

Study of Estrogen, Progesterone, Copper, Zinc and some Antioxidants in Sera of Ovarian Cancer Patients

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(Received 17 / 10 / 2012 ; Accepted 28 / 1 / 2013)

ABSTRACT

The research was concerned with a study of the relationship between patients with ovarian cancer (OCa) and some biochemical parameters.

Serum samples were collected from (25) healthy females and (25) females with ovarian cancer. The measured parameters in sera included the level of estrogen, progesterone hormones, some trace elements (copper and zinc), as well as some antioxidants such as glutathione, ceruloplasmin and malondialdehyde (MDA).

Total protein was also measured in the present study. Compared with the healthy females, the females ovarian cancer exhibited a significant increase in the level of estrogen and progesterone hormones, ceruloplasmin, MDA and copper. They also exhibited a significant decrease in the level of glutathione, total protein and zinc.

Keywords: Ovarian cancer, oestrogen and progesterone hormones, zinc, copper, antioxidant.

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INTRODUCTION

Cancer is a disease in which cells in the body grow out of control. As the cells grow and multiply, they form masses called tumors (Doll and Peto, 1988). The ovaries are the main source of the females' hormones estrogen and progesterone. Estrogens (estradiol, estrone, estriol) are predominately female hormones, and in adults they are important for maintaining the health of the reproductive tissues, breast, skin and brain (Maker, 2000).

Progesterone is a hormonal balancer, particularly of estrogen. It enhances the beneficial effect of estrogens while mitigating the problems associated with estrogen excess, natural progesterone helps regulate apoptosis and also helps create a balance of all other steroid hormones (Shuk- Mei, 2003).

Ovarian cancer is a cancer that begins in one or both of woman's ovaries, it is the leading cause of death from all gynecology malignancies (Maker, 2000).

Most (more than 90%) ovarian cancers are classified as "epithelial". These tumors arise from a layer of cells that surrounds the outside of the ovary called the germinal epithelium mostly common in women over 45 years (Cannistra, 2004).

The major role in ovarian cancer initiation and progression is DNA damage, mostly caused by oxidative stress, which is an imbalance between the formation of reactive oxygen species (ROS) and antioxidative defense mechanisms (Sanjyotoi and Melinkeri, 2011 ; Gina *et al.*, 2009). Inadequate antioxidant production or excess production of free radicals, particularly oxygen radicals creates oxidative stress, which causes cell damage and plays a fundamental role in various diseases like cancer (Naidu *et al.*, 2007; Pijic *et al.*, 2006).

The majority of ovarian cancers are thought to be originated from a surface epithelial cell perturbed by ovulation (William and James, 2004). The reactive oxidants (hydroxyl radical, superoxide radicals, hydrogen peroxide and singlet oxygen) which are generated during the mechanics of ovulatory follicular rupture damage the DNA of ovarian surface epithelial cells that are located within limited diffusion radius (Sanjyotoi and Melinkeri, 2011). Recent molecular studies have shown that ovarian cancer has acquired genetic alteration of oncogenes and tumor suppressor genes such as BRCA₁, P53, nm23 and K-ras, which may be due to inflammation and oxidative stress (Barrt, 2007; Sanjyotoi and Melinkeri, 2011).

The present research deals with studying the effect of ovarian cancer on the levels of some hormones, trace elements, antioxidant and total protein in ovarian cancer patients compared to control females.

MATERIALS AND METHODS

Subjects

The subjects included two groups, patient and healthy, aged (49-69). The patient group consisted of (25) females with ovarian cancer who were diagnosed by specialist doctors. The collected samples were obtained at tumor and nuclear medicine hospital (Al-Hafith Hospital) in Mosul City. The healthy (control) group consisted of (25) healthy females.

Both groups (patient and healthy) were in postmenopausal period, and free from any other gynecologic malignancies like breast, cervical, uterine cancers and also any concomitant illnesses such as diabetes mellitus and cardiovascular diseases.

Sample Collection and Analysis:

Venous blood samples were drawn from each patient then transferred immediately to a

clean dry plastic tubes. The blood was allowed to clot for at least (10-15) min. at room temperature and then centrifuged for (10) min. at (3500 rpm). Serum was collected and used to measure some biochemical parameters (Burits and Ashwood, 1999).

Methods:

Estrogen and progesterone hormones were determined by mini VIDAS analyzer (Bio Merieux Vitek, USA) using Enzyme Linked Fluorescent Assay (But and Blunt, 1988 ; Diver, 1987).

Ceruloplasmine level was determined by oxidization method based on the ability of ceruloplasmine to catalyse the oxidation of colorless para-phenylene diamine to a blue-violet complex, which can be spectrophotometrically estimated (Sunderman and Nomato, 1970).

Glutathione level was determined by using the modified method described by (Sedlak and Lindsay, 1968) based on using Ellman's reagent (DTNB).

Serum lipid peroxidation was determined by thiobarbituric acid modified procedure (Guidet and Shah, 1989).

