

SHORT COMMUNICATION

SERUM COPPER, CERULOPLASMIN AND THIOBARBITURIC ACID REACTIVE SUBSTANCE STATUS IN PATIENTS WITH OVARIAN CANCER

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Abstract : Serum copper, ceruloplasmin and thiobarbituric acid reactive substances (TBARS) were estimated in 30 patients of ovarian cancer. The copper to ceruloplasmin ratio was moderately increased ($P < 0.05$) but the copper ($P < 0.01$) and ceruloplasmin ($P < 0.001$) levels were significantly increased in ovarian cancer patients as compared to controls. TBARS levels were also found to be highly significant ($P < 0.001$). Trace elements and free radicals have been implicated in the etiology of cancer. Hence the estimation of antioxidants like ceruloplasmin and TBARS along with the trace element like copper may be of value in the early diagnosis of cancer.

Key words : ovarian cancer copper thiobarbituric acid reactive
ceruloplasmin substance CA 125

INTRODUCTION

Of all the gynecologic cancers, ovarian cancer is the most common cause of mortality. More than 60% of patients with ovarian cancer do not present until they are at an advanced stage and the average 5-year survival rate is reported to be lower than 20% (1). A balance between oxidant carcinogens and endogenous antioxidant defence is of particular relevance to the carcinogenesis. Oxidative stress is an imbalance between free radical damage and antioxidant protection in the body.

Cancer antigen 125 (CA 125) has been described as a useful marker in patients monitoring for ovarian malignancy (2).

Although zinc and iron levels remain unaltered, serum copper was significantly increased. Ceruloplasmin, the copper binding protein was also increased (3). Oxidative alterations were assessed by determining thiobarbituric acid reactive substance (TBARS), mainly malonaldehyde (as a measure of lipid peroxidation) (4). Data indicated that there was a substantial increase in thiobarbituric acid reactive substances. Hence this study was planned to assess CA 125, copper, ceruloplasmin and thiobarbituric reactive substances in patients with ovarian cancer.

METHODS

Serum samples were obtained from 30

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patients (Mean age: 60 ± 15 years) with clinically and histological verified ovarian cancer. Thirty normal healthy age and sex-matched volunteers were taken as controls. Serum samples were assayed for copper, ceruloplasmin and TBARS. Copper was assayed colorimetrically (5). 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 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. Four molecules of thiobarbituric acid will react with two molecules of malonaldehyde to form a coloured compound which is measured colorimetrically at 532 nm (8). 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).

RESULTS AND DISCUSSION

Table I depicts the serum levels of CA 125, copper, ceruloplasmin, their ratio and TBARS levels in healthy individuals and ovarian cancer patients. The utility of serum CA 125 monitoring in ovarian carcinoma patients was extremely helpful in defining the response to therapy and detecting an early recurrence of the disease (2). The CA 125 was markedly elevated in patients with ovarian carcinoma. Rising trend in serum CA 125 will indicate appearance of early recurrence. Copper and ceruloplasmin levels were also increased significantly in ovarian cancer patients as compared to controls. Copper/ceruloplasmin ratio in serum was significantly increased when compared to normal volunteers. Studies have reported that copper and ceruloplasmin levels were also significantly increased in both prostate and colon cancers (9). However it continues to be used in follow up studies of patients with breast (10) and lung cancers (11) and widely accepted as having prognostic significance.

Sufficient evidence has been noted in the recurrence of the disease by rise in serum CA 125 concentration earlier than any clinical or radiological investigation. Therefore they suggest that patients with ovarian carcinoma should get serial estimation of serum CA 125 during the

TABLE I: Serum copper, ceruloplasmin and TBARS status in patients with ovarian cancer.

Group (n)	Copper (mg%)	Ceruloplasmin (mg%)	Cu: Ceruloplasmin Ratio	TBARS (μ moles/L)	CA-125 (U/ml)
Control (30)	75.11 ± 2.52	39.85 ± 1.49	1.99 ± 0.13	0.001	21.03 ± 1.21
Ovarian Cancer (30)	$148.37 \pm 21.9^{**}$	$50.62 \pm 2.52^*$	$3.31 \pm 0.55^{**}$	$0.337 \pm 0.0266^{**}$	$806.2 \pm 259.80^{**}$

* $P < 0.01$, ** $P < 0.001$

#Among 30 controls value of TBARS was found to be constant and it was equal to 0.001. So the SD was zero for the control group.

course of their treatment.

Cupric ions are reported to inhibit the production of singlet oxygen; this is of particular significance because of the latter's ability to cross the cell membrane and its high reactivity towards various biomolecules (12).

Ceruloplasmin is a copper binding protein, which increases in several carcinomas. Lightman and Brandes (13) 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. Secondary in the liver might be contributory to the high levels of ceruloplasmin.

Lipid peroxidation is a well established mechanism of cellular injury which leads to production of lipid peroxides and their by products. Malonalaldehyde peroxidation of

polyunsaturated fatty acids and related esters provide a convenient index of lipid peroxidation. In the present study we have been a significant increase in TBA reactivity. Plasma lipids are protected from peroxidation by naturally occurring antioxidants. The enhanced chromogen development with acetic acid of plasma and lipid standards (in which peroxidation has not been induced but which nevertheless contains small amount of peroxides) possibly reflects the acids solvent properties towards the less polar peroxidation products characteristic of the early stages of lipid peroxidation (15). Elevation of serum Copper, Ceruloplasmin and their ratios have been reported to be useful in diagnosis and prognosis of other malignancies (16). The results indicate that serum copper and ceruloplasmin may be used as a valuable predictor of the presence of malignant gynecological tumor or specifically indicates the presence of advanced ovarian cancer along with the CA 125.

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