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Measurement of some Biochemical Parameters in Serum of Uterine Cancer

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ABSTRACT

The research includes measurement of some biochemical parameters in serum of uterine cancer patients. Serum samples were collected from (20) healthy females and (20) females with uterine cancer, their ages ranged between (35-68) years. These parameters included: lactate dehydrogenase (LDH) activity and the level of some antioxidants like vitamin C, vitamin E, glutathione (GSH), malondialdehyde (MDA) and peroxynitrite. Some trace elements (copper and zinc) were also tested in the present study.

In comparison with the healthy females, the uterine cancer exhibited a significant increase in the level of MDA, peroxynitrite, LDH and copper. They also exhibited a significant decrease in the level of vitamin C, vitamin E, glutathione and zinc in comparison with healthy females.

Keyword: Lactate dehydrogenase, uterine cancer, antioxidants

قياس بعض المتغيرات الكيموحيوية في مصل المصابات بسرطان الرحم

الملخص

تضمن البحث قياس بعض المتغيرات الكيموحيوية في مصل المصابات بسرطان الرحم. جمعت نماذج لمصل الدم من (20) امرأة سليمة و(20) امرأة من المصابات بسرطان الرحم، وتراوحت اعمارهن ما بين (35-68) سنة. تضمنت المتغيرات الكيموحيوية المقاسة: فعالية انزيم اللاكتيت ديهايدروجينيز وتقدير مستوى بعض مضادات الأكسدة مثل فيتامين C، فيتامين E والكلوتاثايون فضلاً على التعرف على مستوى المالونداليالديهايد والبيروكسي نتريت. كما تم تقدير مستوى بعض العناصر النادرة (النحاس والخارصين).

مقارنة بالنساء السليمات، أظهرت النساء المصابات ارتفاعا معنوياً في مستوى المالونداليالديهايد، البيروكسي نتريت، فعالية انزيم اللاكتيت ديهايدروجينيز والنحاس. كما أظهرت النساء المصابات انخفاضاً معنوياً في مستوى كل من فيتامين C، فيتامين E، الكلوتاثايون و الخارصين بالمقارنة مع النساء السليمات.

الكلمات الدالة: لاكتيت ديهايدروجينيز، سرطان الرحم، مضادات الاكسدة.

INTRODUCTION

Cancer is a complex disease, involving numerous tempospatial changes in cell physiology, which ultimately lead to malignant tumors, it is characterized by an increase in the number of abnormal cells and an invasion of adjacent tissue by these cells, followed by a spread of malignant cells to distant sites (Seyfried and Shelton, 2010; Gillham *et al*., 2000).

Uterine cancer is the most common gynaecological cancer, it is also called womb cancer or endometrial cancer (Buchanan *et al*., 2009). Nearly all cancers of the uterus start in the lining of the uterus (The endometrium), it is more common in women over 45 year than younger women. Most (over 80%) of endometrial cancers are typical adenocarcinomas (Allen *et al*., 2011; Giordano *et al*., 2007).

The causes of the uterine cancer may be attributed to the carcinogenic chemicals, radiation and oxidative stress (Sun, 1990). Oxidative stress is defined as an imbalance between the formation of oxidant compounds and antioxidants defense mechanisms (Naidu *et al.,* 2007). Inadequate antioxidant protection or excess production of free radicals, particularly oxygen radicals create oxidative stress, which causes cell damage and plays a fundamental role in various diseases like cancer (Naidu *et al*., 2007; Pejic *et al*., 2006).

Severe oxidative stress is not only known to cause DNA damage and mutations of tumor suppressor genes, which are initial events in carcinogenesis (Kang, 2002), but can also play an important role in the promotion of multistep carcinogenesis (Ahmed *et al*., 1999).

Antioxidants are the first line of defense against the free radical damage. Human cells are equipped with both enzymatic and non- enzymatic antioxidant defense mechanisms to minimize the cellular damage resulting from the interaction between the cellular constituents and the reactive oxygen species (ROS) (Mila-Kierzenkowska *et al*., 2004). Epidemiological evidence consistently relates low antioxidants intake or low blood levels of antioxidants with an increased cancer risk (Block, 1992).

The present study discusses the role of oxidative stress in causing uterine cancer by determining the level of: antioxidants, LDH and trace elements in females of uterine cancer compared with healthy ones.

MATERIALS AND METHODS

Subjects

The females included two groups, patient and healthy. The patient group consisted of (20) females with diagnosed uterine cancer aged (35-68) years. The samples collected is from tumor and nuclear medicine hospital (Al-Hafith Hospital) in Mosul city. The healthy (control) group consisted of (20) healthy females aged (35-68) years.

Samples Collection and Analysis

Venous blood samples were drawn from each patient then transferred immediately to a clean dry plain tube. After removing the needle, 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 to measure some biochemical parameters in the laboratory of Chemistry Department, College of Education.

