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CA-125 and Ceruloplasmin Levels in Ovarian Cancer Patients

Over Kanserli Hastalarda CA-125 ve Seruloplazmin Düzeyleri

Mangala Hegde[1], Yousef Rezaei Chianeh[1], Jeevan Shetty[1], Donald J Fernandes[2], Pragna Rao[1]

[1]Kasturba Medical College, Manipal University, Department of Biochemistry, [2]Department of Radiotherapy Manipal, Karnataka,INDIA.

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ABSTRACT

Purpose: The initial stage of proliferation of epithelial ovarian carcinoma (EOCa) is usually asymptomatic. Due to the lack of sensitive and reliable markers in majority of patients the disease is widespread at the time of diagnosis. The reliable serum biomarkers currently accepted is CA125 but there is limitation in case of sensitivity of CA125 as it is detectable only in 50% of patients in stage I and 80% of patients with advanced stage. We have investigated a correlation between serum CA125 and ceruloplasmin (as a marker of angiogenesis) in ovarian cancer in pre-treatment and post-treatment patients, compared with controls and found to be a significant marker for diagnosis.

Material and Methods: A study was done in age group between 18-45 years diagnosed with ovarian cancer. (cases: n=50, controls: n=50). Cancer was diagnosed based on biopsy and histopathological examination. Serum Ceruloplasmin and CA 125 were estimated in pre-treatment and post-treatment patients and statistically significant decrease of these biomarkers observed in post treatment when compared with pre treatment patients.

Result: We found that serum CA 125 to ceruloplasmin ratio was moderately increased in pre-treatment ovarian cancer patient. The serum ceruloplasmin ($p<0.0001$) level was significantly increased in ovarian cancer patients as compared to controls.

Conclusion: Serum ceruloplasmin as well CA-125 level decline after treatment, and have been associated with efficacy and safety of novel therapeutic strategy to improve diagnosis and treatment for cancer.

Key words: Epithelial ovarian carcinoma (EOCa), Ceruloplasmin, Carbohydrate antigen 125 (CA-125).

ÖZET

Amaç: Epitelial ovarian karsinomunda proliferasyonunun başlangıç aşaması genellikle asemptomatiktir. Hastaların büyük kısmında hassas ve güvenilir belirteçlerin eksikliği nedeni ile hastalık teşhis aşamasında artık yayılmış bulunmaktadır. Güvenilir olarak kabul edilen serum belirteci CA-125'tir ancak evre I hastalarının sadece %50'sinde ve ilerlemiş evre hastaların %80'ninde saptanabilmesinden dolayı, CA-125'in hassasiyeti konusunda bir sınırlama mevcuttur. Bu çalışmada, tedavi öncesi ve tedavi sonrası over kanserli hastalarda CA-125 ve seruloplazmin'in (anjiyogenez belirteci) serum seviyeleri arasındaki korelason araştırılmış, kontroller ile karşılaştırılmış ve teşhis için anlamlı bir belirteç olduğu bulunmuştur.

Materyal ve Metod: Çalışma 18-45 yaş arası over kanseri teşhisi alan hastalar ile yapılmıştır (vaka n=50, kontrol n=50). Kanser teşhisi, biyopsi ve histopatolojik değerlendirme sonucu konulmuştur. Tedavi öncesi ve tedavi sonrası serum seruloplazmin ve CA-125 seviyeleri değerlendirilmiş ve tedavi öncesi ile karşılaştırıldığında, bu belirteçlerin tedavi sonrası hastalarda anlamlı derecede düştüğü gözlenmiştir.

Sonuç: Serum CA-125 seruloplazmin oranı tedavi öncesi over kanserli hastalarda kısmen artmıştır. Serum seruloplazmin seviyesi ($p<0.0001$) kontrollerle karşılaştırıldığında over kanserli hastalarda anlamlı olarak yüksek bulunmştur.

