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Serum lipid peroxidation and trace elements levels in ovarian cancer patients before and after cisplatin and doxorubicin chemotherapy

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Abstract:
Background: chemotherapy and radiation therapy are associated with formation of reactive oxygen species and depletion of critical serum and tissue antioxidants. Although the oxidative statues in patients undergoing chemotherapy treatment were investigated in many studies, but their results were heterogeneous.
Objective: This is a preliminary study conducted to explore the effects of cisplatin and doxorubicin on serum malondialdehyde levels (MDA) (as an oxidative stress indicator) together with the changes in levels of certain trace elements: Zinc (Zn), Copper (Cu), Manganese (Mn), and Iron (Fe) , Cu/Zn ratio, and protein concentration in ovarian cancer patients.
Methodology: This is a case control study conducted in Chemistry Department/ College of Science / University of Baghdad. Six patients having histologically confirmed ovarian cancer(stage III) were participated in this study before and after chemotherapy with cisplatin and doxorubicin. Serum MDA was measured by Hirayama et.al spectrophotometry method, and the levels of certain serum trace elements: Zinc (Zn), Copper (Cu), Manganese (Mn), and Iron (Fe) were estimated by using flame atomic absorption spectrophotometry .

Also serum protein concentration was measured in both mentioned groups. Three of our six patients were followed –up after the second course of the same chemotherapy where the same parameters were measured.

Results: A significant increase ($p<0.05$) in the levels of MDA, Cu, Fe, and a significant decrease ($p<0.05$) in Zn level was observed after the 1st course of chemotherapy when compared with those before treatment. On the other hand, serum Mn level and protein concentration show no significant differences before and after the first course of chemotherapy. Cu/Zn ratio was significantly increased in ovarian cancer patients after treatment when compared with that of the pre-treatment group.

Among the six patients under treatment, we manage to follow-up only three patients after the 2ed course of chemotherapy. An increase in MDA, Cu, Fe levels, and depletion in Zn, Mn levels, and protein concentration were observed when compared with that of the 1st course of treatment.

Conclusion: The results of the present study support the suggestion that treatment with cisplatin and doxorubicin gives rise to an increase in the oxidative stress through the increase in reactive oxygen species.

Keywords: Malondialdehyde; Zinc; Copper; Iron; Manganese; Chemotherapy; Ovarian cancer.

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)] . Surgery has to be followed by chemotherapy in most cases of ovarian cancer because of the advanced stage of the disease [(2)].

Resistance of ovarian cancer cells to cytostatic drugs represents the principal cause of therapeutic failure and, consequently, the principal cause of death in cases of ovarian cancer [(2)]. A balance between oxidant carcinogens and endogenous antioxidant defence is of particular relevance to the carcinogenesis [(3)] .

Reactive Oxygen Species (ROS) result from cell metabolism, as well as from extracellular processes. ROS exert some functions necessary for cell homeostasis maintenance, but when produced in excess a state of oxidative stress results (Oxidative stress is an imbalance between free radical damage and antioxidant protection in the body) and an intracellular environment more favorable for macromolecule damage, mutations, and cancer progression will result [(4,5)]. There is ample evidence showing that patients with malignancies

have a higher incidence of reactive oxygen species (ROS), and a lower concentration of antioxidants [6,7], further decreasing levels of antioxidant was reported as the spread of the malignancy increases [6]. The increase in ROS leads to an increase in DNA mutations, as well as to immunosuppression [8,9].

It has been suggested that ROS, mediated lipid peroxides, are of critical importance since they participate in chain reactions that amplify damage to biomolecules including DNA. This will give rise to mutations that may involve tumor suppressor genes or oncogenes, and this is an oncogenic mechanism [10].

On the other hand, ROS production is a mechanism shared by many chemotherapeutic drugs due to their implication in apoptosis control. The ROS mediated cell responses depend on the duration and intensity of the cells exposing to the increased ROS environment. Thus the status redox is of great importance for oncogenetic process activation and it is also implicated in tumor susceptibility to specific chemotherapeutic drugs [11,12].

