastasis by favoring cell-cell interaction with mesothelin of peritoneum, suppressing the activity of Natural-Killer cell and improving the motility of tumor cell [7-9].

Serum Amylase contains parotid (Salivary) Amylase , pancreatic Amylase and genitourinary Amylase. It was found that fluid from serous ovarian tumor contains genitourinary amylase called ovarian tumor amylase [10]. Isoelectric focusing and diethylaminoethyl-sephadex chromatography differentiate tumor amylase from salivary and pancreatic amylase. Ovarian Tumor amylase is similar to salivary amylase except slight acidic nature. But it is totally different from pancreatic amylase [11]. Acidic nature of ovarian tumor amylase

may be due to Post-translation modification like Deamination and Glycosylation that occur during ovarian tumor development [12]. So this isoenzyme of salivary amylase is similar to salivary amylase (Except location) can be measured in serum and used as a tumour marker to predict malignant ovarian cancer preoperatively.

Carcinoembryonic antigen (CEA) is Glycosylphosphatidyl inositol (GPI) cell surface glycoprotein. It is synthesize by fetal gastrointestinal tissue and in some carcinoma like colon, lung, breast and gynecological cancer. 35-40% epithelial ovarian cancers patients have high serum CEA level [13-14]. We hypothesized that combination of CA-125 & salivary amylase and CA-125 & CEA may be more sensitive and specific than independent use of tumor marker to distinguish malignant ovarian tumour. Based on this background, the aim of our study is to determine the serum level of CA-125, salivary amylase and CEA as a tumor marker in both benign and malignant cases of ovarian tumor.

MATERIALS AND METHODS

Study population

The study was conducted in Department of Biochemistry, Maulana Azad Medical College in collaboration with department of Obstetrics and Gynecology, Lok Nayak Hospital, New Delhi. It was a hospital based cross sectional study. A total of 50 malignant epithelial ovarian cancer (EOC) patients and 50 benign cases of epithelial ovarian cancer were included in the study were included in the study. Patients were assessed on the basis of clinical and pathological parameters. Diagnosis of all tumors was verified by two senior pathologists. The cancer was staged in according to the International Federation of Gynecology and Obstetrics surgical staging system (FIGO). Informed consent form signed by all patients and research protocol was approved by the Local ethical committee.

Exclusion criteria

Patients with Metastatic Ovarian cancer, any other cancer, patients who has received radio/chemotherapy, pancreatitis, salivary gland disease, bowel ischemia, ectopic pregnancy, pelvic inflammatory disease, perforated peptic ulcer, endometriosis, AIDS were excluded from study.

Sample collection

About 4-5 ml of fasting venous blood was collected from benign and malignant patients in plain vial after written informed consent. Blood allowed to clot and centrifuges 3000 rpm for 5 min for separation of serum. Serum was used for measure CA-125, salivary amylase and CEA. All samples were collected preoperatively and before the starting of chemotherapy.

Serum salivary Amylase estimation

Serum pancreatic amylase was precipitated by using monoclonal anti pancreatic antibody on solid phase support. Then sample mixture was centrifuged and supernatant was used for salivary amylase estimation by commercially available SBio α AMYLASE (Direct Substrate Method) kit (Biosciences PTE Ltd, Singapore). Chloronitrophenol (CNP) produced by hydrolysis of 2-chloro-4-nitrophenol in the presence of α-amylase in sample. The Activity of α amylase is directly proportional to production of CNP and measured absorbance.

Serum CA-125 and CEA estimation

The serum CA-125 and CEA were measured by electrochemiluminescence immunoassay method using CA-125 and CEA kits custom-made to ELECSYS 2010 (Roche diagnostics, Germany)

Statistical analysis

All statistical analysis was performed using SPSS software version 17.0. Chi-square test was used to examine the differences in Serum level of above three analyte between the study group. Kruskal wallis test .and Mann Whitney U test were used for non-parametric data. ROC curve was used to determine the cut-off value of salivary amylase. p-value <0.05 was considered statistically significant.

