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Oxidative Stress and antioxidants in Uterus Cancer Patients

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Abstract

The present study was undertaken to assess oxidative stress by measuring the level of plasma malondialdehyde (MDA) and total thiol, and relate them to the level of some antioxidants such as β-carotene, vitamins (A, E & C), trace elements (Cu, Zn, & Se), albumin in plasma of uterus cancer patients before starting treatment and after remission.

Blood specimens were taken from 20 newly diagnosed uterus patients. Only 10 patients of those were followed up.

The results were compared with those obtained from 20 healthy individuals matching in age and sex.

The uterus cancer patients showed a significant reduction in plasma β-carotene, vitamins (A, E & C), albumin, total thiol, Zn, & Se. While plasma MDA, Cu & Cu / Zn ratios were significantly elevated.
All above-mentioned parameters were normal or near to normal at remission.

Introduction

Any physiological state that is associated with increased production of reactive oxygen species (ROS) is a state of oxidative stress [1,2]. Oxidative stress has been frequently implicated in the initiation and promotion phases of carcinogenesis [3,4].

MDA, a marker of lipid peroxidation, is one of the several products formed in radical induced peroxidation of polyunsaturated fatty acids. The increase in the rate of lipid peroxidation will lead, in turn, to increase of MDA level which is an indirect indicator of elevated ROS free radicals and their toxicity to the tissue [5,6].

Fortunately, ROS formation is controlled by various beneficial compounds known as "antioxidants". Antioxidant micronutrients are important components of fruits and vegetables, and such foods are ideal candidates to increase the antioxidant capacity and hence the oxidative resistance of LDL [7].

Micronutrients generally function as coenzymes or cofactors in metabolic reactions, gene activators (as in the case for retinol), or as free radical scavengers (vitamin A, E & C) [8].

Deficiency of most vitamins and minerals have been associated with depressed immunity [9,10] and epidemiological evidence on the relationship between nutrition and cancer, were reviewed in which diet appeared to play an important role in carcinogenesis [8].

Antioxidants represent one such set of protective factors, which have recently received a great deal of attention because of their ability to reduce oxidative stress [11,12].

The antioxidants, which are available in the body work as one system and are affected by the level of each other [11], and this system has a biological defence properties which oppose the toxic actions of lipid peroxides and ROS, and it limits the amount of lipid peroxides that are formed. The antioxidants are derived either from endogenous synthesis or from the diet, which contains numerous compounds exhibiting antioxidant activity [4]. The antioxidants are present in cells and in extracellular fluids [13], so their intake is easily modifiable by supplementation or dietary changes [12].

Material and Methods

1. Subjects:

Twenty patients with malignant uterus tumors with age range of 40-56 years, and 20 healthy normal individuals of matched age and sex were involved in the study.

These patients were admitted to the Medical City Hospital, they were newly diagnosed and did not underwent any type of therapy and they did not suffer from any other disease that may interfere with our study. Only 10 of these patients were followed up after treatment with surgery.

2. Blood Specimens:

A total of 10 ml venous blood was aspirated from each patient and control subject in EDTA containing tubes. Plasma was separated by centrifugation and stored at −20° C.

3. Methods :

Malondialdehyde (MDA) was assayed according to the methods of Ohkawa et al. [14] with minor modification from Hiraysma [15].

Measurement of plasma β-carotene was done by extraction with ethanol, petroleum ether and chloroform according to the method described by Pesce & Kaplan (1987) [16], while vitamin A was

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measured by trifluoro acetic acid reagent after extraction with chloroform, petroleum ether and acetic anhydride mixture [19].

Vitamin E was measured according to Emmerie-Angle procedure using $FeCl_3$ reagent after removal of interferences by absolute ethanol and n-heptane [20].

Measurement of plasma vitamin C was done by oxidation with Cu^{+2} ions and formation of colored complex with 2,4 dinitro-phenyl hydrazine in sulfuric acid after deproteinization of plasma sample by metaphosphoric acid [18,19].