Lipid peroxidation (LPO) is the oxidative conversion of polyunsaturated fatty acids (PUFA) to MDA ; which is cytotoxic and acts as tumor promoter and co-carcinogenic agent [13,14].Damage caused by LPO impairs the functioning of the biological membrane and the continued damage leads to loss of membrane integrity which is one of the main characteristics of tumor cell[15].

The most frequently applied drug in the therapy of ovarian cancer is platinum analogues. The mechanism of action of cisplatin and of other platinum analogues involves binding drug molecules to DNA (the formation of so-called DNA adducts). Cells may react to the formation of such adducts in various ways, depending upon the efficacy of their regulatory systems, proliferative activity and the number of adducts formed. One of the possible cellular reactions involves DNA repair and the other mobilization of the process of cell apoptosis. The efficiency of a therapy incorporating platinum analogues thus reflects the efficacy of the mechanisms which control apoptosis. The best recognized apoptosis regulators of predictive value in ovarian cancers are p53, p21, p27 and BCL-2 [16,17].

Among the drugs used in ovarian cancer treatment is doxorubicin, which is an antitumor antibiotic, made from natural products, and act during multiple phases of cell cycle through intercalation in the DNA strand, free radical formation are among the mutagenic mechanism of this drug [18].

Recent studies draw attention to trace elements levels which act mainly as basic components of essential enzymic systems or other proteins that plays major roles in human physiology. Zn is involved in the function of approximately 80 enzymes in body [19]. Also it serves as an integral part of biomembrane; where it may be involved in the control of membrane integrity, membrane stability, and lipid peroxidation- related injuries. An important role of Zn was suggested in carcinogenesis since it has an inhibitory effect on phosphodiesterase as well as it has role in RNA and DNA polymerase and has an activating effect on membrane bound adenyl cyclase [20]. Zn, and Mn are important elements in the preservation of immune resistance and both Zn and Cu are required for numerous biochemical functions and for optimal activity of the immune system [21].

On the other hand, Cu and Fe can catalyze the formation of the highly reactive hydroxyl radicals from H_2O_2 via the Haber-Weiss reaction and decompose lipid peroxides to peroxyl and alkoxyl radicals, which favor the propagation of lipid oxidation. The competition of Zn for Fe binding sites is particularly relevant taking into account that Zn deficiency facilitates intracellular Fe accumulation [22].

A decrease in trace elements concentration may facilitate the malnutrition process that takes place in cancer patients. Negative acute phase reactants like selenium and zinc decreased in cancer patients while copper serum levels increased. Whereas manganese deficiency may interfere with free radical-mediated damage [23].

This study aimed to explore the effects of cisplatin and doxorubicin on oxidative stress parameter (expressed as lipid peroxidation products, MDA),some trace elements levels, Cu\Zn ratio, as well as sera protein levels in ovarian cancer patients during chemotherapy.

Materials and methods:

Chemicals:

All laboratory chemicals and reagents used were of analar grade.

Patients:

A total of (6) patients suffering from ovarian cancer tumors (stage III) aged (35-50) years were used in this study. All patients were admitted for management to nuclear medical hospital and Al-khadumia teaching hospital. The diagnosis was proven by cytological histopathological examination. Patients were given treatments and the dosage of drug was adjusted according to weight on each follow-up.

There was no dietary or supportive antioxidant medication given to patients which could affect the status of free radicals and trace elements levels. At the time of blood collection, there was no evidence of infections, tissues injuries or any inflammatory manifestation in the patients. Blood samples were taken from patients following an overnight fasting period. Thus, avoiding the possible influence of dietary factors on ROS level.

Blood samples were taken from the cancer patients group after the first and second course of chemotherapy that included cisplatin (20 mg/ m^2) and doxorubicin (60 mg/ m^2), which were given every 21 days.

Blood samples:

Five milliliters of blood sample were obtained from each participant in this study, by vein puncture, left for (15) minute at room temperature for coagulation. Then the sera were separated by centrifugation at (3000xg) for (10) minutes and stored frozen at (- 20) ^oC until being used.

Determination of total protein concentration:

Lowry et.al. method [24], was used for the determination of total protein concentration. Bovine serum albumin (BSA) was used as a standard protein.