RESULTS

Study population

The baseline characteristics of subjects are summarized in Table 1. Study groups were divided according to Menopausal status, the FIGO staging of ovarian cancer, histopathological types and histopathological grade. In this study, highest number of cases was in stage III (60%) as compared to stage IV (16%), stage II (12%) and stage I (12%). According to histopathological types highest number of cases was in mucinous (48%) and serous adenocarcinoma (46%), endometroid adenocarcinoma (4%) and clear cell adenocarcinoma (2%). In histopathological grade highest number of cases was in moderately differentiated (74%) as compared to poorly differentiated (16%) and well differentiated (10%). No patients had a family history of epithelial ovarian cancer. Mean age were 43 ±8.04 and 42.7 ±11.82 in malignant and benign ovarian tumour respectively (Table 1).

Table 1: Baseline characteristics of subjects involved in the study

Variables	Malignant Ovarian tumour n (%)	Benign Ovarian tumour n (%)
Age in years (Mean ± SD)	43 ±8.04	42.7 ±11.82
Menopausal status		
Reproductive age group	16(32)	15(30)
Post menopausal group	34(68)	35(70)
Staging		
I	6(12)	
II	6(12)	
III	30(60)	
IV	8(16)	
Histopathology		
Mucinous AC	24(48)	
Serous AC	23(46)	
Endometroid AC	2(4)	
Clear cell AC	1(2)	
Grading		
Well differentiated	5(10)	
Moderately differentiated	37(74)	
Poorly differentiated	8(16)	

Table 2: Serum level of CA-125, CEA and salivary amylase in study groups

	Malignant ovarian tumour	**Benign ovarian tumor**	
CA-125			Sensitivity-84% Specificity-80% PPV-80.76% NPV-83.34%
>35 U/ml	42(84%)	10(20%)	
<35 U/ml	8(16%)	40(80%)	
	Chi square-38.50, df-1, p<0.0001		
Salivary Amylase			Sensitivity-78% Specificity-74% PPV-75% NPV-77.08%
>70 U/L	39(78%)	13(26%)	
<70U/L	11(22%)	37(74%)	
	Chi square-19.04, df-1, p<0.0001		
CEA			Sensitivity-32% Specificity-60% PPV-44.45% NPV-46.87%
>3.4 ng/ml	16(32%)	20(40%)	
<3.4 ng/ml	34(68%)	30(60%)	
	Chi square-0.39, df-1, p=0.53		
CA-125 >35 U/ml & Salivary amylase > 70 U/L	44 (88%)	8 (16%)	Sensitivity-88% Specificity-84% PPV-84.61% NPV-87.50%
	Chi square-52.07, df-1, p<0.0001		
CA-125>35 U/m l & **CEA** > >3.4 ng/ml	35(70%)	12 (24%)	Sensitivity-70% Specificity-76% PPV-74.46% NPV-71.69%
	Chi square-25.36, df-1, p<0.0001		

Table 3: Distribution of markers according to Menopausal status, Staging and histopathology (by Mann Whitney U test & * by Kruskal wallis test)

Malignant tumour	CA-125 U/ml Median (Range)	Salivary Amylase U/L Median (Range)	CEA ng/ml Median (Range)
Menopausal status			
Reproductive age group	74.2(10.2-54.6)	91.5(50.2-400.8)	3.42(0.50-30.2)
Post menopausal	80.2(42.8-210.2)	87(20.2-122.2)	2.54(0.20-0.50)
	p>0.05**	p>0.05**	p>0.05**
Staging			
Early (Stage I+II)	32.7(10.2-55.2)	75.1(20.2-100.2)	3.36(0.20-20.7)
Stage III	80.3(58.2-210.2)	145.2(57.2-395.5)	4.21(0.50-30.2)
Stage IV	120.2(50.4-180.7)	190.7(60.5-400.8)	5.82(2.10-25.4)
	p <0.0001*	p <0.001*	p>0.05*
Histological type			
Mucinous	73.2(20.5-160.2)	70.5(20.2-105.6)	5.2(0.9-30.2)
Serous	67.3(10.4-210.2)	90.4(50.2-400.8)	1.7(0.2-2.9)
Endometroid	69.2(58.2-69.5)	69.7(57.2-82.2)	2.8(1.1-2.3)
	p>0.05*	p>0.05*	p>0.05*