Tartışma: Seruloplazmin ve CA-125'in serumdaki seviyeleri tedaviden sonra azalmaktadır, buna bağlı olarak kanser tanı ve tedavisinde yeni tedavi stratejilerinin geliştirilmesinde güvenli ve etkili olabileceği düşünülmektedir.

Anahtar kelimeler: Epitelial Over Karsinom, Seruloplasmin, Karbohidrat antijen 125 (CA-125)

INTRODUCTION

Mortality due to ovarian cancer remains unchanged during the last decades, despite an advancement in cancer therapy[1,2]. The probable reason could be, diagnosis at the late stage approximately in more than 80% of patients[3] and estimate survival rate is 5 years in almost 35% in these patients. If the diagnosis is in early stage the 5 year survival rate would increase to 90% of those patients and presently surgery is a selective therapy[4]. Cancer antigen 125 (CA125) is considered as one of the reliable markers for ovarian cancer[5]. The diagnostic value of CA125 alone as a single marker, is less than 10%, but ultrasound screening methods can increase the diagnostic value[6].

The use of serum markers for early detection has largely focused on CA-125, a high-molecular-weight mucin (MUC 16) that was initially detected with a homologous double-determinant radioimmunoassay[7]. The OC-125 murine monoclonal antibody was initially used to bind CA-125 antigen from serum on a bead[8]. Because multiple epitopes are present in each CA-125 molecule, the same OC-125 antibody labelled with iodine-125 could be used to detect identical determinants on CA-125 molecules that had been bound. However, the CA-125 assay exhibits a sensitivity of 50% to 60% for stage I disease[9,10]. Antigen levels can increase exponentially 10 to 21 months before diagnosis[11-13]. Specificity of CA-125 is not suitable for screening, particularly in a premenopausal population in which endometriosis, adenomyosis, and retrograde menstruation can produce false-positive elevations of antigen levels. Specificity can, however, be improved by combining CA-125 with ultrasonography in a two-stage strategy and by sequential monitoring of CA-125 values over time.

The ceruloplasmin serves as a cofactor in various physiological enzymatic reactions, including a role in copper transport[14], maintenance of vessel tone[15-17], and antioxidant properties, which has implications in disorders like Alzheimer's diseases[18]. High levels of ceruloplasmin expression have been demonstrated in various cancers and non-cancerous condition such as thyroid carcinoma[19],melanoma[20] and patient with dysfunctional uterine bleeding[21,22]. Dysregulation of copper transport due to ceruloplasmin expression in tumors has been studied by suppressing copper with tetrathiomolybdate in head and neck tumors in clinical trials.This study has designed to identify a possible role and correlations between serum ceruloplasmin level and CA125 in ovarian cancer that indicate the role of ceruloplasmin in the pathophysiology of ovarian cancer.

MATERIALS and METHODS

Serum samples were obtained from 50 patients (age between 18-45 years) with clinically and histologically verified ovarian cancer. 50 normal, healthy age and sex matched volunteers were taken as controls. Serum samples were assayed for ceruloplasmin and CA 125. Ceruloplasmin level was determined by the diamine oxidase method [6] based on the property of ceruloplasmin to catalyse the oxidation of colorless para-phenylene diamine to a blue violet complex, which can be estimated spectrophotometrically. CA 125 levels were assayed by autoanalyzer based on the microparticle enzyme immunoassay (MEIA)

supplied by Abbott Laboratories, USA [7]. Appropriately diluted blood samples were incubated in reaction vessel well along with antibody-coated microparticles. CA–125 reacts with anti CA-125 forming an antigen antibody complex. The excess reaction mixture is washed off and the antigen antibody complex is treated with 4-methyl umbelliferyl phosphate to give a fluorescent product, which is measured by the analyzer's optical assembly. Appropriate standard curves were made similarly.

ETHICAL APPROVAL

This study was approved by the Kasturba Medical College, Manipal University Ethics Committee, under reference number (IEC150-2012). Written, informed consent was obtained from all participants.

RESULT

Statistical analysis was done using t-test for equality of means and one-sample test for TBARS. Significance was determined by Mann-Whitney U test ($P<0.01$, and $P<0.001$, were taken as significant and highly significant, respectively).