Lipid peroxidation determination:

Hirayama et.al. [25] method was used for the determination of lipid peroxidation. In this method, MDA, the product of poly unsaturated fatty acid oxidation (PUFA), react with thiobarbituric acid (TBA) to give a pink color chromophore absorbing at (535) nm.

MDA concentrations were calculated using its molar absorbtivity coefficient of (1.56×10^5 M^{-1} cm^{-1}) and the results was expressed as nmole MDA /mg protein.

MDA (nmol / mg) =A x Vt x 10^9 / Vs x 1000 x 1.56 x 10^5 x protein concentration

Where:

A= Absorbance.

Vt = Total volume.

Vs= Sample volume.

Determination of trace elements in serum:

Some trace elements: Iron (Fe), Copper (Cu),Manganese (Mn), and Zinc (Zn) levels were determined in sera of healthy women, and patients with ovarian cancer before and after chemotherapy, using

flame atomic absorption spectrophotometer. The serum was diluted five folds with de-ionized water. Fe was determined at a wave length of (284.3) nm, Cu at (324.7) nm, Mn at (279.5) nm, and Zn at (319.9) nm. The concentrations of Fe, Cu, Mn, and Zn were calculated from their corresponding standard.

Statistical Analysis:

Statistical analysis between group 1 (pre-treatment), and group 2(post- the 1st course of treatment), was performed using student's t-test. The data were expressed as mean ± SD. Differences between means giving a probability of less than 5% ($p < 0.05$) was considered statistically significant.

Results:

The levels of well- known marker of oxidative stress were measured in term of MDA ,a circulating serum lipid peroxide, in sera of ovarian cancer patients before and after two courses of chemotherapy. A significant increase ($p < 0.05$) was found after treatment group when compared with that of before treatment group (table 1).

Trace elements levels were estimated in sera of two groups: ovarian cancer patients before and after chemotherapy using flame atomic absorption spectrophotometer. The results revealed a significant increase ($p < 0.05$) in sera Cu and Fe and a significant decrease ($p < 0.05$) in Zn levels in sera of ovarian cancer patients after the 1st course of treatment compared to that before treatment. While there were no significant differences in sera Mn levels in the above groups (table 2).

Cu/Zn ratio was also found to be substantially higher ($p < 0.05$) in cancer group after treatment when compared with that of pre-treatment group (table 2).

It is worth to mention that we were able to estimate the measured parameters in only 3 cases after the 2ed course of chemotherapy were a continues increase in their sera Cu and Fe levels and a decrease in Zn level was observed when compared with that of the 1st course. Although Mn levels were unchanged in all mentioned groups, but we noticed a decrease in its level after the 2ed course of chemotherapy. An increase in Cu / Zn ratio was found after the 2ed treatment when compared with that of the 1st course.

Insignificant differences were found in serum protein concentration between the ovarian cancer patients before and after the 1st course of chemotherapy (table 1), while there was a decrease in its level after the 2ed course of treatment when compared to other groups.

Discussion:

Cancer is thought to occur through a multisteps carcinogenesis pathway that includes damage to DNA [26]. This damage may lead to structural modifications in DNA [27], which over time and without repair can lead to increased cancer incidence.

ROS play an effective role in pathogenesis of different pathological diseases including cancer. Some chemotherapeutic agents have been shown to increase lipid peroxidation and thus increase generation of ROS[28].

It was shown that radiation therapy and several chemotherapeutic agents exert their antitumor effects through increased formation of reactive oxygen species [29], leading to increased oxidative stress and provoking cell death as a result of massive cellular damage. So, although the malignant neoplasms undergo apoptosis, damage may also occur to normal, healthy cells [30].

Since generated ROS causes peroxidation of PUFA of the membrane, they interrupts the membrane integrity and may be one of the possible reasons of cancer progression. Direct measurement of ROS is not reliable due to short half-life of ROS and none of the methods are sensitive enough for quantification of ROS. The indirect methods for estimation of ROS or oxidative stress include estimation of oxidized products of lipid, protein and status of endogenous antioxidants. Lipid oxidation is evaluated in term of total lipid hydroperoxides (LOOH), and MDA, which was used as a surrogate marker for oxidative damage to tissues [31,32,33].