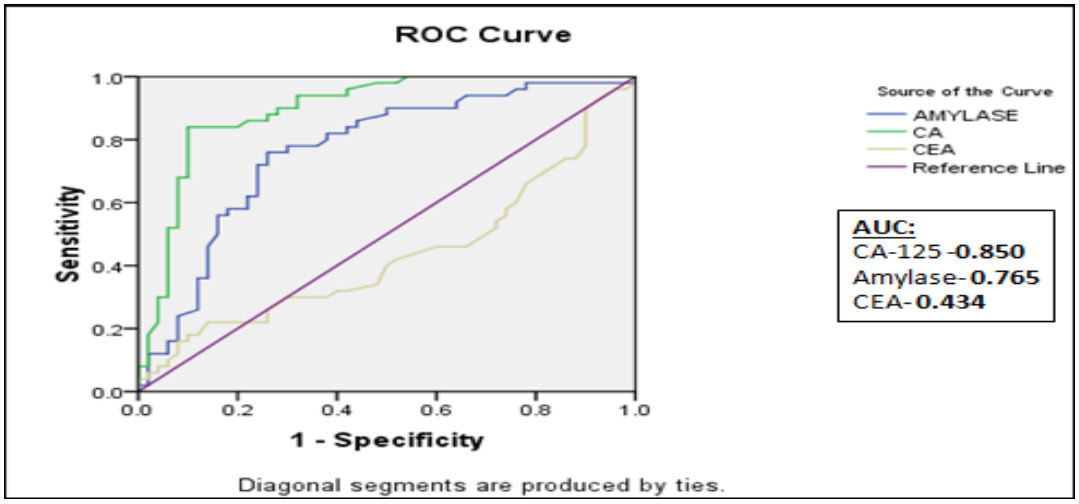

Fig. 1: ROC curve for serum CA-125, salivary amylase and CEA. ROC: Receiver Operating Chracteristics Curve, AUC: Area Under Curve