The patients (n = 50) of ovarian cancer presented with either stage 1 and 2 in one group and 3 and 4 in another group. On stage-wise comparison of pre treatment and post treatment serum ceruloplasmin and CA125 levels using mean±SD, a significant ($P<0.0001$) difference was observed in each group when comparison is done pre and post treatment of each parameter. There is a significant decrease in the level of CA125 and ceruloplasmin after treatment as shown in Table 1.

Table 1. Pre and Post treatment Levels of CA-125 and ceruloplasmin expressed as mean ± SD in different stages of ovarian cancer.

Stage of Cancer	CA125 (units/liter)		ceruloplasmin (mg/dl)	
	Pre treatment	Post treatment	Pre treatment	Post treament
Stage I & II	43.34±20.50*	28.09±13.22*	75.66±22.35***	37.32±15.13***
Stage III & IV	274.10±113.12**	128.39±36.55**	110.59±22.18****	51.35±15.19****

P<0.005*, P<0.0001** , P<0.0001*** , P<0.0001****

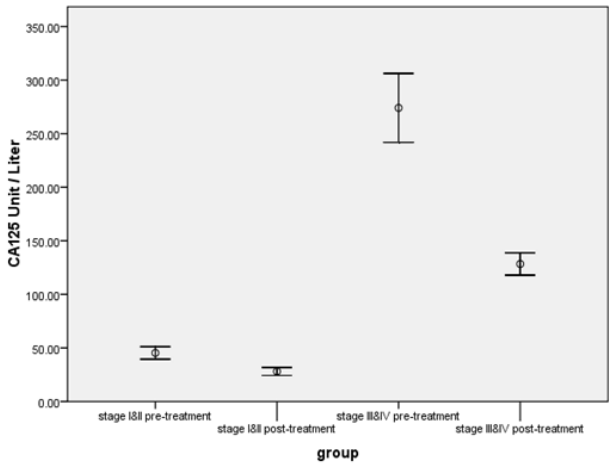


Figure 1. Representing a summary of serum level CA 125 in pre-treatment and post-treatment in patients with ovarian cancer.

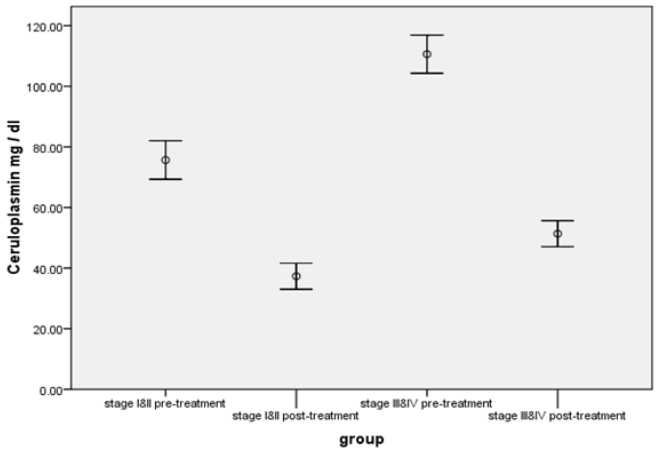


Figure 2. Representing a summary of serum level of Ceruloplasmin in pre-treatment and post-treatment in patients with ovarian cancer.

DISCUSSION

Cancer is one of the leading causes of death worldwide. Despite progress in cancer therapy, ovarian cancer mortality has remained virtually unchanged over the past two decades[23]. Annually in the United States alone, ~23,000 women are diagnosed with the disease and almost 14,000 women die from ovarian cancer[23]. Given our knowledge about the steep survival gradient relative to the stage at which the disease is diagnosed, it is reasonable to suggest that early detection remains the most promising approach to improve the long-term survival of ovarian cancer patients.