Methods: Serum vitamin C and vitamin E were measured spectro-photometrically. Vitamin C was determined by treatment with 2,4 – dinitro phenylhydrazine (DNPH) (Stanley *et al*., 1979). The method determination of vitamin E in serum is based on the oxidation reduction reaction using Emmerie – Engle Reaction (Varley *et al*., 1980).

The level of serum GSH was determined by using modified method described by (Sedlak and Lindsay, 1968) using Ellman's reagent (DTNB)[5,5-dithiobis(2-Nitrobenzoic acid)].

Serum MDA was measured by a modified method described by (Guidet and Shah, 1989) using thiobarbituric acid.

Peroxynitrite ($ONOO^-$) was measured by the modified method described by (Vanuffelen *et al*., 1998) based on the ability of peroxynitrite to convert the phenol to nitrophenol which can be estimated spectrophotometrically.

LDH was assayed using fortress kit (United Kingdom), by measuring the rate of absorbance change at 340 nm due to the reduction (Tietz, 1976).

The levels of serum trace elements (Cu^{++} and Zn^{++}) were determined using the atomic absorption spectrophotometry (Willard *et al*., 1974) in the laboratory of Department of Biology, College of Science.

Statistical Analysis

The data were subjected to a statistical analysis using the student unpaired t- test for comparison of means between patients and controls. All the data were expressed as mean ± standard deviation (SD) of the mean. P–values ≤ 0.05 were considered significant (Kirkwood, 1988).

RESULTS

The results of the measured biochemical parameters are summarized in the table (1). The result showed a significant increase ($p \leq 0.05$) in the level of MDA, peroxynitrite, LDH and copper in serum of uterine cancer females compared to healthy females. There was a significant decrease ($p \leq 0.05$) in vitamin C, vitamin E, GSH and zinc levels in serum of females with uterine cancer compared to healthy ones.

Table 1: Values of biochemical parameters in females of uterine cancer and healthy females

Biochemical parameters	Healthy females (n: 20)	Uterine cancer females (n:20)
	Mean ± SD	Mean ± SD
Vitamin C (µmol/ L)	39.84 ±8.53	25.96 ± 5.68*
Vitamin E (µmol / L)	18.33± 2.22	11.88 ±2.65*
Glutathione (µmol / L)	12.34± 2.77	8.01 ± 2.85*
Malondialdehyde (µmol / L)	2.30± 0.75	5.64± 1.98*
Peroxynitrite (µmol /L)	72.66± 12.05	93.06± 10.07*
Lactate dehydrogenase (U/L)	143.03± 18.69	253.77± 33.76*
Copper (µmol /L)	17.56 ± 2.43	22.09± 2.39*
Zinc (µmol / L)	15.58± 2.41	11.05± 1.76*

*Significant differences at $P \leq 0.05$

DISCUSSION

Vitamin C:

The results in Table (1) showed a significant decrease in vitamin C level in serum of uterine cancer females ($p \leq 0.05$) when compared with healthy females. Similar results showed that the vitamin C concentrations decreased in the serum of patients with cervical cancer (Manju *et al*., 2002).

The decreased in the vitamin C levels may be associated with its act as antioxidant where it gets utilized, vitamin C reduces the tissues damage and removes free radicals, it directly react with $O_2^{\cdot}$ and $OH^{\cdot}$ (Koechlin, 1998; Niki, 1991) and reformation of vitamin E from tocopherol radical which form as a result of lipid peroxidation, so that vitamin C contributes with vitamin E in protecting cells from damage (Stahl and Sies, 1997).

Vitamin E:

Table (1) showed a significant decrease in vitamin E level in serum of uterine cancer females ($p \leq 0.05$) when compared with healthy females. The obtained results were in agreement with those reported by other (Manju *et al*., 2002; Bhuvarahamurthy *et al*., 1996) which showed a decrease in vitamin E level in cervical cancer patients. The reasons for the decreasing of vitamin E concentrations in uterine cancer patients might be attributed its ability to scavenge lipid peroxides and prevents the cellular damage by the free radicals (Manju *et al*., 2002). Vitamin E is the major antioxidant in cell membranes where it protects membrane structures from lipid peroxidation and severe oxidative damage, which tends to be initiated at polyunsaturated fatty acids (Gillham *et al*., 2000).

The results of several studies suggest that the intake of vitamins, including vitamin C and vitamin E from foods or supplements, may be reduce the risk of endometrial cancer (Bandera *et al*., 2009).