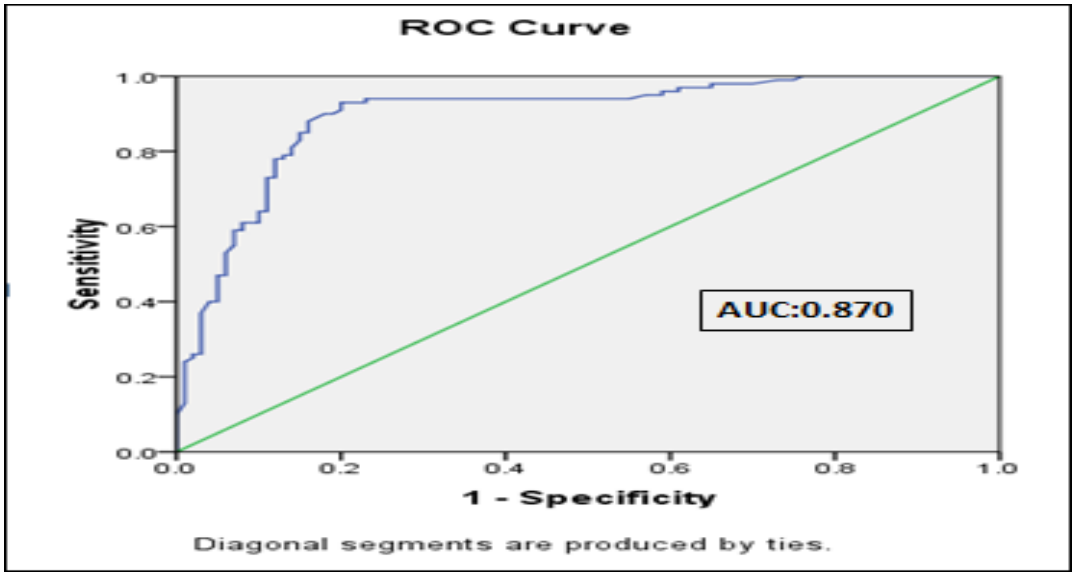

Fig. 2: ROC curve for CA-125 >35 U/ml & Salivary amylase > 70 U/L

Serum CA-125

Cut off value for ca-125 was 35U/ml as per Roche diagnostics ELECSYS 2010 to detect malignant ovarian tumor [16]. There was a significant difference of CA-125 level in malignant and benign ovarian tumor ($p< 0.0001$). CA-125 has a sensitivity of 84%, specificity of 80%, PPV of 80.76% and NPV of 83.34% to differentiate malignant ovarian tumour (Table.1). AUC in ROC curve was 0.850 (Fig. 1).

Serum salivary Amylase

Cut off value of salivary amylase was 70 U/L (Calculated from ROC). There was a significant difference of salivary amylase level in malignant and benign ovarian tumor ($p< 0.0001$). Salivary amylase has a sensitivity of 78%, specificity of 74%, PPV of 75% and NPV of 77.08% to differentiate malignant ovarian tumor (Table.1). AUC in ROC curve was 0.765 (Fig. 1).

Serum CEA level

Cut off value for CEA was 3.4 ng/ml as per Roche diagnostics ELECSYS 2010 to detect malignant tumour [17]. There was no significant difference of CEA level in malignant and benign ovarian tumor ($p=0.53$). CEA has a sensitivity of 52%, specificity of 60%, PPV of 44.45% and NPV of 45.87% to differentiate malignant ovarian tumor (Table.1). AUC in ROC curve was 0.434 (Fig. 1)

CA-125 >35 U/ml & Salivary Amylase > 70 U/L

There was no significant difference of both marker in malignant and benign ovarian tumor ($p=<0.0001$). It has a slight higher accuracy to differentiate malignant ovarian tumour as a sensitivity of 88%, specificity of 84%, PPV of 84.61% and NPV of 87.5% to differentiate malignant ovarian tumor (Table 1). AUC in ROC curve was 0.870 (Fig. 2).

CA-125 >35 U/ml & CEA >3.4 ng/ml

There was a significant difference of CA-125/CEA >25 in malignant and benign ovarian tumor ($p=<0.0001$). It has a sensitivity of 70%, specificity of 76%, PPV of 74.46% and NPV of 71.96% to differentiate malignant ovarian tumor. It has a same specificity as combination use of CA-125 & salivary amylase (Table.1).

There was a significant difference of CA-125 and salivary amylase in different staging of cancer. More concentration was found for CA-125 and salivary amylase in advanced stage of cancer compared to early stage of ovarian tumuor. Concentration of CA-125 and CEA were higher in mucinous type and salivary amylase was in serous type of ovarian cancer. But it was statistically insignificant. No significant difference was observed with respect to menopausal status and histopathological grading (Table 2).

DISCUSSION

Maximum patients of ovarian cancer in early stage are remaining asymptomatic and more than 75% -80% cases are diagnosed at late stage. Treatment approach for late stage of ovarian cancer is mainly cytoreductive surgery [18]. It is necessary to differentiate malignant ovarian tumour from benign variety to avoid unnecessary surgery and its complication in benign cases. Many studies were reported on tumour marker for prediction of ovarian tumor but results were conflicting and no standard reference range is available till date particularly for amylase. So we tried to establish reference range for salivary amylase and compare with CA-125 and CEA.