Previous studies have however reported controversial and conflicting results with regard to the levels of lipid peroxidation by-products and antioxidants in human cancer. MDA levels were reported to decrease in patients with colorectal cancer [34], and patients with breast cancer [35]. MDA was reported to be an unstable intermediate in the peroxidation sequence of unsaturated fatty acids which may be metabolized further or be transported.

Throughout the current study, after chemotherapy, a significant increase in the levels of MDA was found, which was in agreement with those in patients undergoing chemotherapy with stem cell transplantation [36], leukemia [37], cervical cancer [38], and similar result was found in ovarian cancer patients treated with selenium combined with chemotherapy [39]. While there was a decrease in MDA levels in sera of colorectal cancer patients before chemotherapy and no significant differences was found after treatment [40]. Also treatment

with tamoxifen was reported to reduce the increase in lipid peroxidation in postmenopausal women with breast cancer [41].

The effect of chemotherapy on serum lipid peroxidation end-products was also studied in patients with small cell lung cancer SCLC. Serum thiobarbituric acid reactive substances (TBARs) and Schiff's bases (SB) levels rose 24 h after 1st chemotherapy in the whole group, and the highest increments were in patients with complete or partial response after 1st, 3rd and 6th cycles. They speculated that monitoring of circulatory TBARs and SB may be helpful for screening of SCLC patients with high risk of early disease progression and chemotherapy failure [42].Also increased formation of lipid peroxidation products following chemotherapy (adriamycin, mitomycin C, bleomycin) was observed. These results confirm that many anti-cancer drugs augment free radical generation and lipid peroxidation even in vivo situation [43].

It was suggested that the observed increase in serum TBARS is probably due to leakage from cells, or its overproduction and diffusion from tumor tissues [44]. It has been observed that the activities of enzymatic antioxidants tended to decline to the minimum point when the TBARS values are at peak or vice versa [45]. Kim et.al. [46], and Geum [47] et.al reported that the degeneration in the antioxidative system and overproduction of peroxidant may be the cause for higher MDA concentration in women with cervical intraepithelial neoplasia than in control subjects.

Throughout the present study, serum Cu level was found to elevate while serum Zn level was found to decrease. These data confirm the earlier studies in other cancers [20,48]. Contrarily, unchanged serum Zn and Cu were determined in cases of gastrointestinal cancer and cervical cancer [49,50]. As both metals, together with Mn, are the prosthetic group of Superoxide dismutases (SODs), any alterations in their levels may affect activities of this enzyme. Nevertheless, this may cause oxidative stress or may further increase the existed stress [20]. Zinc, is an essential trace elements, involved in protection against oxidative stress in cells, and the decrease in serum levels of Zn may be due to the overproduction of ROS in cells during chemotherapy so, Zn may be taken up by cells to bring down the oxidative stress [3]. It was speculated that this decrease in Zn level in patients with colorectal carcinoma [22] may be due to the increased turnover of Zn for preventing oxidative damage in those patients. Similar reports of lowered Zn levels in cancers have been reported earlier in patients with cervical cancer [51].

It is worth to mention that the biosynthesis of macronucleotides and their incorporation into nucleic acid had been found to be impaired during Zn deficiency [52].

Serum Cu was suggested as a useful index for the extent of leukemia and malignant lymphoma, and may predict response to chemotherapy [53]. It might indicate that essential trace elements alteration in leukemia patients were mostly dependent on tumor activity [54].

High-dose chemotherapy gives rise to an increase in the oxidative stress and the reactive oxygen species, where a significant increase in the levels of MDA, glutathion peroxidase GSH-Px, and SOD were observed, on the other hand, Cu levels remained the same while the levels of Zn and Fe were increased [36].

Decrease in Cu, Zn, and Mn was reported during platinum treatment and it was suggested that this decrease in serum could be due to increased urinary excretion caused by impaired cellular metabolism [55,56].