Plasma total thiol was measured according to Ellmans method [21], and plasma albumin by bromocresol green in acidic pH [22].

For trace elements (Zn & Cu) flame atomic absorption spectrophotometric method after 1:10 dilution with deionized water was used [15,23,24], while plasma Se was measured by flameless atomic absorption spectrophotometer directly [15,24].

Results and Discussion

Tables (1-11) show a significant decrease in each of plasma β-carotene, vitamins (A, E & C), albumin, total thiol, Zn, & Se, and a significant increase in each plasma MDA Cu & Cu / Zn ratios in all measured patients and all returned to normal after complete remission.

Many studies indicated that oxidative stress mediated by increased level of (ROS) had a causative role in the pathogenesis of several types of cancer. Potentially toxic oxygen free radicals are generated continuously in humans [13].

Vitamins (A, E, C, and carotenoids) are the most prominent dietary antioxidant [25]. They have the highest protective action, in cooperation with the scavenging enzymes, in reducing the toxicity of ROS [26], while Cu, Zn & Se are essential nutrients in the enzymatic reactions of antioxidants against free radicals [14].

For all what mentioned above cancerous patients may need to be provided by antioxidants so to overcome the effects of lipid peroxidation and ROS.

The present results revealed a highly significant increase in plasma malondialdehyde level, which returned back to normal after successful treatment and at remission state. This is in agreement with the results of several authors, who reported an increase in MDA in other forms of cancers, they attributed this increase in the level of serum MDA to the increase in the rate of lipid peroxidation of PUFA, which is an indication to the increase in free radicals, lipid peroxidation of polyunsaturated fatty acids and tissue injury [27-31].

The present results also, revealed a significant lowering in the level of beta-carotene in plasma of uterus cancer patients when compared with their controls, which returned back to normal after successful treatment and at remission state.

Each of β carotene and total carotenoids were thought to associate reduced risk of coronary heart disease and some types of cancer [32], and its reduction may be attributed to its role in scavenging superoxides in cultured cells [33], either by chemical interaction or physical quenching [34]. It is also believed that β-carotene could play a role in limiting LDL oxidation as it is transported in plasma mainly in LDL [32].

The low level of vitamin A in plasma of the uterus cancer patients of the present study is in accordance with other reports in other types of cancer [35], and maintaining adequate vitamin A status was found to be important since it can minimize the bioconversion of β carotene to vitamin A and thereby maximizes its recovery in serum as β-carotene [34].

The decrease in vitamin E level with increased oxidative stress as presented by low plasma thiol confirms other reports [31,37].

Vitamin E appears to be at the first line of defense against peroxidation of polyunsaturated fatty acids contained in cellular and sub-cellular membrane phospholipids [18,39].

The present results also showed a marked significant reduction in plasma vitamin C in uterus cancer patients collectively when compared with the controls which also returned to normal or near normal values at remission, a finding which agrees with previous report that showed the presence of an inverse association between various micronutrients, mostly vitamins C, E and beta-carotene and the risk of cancer [40-43].

Reduction in plasma thiol group in the patients of the present study agrees with many previous reports on other types of malignancies [15,44], and glutathion GSH has been suggested, by many workers, to be a critical factor in protecting organisms against toxicity and diseases with maintaining membrane integrity. Its reduction is believed to be due to the increase in its consumption as a result of increased free radical generation [17]. The inhibitory action of glutathion on peroxidation depends on vitamin E and both were seen to act in a synergestic way in this respect [45].

Hypoalbuminemia of cancerous patients could be ascribed to a defective synthesis, intravascular dilution by increased plasma volume or gastrointestinal loss [46]. This low albumin was found to be associated with a decrease in total peroxyl trapping capacity of plasma [30].