Our findings are biologically credible and support the previously reported observation.CA-125 is considered as a gold standard tumour marker for ovarian cancer. It is elevated in 50% cases of early stage and 80-90% cases of advanced stage of ovarian tumour [19]. It also used to evaluate therapeutic efficacy and for monitoring of treatment [20]. Sensitivity and specificity of CA-125 is 84% and 80% respectively in our study. Similar observation was found in study of Meyer *et al*. [21] and Bast *et al*. [22]

Apart from salivary gland and pancreatic tissue Amylase is also secreted from endosalpingeal epithelium of fallopian tube, semen and tears [23]. Amylase spot in serous ovarian tumor and in fallopian tube was confirmed by immunohistochemistry [24, 25]. So increased amylase activity in malignant ovarian tumor may be due to existence of active endosalpingeal like epithelial tissue in malignant ovarian tumour. Overgrowth of enosalpingeal epithelium and active inflammatatory response in ovarian carcinogenesis further increased release of amylase in serum.

We reported high serum amylase activity in ovarian malignant tumour in the absence of salivary gland disorder and pancreatic disorder. Similar finding was observed in study of Luka F *et al*. [25] and case report Tanaka Y *et al*. [26]. Sensitivity and specificity of salivary amylase is 78% and 74% respectively in our study at cut off value of 70 U/L and AUC in ROC is 0.765.So it is quite good tumor marker to detect malignant ovarian tumor.

Sensitivity and specificity of CEA is only 32% and 60% respectively in our study. It should not use for screening of ovarian malignancy. Combine use of CEA and CA-125 lead to increase in sensitivity and specificity but less than CA-125 alone. It is better to avoid use of CEA in Ovarian cancer screening.

We get sensitivity of 88% and specificity of 84% by Combine use of CA-125 and Salivary Amylase. AUC in ROC 0.870 is also more for combine CA-125 & salivary amylase than CA-125 (0.850) and salivary

amylase (0.765) alone. 0.870 AUC suggests they are good diagnostic marker [28] for ovarian malignancy. So it is better to use both CA-125 and Salivary amylase pre operatively to predict malignant ovarian tumor. It is cost effective and simplest way for screening of ovarian cancer preoperatively. Study on larger group (population based) is required to validate our findings.

CONCLUSION

The study concludes higher concentration of CA-125 and salivary Amylase was found in malignant ovarian Cancer compared to Benign Ovarian Cancer. Pre operatively integrate use of CA-125 and serum amylase can serve as superior marker for screening and progression of malignant ovarian cancer.