Advances in screening methods significantly improved early diagnosis with consequent enhancement of prognosis, survival and treatment efficacy. Unfortunately, some tumors are difficult to diagnose before the disease is in advanced or metastasizing state. Therefore, there is an urgent need to discover novel biomarkers which provide

sensitive and specific disease detection. Over the past decade, serum biomarkers have been identified in sera from cancer patients by using powerful high-throughput technologies.

As an important biological indicator of cancer status and progression from the physiological state of the cell at a specific time, biomarkers represent powerful tools for monitoring the course of cancer and gauging the efficacy and safety of novel therapeutic agents. They can have tremendous therapeutic impact in clinical oncology, especially if the biomarker is detected before clinical symptoms or enable real-time monitoring of drug response. There is a critical need for expedited development of biomarkers and their use to improve diagnosis and treatment for cancer.

Studies have reported that copper and ceruloplasmin levels were also significantly increased in both prostate and colon cancers[24]. However, it continues to be used in follow up studies of patients with breast[25] and lung cancers[26] and widely accepted as having prognostic significance. Ceruloplasmin is a copper binding protein, which increases in several carcinomas. Lightman and Brandes reported that decreased concentrations of zinc and the increased concentrations of copper in serum do not seem to result from a shift of zinc into or release of copper out of the malignant tumor tissue[27]. Secondary in the liver might be contributing to the high levels of ceruloplasmin. Elevation of serum Copper, Ceruloplasmin and their ratios have been reported to be useful in the diagnosis and prognosis of other malignancies.

The results of our study indicate that serum ceruloplasmin along with CA125 may be used as a valuable predictor of the presence of malignant gynaecological tumor or specifically indicates the presence of advanced ovarian cancer.

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REFERENCES

1. Ginath S, Menczer J, Fintsi Y, Ben-Shem E, Glezerman M, Avinoach I. Tissue and serum CA125 expression in endometrial cancer. Int J Gynecol Cancer. 2002;12:372-5.
2. A Jemal, R Siegel, J Xu, E Ward. Cancer statistics, CA: A Cancer Journal for Clinicians. 2010;60:277–300.
3. U Menon, IJ Jacobs. Recent developments in ovarian cancer screening. Current Opinion in Obstetrics and Gynecology. 2000;12:39–42.
4. S. A. Cannistra. Cancer of the ovary. New England Journal of Medicine. 2004;351:2519–65.
5. Nossov V, Amneus M, Su F, Lang J, Janco J. MT Reddy, ST Farias-Eisner R. the early detection of ovarian cancer: from traditional methods to proteomics. Can we really do better than serum CA-125? American Journal of Obstetrics and Gynecology. 2008;199:215–23.
6. LS Cohen, PF Escobar, C Scharm, B Glimco, DA Fishman. three-dimensional power doppler ultrasound improves the diagnostic accuracy for ovarian cancer prediction. Gynecologic Oncology. 2001;82:40–8.
7. Bast Jr, R. C., Klug, T. L., John, E. S., Jenison, E., Niloff, J. M., Lazarus, H.,Knapp, R. C.. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. N Engl J Med. 1983;309:883-7.
8. BAST RC. Reactivity of a monoclonal antibody with human ovarian carcinoma. J Clin Invest. 1981;681:1331-7.

9. Jacobs I, Bast RC Jr. CA125 tumour-associated antigen: A review of the literature. Hum Reprod. 1989;4:1-12.

10. van Haaften-Day, Carolien, Y U Shen, Fengji Xu, Yinhua Yu, Andrew Berchuck, Laura J. Havrilesky, HWA de Bruijn, Ate GJ van der Zee, Robert C. Bast, Neville F. Hacker. OVX1, CA 125 and M-CSF as tumor markers for surface epithelial-stromal tumors of the ovary: A critical appraisal. Cancer. 2001;92:2837-44.

11. Skates SJ, Jacobs IJ, MacDonald N, Menon U, Rosenthal AN. Estimated duration of pre-clinical ovarian cancer from longitudinal CA125 levels. Proc Am Assoc Cancer Res. 1999;43.