Glutathione:

The statistical analysis showed that there is a significant decrease in GSH level in serum of uterine cancer females ($p \leq 0.05$) when compared with the healthy females. This finding is in agreement with those reported by other investigators (Iyoti *et al*., 2009), which showed that, the level of glutathione is lower in uterine cancer patients. Several studies showed that GSH concentration decreased in cervical, ovarian and colorectal cancer (Iyoti *et al*., 2009; Skrzydlewska *et al*., 2005). The reduction in the GSH level may be resulted in increasing the defense against oxidant damage in cancer. GSH can play directly as an antioxidant in many ways as a free radical scavenger or as a co-substrate of some enzymes like glutathione peroxidase, lower levels of glutathione may favor an overproduction of free radicals and lipid peroxides which in turn may induce damage to the DNA and the cell membrane (Iyoti *et al*., 2009; Suleyman *et al*., 2003).

Malondialdehyde:

Table (1) showed that there is a significant increase in MDA concentration in serum of uterine cancer females ($p \leq 0.05$) when compared with the healthy females. These results were in agreement with other investigations (Pejic *et al*., 2009; Pejic *et al*., 2006), which showed an increase in concentration of lipid peroxidation products, as markers of oxidative stress whereas the level of antioxidants decreased in uterine cancer patients. The rise in MDA concentration could be due to increased generation of (ROS) due to the excessive oxidative damage generated in uterine cancer patients, oxygen radical production, which increases with clinical progression of diseases, involves increased lipid peroxidation, as a

result of which there are cellular membrane degeneration and DNA damage (Pejic *et al*., 2006).

Finally, the increase in the levels of MDA indicated the upsurged lipid peroxidation as a consequence of the increase in free radical generation, these free radical may cause profound alterations in the function of the cell membrane and also structural organization of DNA leading to mutations, therefore, it can be stated that lipid peroxidation product is one of the possible causes of uterine cancer progression (Wagner *et al*., 1998).

Peroxynitrite:

The increase in the levels of the free radicals nitric oxide ($NO^{\cdot}$) and super oxide ($O_2^{\cdot -}$) produced $ONOO^-$ may be occur in malignancies *in vivo* (Cobbs *et al*., 2003).

The results in Table (1) showed a significant elevation in peroxynitrite in the serum of uterine cancer females compared with the healthy females. The increase of peroxynitrite might be due to the excess of $O_2^{\cdot -}$, which is formed as a result of reaction between $NO^{\cdot}$ and $O_2^{\cdot -}$ (Cobbs *et al*., 2003; Paul *et al*., 1998).

Lactate Dehydrogenase:

Lactate Dehydrogenase is an enzyme that is found in almost all body tissues, it's one of the enzyme systems preferentially produced and retained by cancer cells, being necessary to maintain tumor growth. LDH is released into the blood stream when cells are damaged or destroyed and the activity of the enzyme will change (Rijke and Trienekens, 1985).

The results in Table (1) showed a significant increase ($p \leq 0.05$) in LDH activity in the serum of uterine cancer females compared with the healthy females. The obtained results were in agreement with those reported by other investigators (Subramanian *et al*., 2009; Kumar *et al*., 1988), which showed a higher activity of LDH in cervical cancer patients.

On the other hand, Subramanian *et al*., (2009) recorded changes in the total LDH activity in serum and tissues of cervical carcinoma patients, and they suggested that LDH isoenzyme is a useful biochemical tumor indicator for diagnosis and treatment monitoring of the disease, and to assess the grade of malignancy. The increase of LDH activity may be attributed to the genomic changes during malignant transformation, the main reason for the increase of LDH was due to increased tumor cells also produce more amount of lactic acid, and mainly due to the breakdown of glycoprotein into lactic acid formation (Subramanian *et al*., 2009).

Copper:

The level of copper in serum of females with uterine cancer are found to be significantly increased ($p \leq 0.05$) when compared with the healthy females as shown in Table (1). A similar result showed that the copper concentration increases in serum of patients with cervical cancer (Naidu *et al*., 2007). Copper can interact directly with the bases of DNA. The addition of copper to DNA in vitro mediates more extensive DNA base damage inducing more mutations, therefore, the inactivation or loss of certain tumor suppressor genes can lead to initiation and progression of carcinogenesis (Singh *et al*., 1990).

Zinc:

The statistical analysis showed a significant decrease ($p \leq 0.05$) in zinc level in serum of uterine cancer females compared with the healthy females. The obtained results were in agreement with those reported by others (Naidu *et al*., 2007) which showed that there is lower in zinc levels in cervical cancer patients.

It was noted by (Al-Taee, 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, and it control the membrane integrity and membrane stability. So, it may be happen that the cancer cell may consume the zinc present in the circulation for tumor growth and maintain its membrane integrity (Beerheide *et al*.,1990). This might be the possible reason for depletion of zinc in uterine cancer patients.

CONCLUSION

It is concluded that the parameters a raised from the increase in levels of MDA, peroxynitrite, LDH and copper, and the decrease in the levels of vitamin C, vitamin E, GSH and zinc which was noted in uterine cancer, may be used in diagnosis of uterine cancer.

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