The increase in the catabolism of major copper containing plasma proteins (ceruloplasmin) or the displacement of copper from ceruloplasmin by the peroxynitrite radicals, or the use of Cu as a co-factor for many antioxidant enzyme systems are possible causes of hypercupremia seen in malignancy [31-33].

Zinc, on the other hand, is decreased as result of malignancy, something that could be attributed to the increased use of this metal by the cell as a protecting agent against free radicals including the superoxide ions that are produced during the disease [34].

As a result of an elevation in serum Cu and reduction in serum Zn results in an increase in Cu/ Zn ratio, which have been considered as a diagnostic and prognostic tool for different types of cancer [35]. It is well known that zinc antagonizes copper and that lowering serum zinc results in more binding sites in the albumin for non specific transport of copper with a consequent increase in the in plasma copper in cancerous patients [36].

The relationship between serum selenium (Se) and malignant disease has been previously reported [37]. Reduction in serum Se at remission may be a reflection of selenium sequestration by tumor cells as a result of poor dietary intake [38].

Table (1): Plasma MDA levels in uterus cancer patients

MDA (nmole/L)	Control	Uterus cancer patients Pre treatment	Post treatment
N	20	20	10
Mean ± SD	2.28 ± 0.34	11.56 ± 3.55	2.84 ± 0.53
SEM	0.075	0.056	0.198
T-test	-	20.33	14.31
P	-	0.0001*	0.0001**

* For comparison between uterus patients before treatment and normal controls.

** For comparison between uterus patients before and after treatment.

Table (2): Plasma β carotene level in uterus cancer patients

β-carotene (mg/dL)	Control	Uterus cancer patients Pre treatment	Post treatment
n	20	20	10
Mean ± SD	0.057 ± 0.017	0.073 ± 0.005	0.143 ± 0.006
SEM	0.004	0.011	0.002
T-test	-	13.49	34.323
P	-	0.0001*	0.0001**

* For comparison between uterus patients before treatment and normal controls.

** For comparison between uterus patients before and after treatment.

Table (3): Plasma vitamin A level in uterus cancer patients

Vitamin A (mg/dL)	Control	Uterus cancer patients Pre treatment	Post treatment
n	20	20	10
Mean ± SD	0.057 ± 0.010	0.021 ± 0.007	0.057 ± 0.006
SEM	0.002	0.001	0.002
T-test		14.16	28.54
P	-	0.0001*	0.0001**

* For comparison between uterus patients before treatment and normal controls.

** For comparison between uterus patients before and after treatment.

Table (4): Plasma vitamin E level in uterus cancer patients

Vitamin E (mg/dL)	Control	Uterus cancer patients Pre treatment	Post treatment
N	20	20	10
Mean ± SD	1.35 ± 0.35	0.45 ± 0.08	1.14 ± 0.12
SEM	0.078	0.018	0.034
T-test		25.05	32.97
P	-	0.0001*	0.0001**

* For comparison between uterus patients before treatment and normal controls.

** For comparison between uterus patients before and after treatment.

Table (5): Plasma vitamin C level in uterus cancer patients

Vitamin C (mg/dL)	Control	Uterus cancer patients Pre treatment	Post treatment
n	20	20	10
Mean ± SD	1.56 ± 0.36	0.43 ± 0.10	1.34 ± 0.33
SEM	0.08	0.02	0.11
T-test	-	13.75	12.78
P	-	0.0001*	0.0001**

* For comparison between uterus patients before treatment and normal controls.

** For comparison between uterus patients before and after treatment.

Table (6): Plasma total thiol level in uterus cancer patients

Total thiol (μmol/L)	Control	Uterus cancer patients Pre treatment	Post treatment
N	20	20	10
Mean ± SD	778.25 ± 13.45	452.00 ± 58.17	64.00 ± 87.51
SEM	3.01	13.01	27.68
T-test	-	22.93	24.75
P		0.0001*	0.0001**

* For comparison between uterus patients before treatment and normal controls.