REFERENCES

1. Parkin DM, Bray F, Ferlay J, Pisani P; Global cancer statistics, 2002. CA Cancer J Clin., 2005; 55(2): 74-108.
2. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T; Cancer statistics, 2009. CA Cancer J Clin., 2009; 59(4): 225-249.
3. Schwartz PE; Current diagnosis and treatment modalities for ovarian cancer. Cancer Treat Res., 2002; 107: 99-118.
4. Mutch D; Surgical manegement of ovarian cancer. Semin Oncol., 2002; 29(1 Suppl 1): 3-8.
5. Duraisamy S, Ramasamy S, Kharbanda S, Kufe D; Distinct evolution of the human carcinoma-associated transmembrane mucins, MUC1, MUC4 AND MUC16. Gene, 2006; 373: 28-34.
6. Bast RC, Xu FJ, Yu YH, Barnhill S, Zhang Z, Mills GB; CA 125: the past and the future. Int J Biol Markers., 1998; 13(4): 179–187.
7. Rump A, Morikawa Y, Tanaka M, Minami S, Umesaki N, Takeuchi M *et al*.; Binding of ovarian cancer antigen CA125/MUC16 to mesothelin mediates cell adhesion. J Biol Chem., 2004; 279(10): 9190–9198.
8. Gubbels JA, Belisle J, Onda M, Rancourt C, Migneault M, Ho M *et al*.; Mesothelin-MUC16 binding is a high affinity, N-glycan dependent interaction that facilitates peritoneal metastasis of ovarian tumors. Mol Cancer, 2006; 5(1): 50.
9. Chang K, Pastan I; Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. Proc Natl Acad Sci U.S.A., 1996; 93(1): 136–140.
10. Van KIey H, Cramer S, Bruns DE; Serous ovarian neoplastic amylase (SONA): A potentially useful marker for serous ovariant tumors. Cancer, 1981; 48(6): 1441-1449.
11. Zakowski JJ, Bruns DE; Improved DEAE-Sephadex column chromatography in measuring amylase in serous ovarian neoplasm, and results for 13 cases. Clin Chem., 1982; 28(10): 2095-2098.
12. Pieper Bigelow C, Strocchi A, Levitt MD; Where does serum amylase come from and where does it go? Gastroenterol Clin North Am., 1990; 19(4):793-810.
13. Hogdall EV, Christensen L, Kjaer SK, Blaakaer J, Jarle Christensen I, Gayther S *et al*.; Protein expression levels of carcinoembryonic antigen (CEA) in Danish ovarian cancer patients: from the Danish MALOVA ovarian cancer study. Pathology, 2008; 40(5): 487-492.
14. Yabushita H, Masuda T, Ogawa A, Noguchi M, Ishihara M *et al*.; Combination assay of CA125, TPA, IAP, CEA, and ferritin in serum for ovarian cancer. Gyn Onc., 1988; 29(1): 66-75.
15. Wallenfels K, Foldi P, Niermann H, Bender H, Linder D; The enzymatic synthesis, by transglucosylation of a homologous series of glycosidically substituted malto-oligosaccharides, and their use as amylase substrates. Carbohydrate Research, 1978; 61: 359-368.
16. Hellstrom I, Raycraft J, Hayden-Ledbetter M, Ledbetter J, Schummer M, McIntosh M *et al*., The HE4 (WFDC2) Protein Is a Biomarker for Ovarian Carcinoma. Cancer Research, 2003; 63(13): 3695–3700.
17. Yamashita K, Watanabe M; Clinical significance of tumor markers and an emerging perspective on colorectal cancer. Cancer Sci., 2009; 100(2): 195-199.
18. Akahira JI, Yoshikawa H, Shimizu Y, Tsunematsu R, Hirakawa T, Kuramoto H *et al*.; Prognostic factors of stage IV epithelial ovarian cancer: a multicenter retrospective study. Gynecol Oncol., 2001; 81(3): 398-403.
19. Markman M; Optimizing primary chemotherapy in ovarian cancer. Hematol Oncol Clin North Am., 2003; 17(4): 957-968.
20. Rustin GJ, Bast RC, Kelloff GJ, Barrett JC, Carter SK, Nisen PD *et al*.; Use of CA-125 in clinical trial evaluation of new therapeutic drugs for ovarian cancer. Clin Cancer Res., 2004; 10(11): 3919-3926.
21. Meyer T, Rustin GJ; Role of tumour markers in monitoring epithelial ovarian cancer. Br J Cancer, 2000; 82(9): 1535-1538.
22. Bast RC Jr, Klug TL, St John E, Jenison E, Niloff JM, Lazarus H *et al*.; A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. N Engl J Med., 1983; 309(15): 883–887.
23. Kipps AE, Whitehead PH; A method for quantitating amylase and its use in the investigation of various body fluids. Ann Clin Biochem., 1974; 11(6): 219-223.
24. Skude G, Mardh PA, Westrom L; Amylases of the genital tract. Isoamylases of genital tract tissue homogenates and peritoneal fluid. Am J Obstet Gynecol., 1976; 126(6): 652-656.

25. Bruns DE, Mills SE, Savory J; Amylase in fallopian tube and serous ovarian neoplasms: Immunohistochemical localization. Arch Pathol Lab Med., 1982; 106(1): 17-20.
26. Frulloni L, Patrizi F, Bernardoni L, Cavallini G; Pancreatic Hyperenzymemia: clinical significance and diagnostic approach. JOP J Pancreas, 2005; 6(6): 536-551.
27. Tanaka Y, Ueda G, Tanizawa O, Miyake H, Kohno M, Nishiyama S *et al*.; A case of amylase-producing ovarian tumor. Gan No Rinsho, 1986; 32(1): 111-116.
28. Pepe MS; Receiver operating characteristics methodology. J Am Stat Assoc., 2000; 95(449): 308–311.