The effect of chemotherapy (5-fluorouracil, methotrexate, and L-folinic acid) on some trace elements levels was studied. Selenium Se and Zn concentration were significantly lower whereas copper Cu concentration was significantly higher in cancer patients than in control subjects. Moreover, at the end of treatment Se and Zn serum levels showed a significant decrease while Cu level was higher compared with base line values. Their conclusion was that in cancer patients an alteration in the serum concentration of trace elements after polychemotherapy occurred, and this alteration is the consequence of a direct effect of antineoplastic drug on the catabolism of trace elements [57].

Since it appears that the serum Cu levels is influenced by the level of Zn, because these metals are physiological antagonists, the Cu\Zn ratio has been advocated as a more useful measure of disease activity [58].

Increased ratio of Cu/Zn found in this study may be due to the significant decrease in Zn and concomitant increase in copper. It was suggested that, as this ratio is altered, this could be considered as risk factor for tumor growth or carcinogenesis. Further, altered Cu/Zn ratio may be considered as a risk for sustained tumor growth [59,60]. Other studies suggest that the use of serum zinc and copper concentration and the copper/zinc ratio (Cu/Zn) may be useful parameters for estimating the presence and prognosis of malignant tumors [61,62]. Also high Cu/ Zn ratio was observed in patients with cervical intraepithelial neoplasia [63].

The decrease in serum Mn level found in our study after the second course of chemotherapy may be due to malnutrition which has been reported to be associated with changes in drug disposition, including changes in absorption, protein binding, hepatic metabolism and renal elimination [64].

It is widely recognized that Fe status is disturbed in cancer. Patients are typically anemic, reflecting the high demands of proliferating tumor cells for the metal. Conversely, serum ferritin – an acute phase protein – is often increased in cancer. In health, the speciation of iron is tightly controlled so as to prevent the catalysis of oxygen radical formation and biomolecular damage, which occur when the metal is liberated from its storage and transport proteins. It was found that serum ferritin levels were elevated, irrespective of chemotherapy status, whereas transferrin saturation levels were generally below the physiological range, but increased in response to chemotherapy. Iron –binding capacity was often increased in patients with testicular cancer, which may result from iron release from liver and tumor cells in response to chemotherapy. This increase may catalyze harmful free radical reactions, thereby promoting damage to non- cancerous tissues, but on the other hand, radical generation may also contribute to tumor cell killing [65].

The increase in Fe concentration in our study before and after treatment was in agreement with other studies [36,66]. In a study, where Non-protein bound iron NPBI was measured, various other iron parameters and antioxidants in cancer patients undergoing cisplatin-based chemotherapy at various time points before and during chemotherapy were estimated. The rise in NPBI was accompanied by a significant rise in total plasma iron and ferritin and a marked decrease in the latent iron-binding capacity were found. Concomitantly, plasma vitamins C and E decreased significantly, indicating consumption of antioxidants. Similar observations were also made during the fourth chemotherapy cycle. Their conclusion was that cisplatin chemotherapy induces oxidative damage which rapidly leads to release of iron from intracellular proteins and the appearance of NPBI. They suggested that the observed high levels of NPBI may be a major causative determinant in chemotherapy-induced toxicity [66].

The decrease in protein concentration, found in this study, may be due to ROS overproduction after treatments which cause an increase in the oxidized proteins and protein carbonyls levels as suggested by previous studies [3,65,67], or may be due to malnutrition where in

malnourished patients, reduced concentrations of plasma proteins may increase significantly the likelihood of toxicity from the administration of agents that are highly protein bound, such as prednisolone, etoposide, teniposide, cisplatinum, paclitaxel and the irinotecan metabolite SN-38 [64].

However due to the circumstances and the bad security situation in Iraq, limited number of cases included in this study, more studies may be required to substantiate the results and arrive at a definite conclusion in terms of the efficacy of adding antioxidant therapy as secondary therapy for the treatment of ovarian cancer.

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Table (1): MDA levels (mean ±SD) in sera of ovarian cancer before treatment (group1), and after the 1st course of chemotherapy (group2).

Groups	No.	Protein(mg/dl)	MDA(nmol/mg)
1	6	7.38 (±0.79)	0.20 (±0.03)
2 (1st course)	6	7.29 ns (±0.11)	0.25 S (±0.06)

S = $p < 0.05$ compared to group (1).
ns = not significant when compared to group (1).