12. Bast Jr RC, FP Siegal, C Runowicz, TL Klug, VR Zurawski Jr, D Schonholz, CJ Cohen, RC Knapp. Elevation of serum CA 125 prior to diagnosis of an epithelial ovarian carcinoma. Gynecol Oncol. 1985;22:115-20.

13. Jacobs IJ, Skates S, Davies AP, Woolas RP, Jeyerajah A, Weidemann P. Karen Sibley, David H Oram. Risk of diagnosis of ovarian cancer after raised serum CA 125 concentration: A prospective cohort study. Br Med J. 1996;313:1355-8.

14. Luza SC, Speisky HC. Liver copper storage and transport during development: implications for cytotoxicity. Am. J. Clin. Nutr. 1996;63:812-20.

15. Cappelli-Bigazzi M, G Ambrosio, G Musci, C Battaglia, di Patti MC Bonaccorsi, P Golino, M Ragni, M Chiariello, L Calabrese.. Ceruloplasmin impairs endothelium-dependent relaxation of rabbit aorta. Am. J. Physiol 1997;273:H2843-H2849.

16. Cappelli-Bigazzi M., Battaglia C., Pannain S., Chiariello M., Ambrosio G. Role of oxidative metabolism on endothelium-dependent vascular relaxation of isolated vessels. J. Mol. Cell. Cardiol. 1997;29:871-9.

17. Bianchini A, Musci G, Calabrese L. Inhibition of endothelial nitric-oxide synthase by ceruloplasmin.J. Biol. Chem. 1999;274:20265-270.

18. Patel B N, Dunn R J, Jeong S Y, Zhu Q, Julien J P, David S. Ceruloplasmin regulates iron levels in the CNS and prevents free radical injury. J. Neurosci. 2002;22:6578-86.

19. Kondi-Pafiti A, Smyrniotis V, Frangou M, Papayanopoulou A, Englezou M, Deligeorgi H. Immunohistochemical study of ceruloplasmin, lactoferrin and secretory component expression in neoplastic and non-neoplastic thyroid gland diseases.Acta Oncol. 2000;39:753-6.

20. Cox C, Teknos T N, Barrios M, Brewer G J, Dick R D, Merajver S D. The role of copper suppression as an antiangiogenic strategy in head and neck squamous cell carcinoma. Laryngoscope. 2001;111:696-701.

21. Khandhadiya P K, Yousef Rezaei Chianeh, R Pragna. "Role of serum copper and ceruloplasmin level in patients with dysfunctional uterine bleeding." Int J Reprod Contracept Obstet Gynecol. 2014;3:333-4.

22. Chianeh Yousef Rezaei, Pragna Rao. Molecular and hormonal regulation of angiogenesis in proliferative endometrium. Int J Res Med Sci. 2014;2:1-9.

23. Zhang Zhen, Robert C Bast, Yinhua Yu, Jinong Li, Lori J Sokoll, Alex J Rai, Jason M Rosenzweig, et al. Three biomarkers identified from serum proteomic analysis for the detection of early stage ovarian cancer. Cancer research. 2004;64 :5882-90.

24. Nayak BS, Bhat VR, Upadhya D, Udupa SL. Copper and ceruloplasmin status in serum of prostate and colon cancer. Indian J Physiol Pharmacol. 2003;47:108–10.

25. Steward AM, Niren D. Carcinoembryonic antigen in breast cancer patients. Cancer. 1974;33:1246–7.

26. Concanon JP, Dawbow MH. Prognostic value of preoperative carcinoma. Cancer. 1978;142:1477–9.

27. Lightman A, Brandes JM, Binur N, Drugan A, Zinder O. Use of the serum copper/zinc ratio in the differential diagnosis of ovarian malignancy. Clin Chem. 1986;32:1788–92.

Yazışma Adresi / Address for Correspondence:
Dr. Pragna Rao
Kasturba Medical College, Manipal University
Department of Biochemistry
INDIA
Email: drpragnarao@gmail.com,
pragna.rao@manipal.edu

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