The level of serum trace elements (Cu and Zn) was determined using atomic absorption spectrophotometer (Willard *et al.*, 1974) in Biology Department, College of Science.

Serum total protein was determined by Biuret method (Plummer, 1978) using kit manufactured by RANDOX (United Kingdom).

STATISTICAL ANALYSIS

The data were subjected to statistical analysis using Student's unpaired t-test for comparison of means between patients and controls.

All the data were expressed as mean \pm standard deviation of the mean p-values. The values of 0.05 or less were considered significant.

RESULTS AND DISCUSSION

The results of the measured biochemical parameters are summarized in table (1).

1. Estrogen and Progesterone Hormones:

The results in Table (1) showed a significant increase ($p \leq 0.05$) in the level of estrogen and progesterone hormones in ovarian cancer patients compared to the healthy control group. The obtained results were in agreement with other investigations which suggest that steroid hormones, primarily estrogen and progesterone, are implicated in ovarian carcinogenesis (Shuk-Mei, 2003).

The epidemiological evidence is strongly suggests that steroid hormones, estrogen and progesterone, are implicated in ovarian cancer. Estrogens favor neoplastic transformation of the ovarian surface epithelium (OSE), while progesterone offers protection against ovarian cancer development. Specifically, estrogens, particularly those present in ovulatory follicles, are both genotoxic and mitogenetic to OSE cells. In contrast, pregnancy-equivalent levels progesterone are highly effective as apoptosis inducers for ovarian surface epithelium and ovarian cancer cells. In this regard, high-dose progestin may exert an exfoliation effect and rid an aged OSE of pre-malignant cells (Maker, 2000; Issa *et al.*, 2009). Many studies suggested that ovarian cancer cells under estrogen control containing estrogen-receptors, which are bound with estrogen hormone to form a complex which is bound to promoter

regions of specific genes to activate the transcription of new mRNA and continue this process (Shuk-Mei, 2003; Issa *et al.*, 2009; David, 2003).

Moreover, ovarian cancer has been linked to an increased amount of estrogen activity in the body. Excess fat can cause the body to retain estrogen and may convert other hormones into a form of estrogen, increasing the risk of ovarian cancer (Wolin *et al.*, 2010).

It was reported (Lee *et al.*, 2009) that 86% of ovarian tumor specimens stained positive for estrogen receptors and 50 % positive for progesterone receptors.

Finally, some recent studies suggest that the use of menopausal hormone replacement therapy (HRT) is associated with the increase of rise ovarian cancer particularly in long-time using and users of sequential estrogen–progesterone schedules (James *et al.*, 2002; Anderson, 2003).

Table 1: The means of biochemical parameters in patient and control groups

Parameters	Mean \pm SD		p-value
	Healthy Women (n= 25)	Ovarian Cancer Women (n= 25)	
1. Estrogen Hormone (pg/mL)	6.60 \pm 1.30	22.14 \pm 2.34	$p \leq 0.05$
2. Progesterone Hormone (pg/mL)	2.26 \pm 0.71	7.08 \pm 1.96	$p \leq 0.05$
3. Ceruloplasmin (μ mol/L)	190.21 \pm 24.83	231.62 \pm 33.17	$p \leq 0.05$
4. Glutathione (μ mol/L)	11.73 \pm 1.53	7.27 \pm 1.33	$p \leq 0.05$
5. MDA (μ mol/L)	0.20 \pm 0.076	0.49 \pm 0.15	$p \leq 0.05$
6. Copper (μ mol/L)	8.50 \pm 2.86	14.13 \pm 3.26	$p \leq 0.05$
7. Zinc (μ mol/L)	16.05 \pm 1.52	10.38 \pm 3.76	$p \leq 0.05$
8. Total Protein (gm/L)	61.42 \pm 14.35	49.94 \pm 10.54	$p \leq 0.05$

2. Ceruloplasmin (CP):

Statistical analysis (Table 1) showed a significant increase ($p \leq 0.05$) in the ceruloplasmin level in serum of ovarian cancer patients compared to control group. This finding agrees with those reported by other investigators (Shivananda *et al.*, 2004 ; Knekt *et al.*, 1992), which showed a high ceruloplasmin level in ovarian cancer patients.

Several studies showed that ceruloplasmin concentration increased in breast, liver, cervix, endometrium and gastrointestinal cancer (Shivananda *et al.*, 2005; Alper *et al.*, 2005; Chan *et al.*, 1993).

Ceruloplasmin increase may due to the fact that it is one of the many acute phase reactant proteins synthesized in the liver in response to various stimuli. Many of these internal and external stimuli resulting in liver disorders may be related to acute or chronic infection, and several other conditions, including cancer, may similarly lead to an increased synthesis of ceruloplasmin (Knekt *et al.*, 1992).