Table (2): Trace elements level (mean ±SD) in sera of ovarian cancer before treatment (group1), and after the 1st course of chemotherapy (group2).

Groups	No.	Fe (µg/dL)	Cu (µg/dL)	Zn (µg/dL)	Mn (µg /dL)	Cu/Zn ratio
1	6	250.79 (±8.25)	221.40 (±5.11)	66.68 (±9.90)	115.1 (±11.5)	3.29 (±0.33)
2 1st course	6	287.71 S (±7.01)	244.59 S (±3.23)	51.05 S (±6.01)	112.2 ns (±7.6)	4.76 S (±0.42)

S = $p < 0.05$ compared to group (1).
ns = not significant when compared to group(1).

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مستويات الاكسده الفوقيه للشحوم والعناصر الضئيله في مصول المصابات بسرطان المبيض قبل وبعد العلاج الكيميائي (doxorubicin & Cisplatin)

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الخلاصه:
خلفية الدراسه: أكدت الكثير من الدراسات أن أحد تأثيرات العلاج بالأشعاع والعلاج الكيميائي هو زيادة تكوين الجذور الحره وأنخفاض مستويات مضادات الأكسده في المصل .وبالرغم ان دراسات كثيره أهتمت بحالة الاكسده في مصول المرضى تحت العلاج الكيميائي الأ أن نتائجها كانت متناقضه.
هدف الدراسه:
دراسة تأثير العلاج الكيميائي(doxorubicin &Cisplatin)على مستويات الاكسده الفوقيه للشحوم(MDA أحد نواتج اكسدة الشحوم)والتي تشير الى مستويات حالة الاكسده, ومستويات بعض العناصر الضئيله (الزنك Zn , النحاس Cu , المنغنيز Mn , الحديد Fe) والتركيز البروتيني في مصول (6)عينات لنساء مصابات بسرطان المبيض قبل وبعد العلاج الكيميائي وذلك بسبب عدم وجود بحوث التي تقيس حالة الاكسده عند مرضى سرطان المبيض بعد العلاج الكيميائي بأستخدام(doxorubicin &Cisplatin) .كما تمت متابعة ثلاث حالات للنساء المصابات بسرطان المبيض بعد الكورس الثاني من العلاج الكيميائي.
طريقة العمل: تم قياس MDA باستخدام طريقة(Hirayama et.al) الطيفيه ,كما تم أستخدام مطيافية الامتصاص الذري اللهبي لقياس مستويات بعض العناصر الضئيله في مصول النماذج تحت الدراسه.
النتائج: أظهرت النتائج وجود زياده معنويه في مستويات كل من MDA, Fe, Cuوأنخفاض معنوي في مستويات Zn في مصول المصابات بسرطان المبيض بعد الكورس الاول من العلاج الكيميائي (doxorubicin &Cisplatin) مقارنة مع تلك قبل العلاج. اما في مايخص مستويات Mn والتركيز البروتيني فلم يلاحظ وجود تغيير معنوي في جميع المجاميع أعلاه.
تم التمكن من متابعة (3) عينات فقط من النساء المصابات بسرطان المبيض بعد الكورس الثاني من العلاج الكيميائي وقد لوحظ وجود زيادة في مستويات كل من MDA, Fe, Cuوأنخفاض في مستوى Zn وأيضا أنخفاض في مستوى Mn والتركيز البروتيني عند مقارنتها مع تلك بعد الكورس الأول من العلاج الكيميائي .
أظهرت الدراسه أيضا وجود زياده معنويه في نسبة النحاس الى الزنك (Cu/ Zn) في مصول المصابات بسرطان المبيض بعد العلاج مقارنة مع تلك لمجموعة قبل العلاج الكيميائي.
الاستنتاج: اظهرت نتائج هذه الدراسه أن العلاج الكيميائي لمرضى سرطان المبيض بأستخدام (doxorubicin &Cisplatin) يؤدي الى زيادة حالة الاكسده والتي سببها زيادة مستويات الجذور الحره.