** For comparison between uterus patients before and after treatment.

Table (7): Plasma albumin level in uterus cancer patients

Albumin (gm/dL)	Control	Uterus cancer patients: Pre treatment	Post treatment
n	20	20	10
Mean ± SD	4.49 ± 0.29	2.74 ± 0.72	4.11 ± 0.46
SEM	0.06	0.16	0.145
T test	-	7.05	26.48
P	-	0.0001*	0.0001**

* For comparison between uterus patients before treatment and normal controls.

** For comparison between uterus patients before and after treatment.

Table (8): Plasma copper level in uterus cancer patients

Copper (ppm)	Control	Uterus cancer patients: Pre treatment	Post treatment
n	20	20	10
Mean ± SD	1.3 ± 0.27	1.78 ± 0.27	1.02 ± 0.17
SEM	0.061	0.060	0.053
T-test	-	29.61	16.42
P	-	0.0001*	0.0001**

* For comparison between uterus patients before treatment and normal controls.

** For comparison between uterus patients before and after treatment

Table (9): Plasma zinc level in uterus patients

Zinc (ppm)	Control	Uterus cancer patients: Pre treatment	Post treatment
n	20	20	10
Mean ± SD	0.928 ± 0.25	0.658 ± 0.14	1.01 ± 0.14
SEM	0.056	3.032	0.045
T-test	-	21.58	22.55
P	-	0.0001*	0.0001**

* For comparison between uterus patients before treatment and normal controls.

** For comparison between uterus patients before and after treatment

Table (10): Plasma Cu/Zn ratio in uterus cancer patients

Cu/Zn ratio	Control	Uterus cancer patients: Pre treatment	Post treatment
n	20	20	10
Mean ± SD	1.07 ± 0.28	2.67 ± 0.64	0.9 ± 0.21
SEM	0.063	0.14	0.07
T test		18.49	17.77
P	-	0.0001*	0.0001**

* For comparison between uterus patients before treatment and normal controls.

** For comparison between uterus patients before and after treatment.

Table (11): Plasma selenium level in uterus cancer patients

Selenium (ppm)	Control	Uterus cancer patients: Pre treatment	Post treatment
n	20	20	10
Mean ± SD	0.11 ± 0.029	0.05 ± 0.013	0.10 ± 0.019
SEM	0.007	0.003	0.006
T-test	-	7.9-1	17.37
P	-	0.0001*	0.0001**

* For comparison between uterus patients before treatment and normal controls.

** For comparison between uterus patients before and after treatment.

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الخلاصة

تهدف الدراسة الحالية إلى تقييم حالة الإجهاد التأكسدي من خلال قياس مستوى المالون داي الديهايد والثايول الكلي (Total thiol) وربطها مع دور بعض مضادات الأكسدة في النساء المريضات المصابات بسرطان الرحم متمثلة بقياس مستويات البيتا-كاروتين وفيتامينات (C, E, A) والألبومين والخارصين و النحاس والسيلينيوم أثناء فترة المرض وبعد إكمال العلاج والوصول إلى حالة الشفاء (remission).

ولغرض المذكور أعلاه تم جمع نماذج دم من 20 مريضة مصابة بسرطان الرحم وتم متابعة حالة 10 منهن. قورنت النتائج مع تلك التي تم الحصول عليها من 20 امرأة طبيعية بنفس العمر والجنس. وأظهرت النتائج انخفاضاً واضحاً في جميع المتغيرات التي تمت دراستها عدا تركيز المالون داي الديهايد والنحاس ونسبة النحاس إلى الخارصين (حيث كانت أعلى بصورة ملحوظة) في دم المرضى.

ولوحظ عودة كل المتغيرات المذكورة إلى مستوياتها الاعتيادية أو إلى ما يشبه ذلك بعد إكمال العلاج والوصول إلى حالة الشفاء.