3. Glutathione (GSH):

The result in Table (1) showed a significant decrease in glutathione level in serum of ovarian cancer ($p \leq 0.05$) when compared with the control group. This finding agrees with those reported by other investigation (Iyoti *et al.*, 2009), which showed a lower glutathione levels in ovarian cancer patients. Several studies showed that GSH concentration decreased in cervical, uterine and colorectal cancer (Iyoti *et al.*, 2009; Skrzydlewska *et al.*, 2005).

Reduction of glutathione level might be due to the increased turnover, for preventing oxidative damage to these patients suggesting an increased defense against oxidant damage in ovarian cancer (Tasf *et al.*, 2005). Glutathione can play as an antioxidant in many different ways directly as a free radical scavenger or as a co-substrate of some enzyme like glutathione peroxidase. The lower levels of glutathione may favor an overproduction of free radicals and lipid peroxides which in turn may induce damage to the cell membrane and DNA (Iyoti *et al.*, 2009 ; Suleyman *et al.*, 2003).

4. Malondialdehyde (MDA):

Table (1) showed a significant increase in MDA concentration in serum of ovarian cancer patients ($p \leq 0.05$) when compared with the control group. The results were in agreement with other investigations (Sanjyotoi and Melinker, 2011), which showed an increase in concentration of MDA produced in serum of ovarian cancer.

The reason for the increased MDA concentration possibly due to excessive oxidation initiators which are increased as the disease transferred from one or both ovaries to other organs while levels of antioxidants are decreased (Anbazhagen and Chellappan, 2009).

Also it might be due to morbidity of ovarian cancer, since ovarian cancer patient undergo oxidative stress which leads to break down cell components by free radical and reactive oxygen species (ROS) as a result of increasing its concentration in the body and decreasing the efficiency of antioxidants system (Sanjyotoi and Melinker, 2011; Weinstein *et al.*, 2000). In most cases of diseases which cause oxidative stress including the cancer tumors, the activity of free radicals increases more than the ability of antioxidants to remove the dangerous effect of free radicals. All of these lead to increase lipid peroxidation and increase the level of MDA resulting in a loss in the balance between the activity of free radical and the activity of antioxidants which cause the oxidative stress, and increase the level of MDA resulting in a loss in the mobility of epithelial membrane (AL-Hassany, 2005).

5. Copper (Cu)⁺⁺:

The level of copper ion in serum of patients with ovarian cancer are found to be significantly increased ($p \leq 0.05$), when compared to control group as shown in Table (1). Similar results were shown by others (Shivananda *et al.*, 2004; Margalioth *et al.*, 2006).

High levels of copper have been found in many types of human cancer as well as ovarian cancer, such as breast, prostate, colon, lung, liver and brain (Shivananda *et al.*, 2005; Nazmus *et al.*, 2008). Copper is an important microelement which is not only regulate the physiological functions of various organs but is also associated in the production of pathological changes in these organs by interacting with the bases of DNA which leads to an extensive mutation in DNA (Nazmus *et al.*, 2008; Singh *et al.*, 1990).

In addition to it's affectivity in the processes of angiogenesis of the tumor (the growth of tumor blood supply), which lead to the growth, invasion and metastasis of tumor, these lead to increase the level of serum copper in patient with cancer (Skibniewska *et al.*, 2010).

6. Zinc (Zn^{++}):

The serum Zn ion level was significantly lower in the ovarian cancer patients ($p \leq 0.05$) than in the control group as shown in Table (1). The obtained results were in agreement with those reported by others (Cetinkaya *et al.*, 1994).

It was noted (Altaee, 2003) that the zinc concentration in serum of patients with different types of cancer was significantly decreased when compared with control groups.

Zinc acts as a cellular growth protector, including growth of neoplastic cells, and its deficiency was demonstrated to be involved in several stages of malignant transformation.

Zinc is known to be essential for more than a hundred different metabolic functions, it induce DNA synthesis. It is also essential for the activation of adenylyl kinase, phosphodiesterase, membrane-bound adenylyl cyclase and lipid peroxidase (Nazmus *et al.*, 2008). Experimentally, zinc deficiency and zinc supplementation have been shown to inhibit or stimulate responses on tumor growth, adding confusion to the role of zinc in human cancer (Arinola and Charles-Davies, 2008).

7. Total Serum Protein (TSP):

The level of total serum protein of patients with ovarian cancer was found to be significantly decreased ($p \leq 0.05$) as shown in Table (1) compared to control group.

Several studies showed that total protein concentration decreased in different types of cancer like breast and respiratory cancer. (AL-Hassany, 2005; Ahmad and Ali, 2007).

The reason for the decreasing of total serum protein might be due to the high level of the oxidative stress whose occurrence due to the ovarian cancer and leads to the consumption of the antioxidant and then to the oxidation of the other biological molecules like protein and form protein hydroperoxides which has a big role to increase the chance for cancer formation due to this reaction with DNA, then causing the genetic mutation in biological molecules (Valko, 2006). It also might be due to the nutritional deficiency and weight loss in association with the psychological distress and lower quality of life (Ovesen *et al.*, 1993).

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