Relationship between L- carnitine and trace metals in Breast cancer female patients

Fathy M. El-Taweel[1], Mammdouh M. El-Sheshtawy[2], Salem Habeeb[1]; Sheren. M. Waly[1]

[1]Chemistry Department, Faculty of Science.New Damietta city, Damietta University, Egypt
[2]Biochemistry – Faculty of Pharmacy, Mansoura University, Egypt
fathyeltaweel@yahoo.com, mshisht@mans.edu, egsheren.waly@yahoo.com

Abstract: Trace metals and metals induced oxidative stress have been known to have a role in breast carcinogenesis, L- carnitine, one of complementary and alternative medicine, play important role in cell energy metabolism and has antioxidant effect. The present study was conducted to investigate the effects of L – carnitine on levels of trace metals and oxidative stress process in breast cancer female patient. The present study included 80 female patients as well as 20healthy control. Trace metals, L – carnitine level, were assessed in patients serum before and after end of chemotherapy. Patients divided into two groups, first group include 40 patients received L – carnitine +chemotherapy and a second group of 40 patients received chemotherapy only. The present results showed significant increase in Iron, Lead, Manganese, Cadmium in breast cancer patient compared with control group except copper and Zinc were higher in control group. The only effect of L- carnitine on trace metals was on iron level which decrease in patients received L- carnitine otherwise no effect. The present study concluded that L-carnitine chemotherapy combination leads to the reduction of iron level in female patients with breast cancer and also has potent antioxidative stress in the form of preserving the level of Glutathione on comparison to other groups.
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Keywords: L- carnitine, trace metals, breast cancer, oxidative stress.

Introduction:

Breast cancer is the most common lethal malignancy accounting for nearly 23% of all female cancers in the world, in which 500,000women worldwide will die of this disease with more than a million new cases each year. The incidence rates of breast cancer very worldwide, with high rates in North America, Northern and Western Europe, and low rates in Africa and Asia [1].

Environmental factors also play a decisive role in breast carcinogenesis together with life – long dietary habits. Chronic exposures to various heavy metals are nearly unavoidable in daily life, such as from airborne particles, soil, water and subsequently food [2]. In Egypt, Recent data indicate that the current levels of copper, chromium, cadmium, iron, lead, manganese, vanadium, arsenic, nickel, antimony and titanium were higher than those considered safe for the general population[3]. Multiple reports show that metallic compounds could function as estrogen disruptors, while other studies underline the connection between the exposure to metals or metal compounds and breast cancer risk [4].

Complementary and alternative medicine (CAM) is becoming increasingly popular, particularly among patients with breast cancer. CAM encompasses a wide range of treatment modalities, including dietary and vitamin supplements, mind – body approaches and herbal medicines. The objectives of CAM treatments are diverse: reduction of therapy – associated toxicity, improvement of cancer – related symptoms, fostering of the immune system and even direct anticancer effects [5].

L – carnitine, is a trimethylated amino acid that plays an important role in cell energy metabolism through mediating the transport of long chain fatty acids across the inner mitochondrial membrane [6]. Human requirements for carnitine are usually met with a combination of diet and endogenous biosynthesis. The main dietary sources of carnitine are red meat, fish and dairy products which can supply 2 -12 µmol/ (day. Kg) of body weight, whereas 1 -2 µmol of carnitine is endogenously synthesized. In comparison, vegetable products provide fairly small amounts [7].

Supplementation of L – carnitine has been demonstrated to be able to improve symptoms of fatigue in patients with cancer. The use of L – carnitine as an antioxidant, cardioprotective, neuroprotective, and immunostimulant nontoxic natural compound suggests its use as an adjuvant therapy. For breast cancer L – carnitine has a dual protective effect by enhancing the energy dynamics of the cell and inhibiting cell membrane hyperexcitability, which make it an ideal nutrient for cancer prevention and treatment[8].

It has been also proved that L – carnitine shows an anti – oxidative effect through its action as a metal chelator, Scavenger of superoxide anion, as well as its agonistic effect on the activity of the antioxidant enzyme, catalase and superoxide dismutase [9].

Glutathione is a tripeptide (L-glutamyl-L-cysteinyl-glycine). It performs a variety of important physiological and metabolic function in all mammalian cells, including the detoxification of free radicals, metals and other electrophilic compounds [10].

The Aim of the work:

The present study was conducted to investigate the relationship between L– carnitine and trace metals in breast cancer female patients treated with chemotherapy.

2. Subjects and Methods:

Patient selection

The study included 100 female patients with pathologically proven breast carcinoma who were planned to receive 6 cycles or more of doxorubicin chemotherapy based regimens. (age 50 year and more).

Exclusion criteria

Concomitant cardiac diseases and any other cardiotoxic agent, previous chemotherapy regimens., Brain metastasis and Pregnant patients.

Study groups:

Patients divided into three groups, first group include 40 female patients received L – carnitine +Doxorubicin chemotherapy, second group include 40 female patients received Doxorubicinchemotherapy only and third group 20 healthy female patients (control group).

Samples collections and serum preparation:

Five ml blood sample were obtained from each patient. Samples were centrifuged at 3000 rpm for 30 min then serum was collected and kept in polyethelene containers and frozen immediately at -20 c until analysis. The withdrawal of blood samples are done according to the ethics of Medical Research Ethical commity

Biochemical assays:

Analysis of the studied heavy metals and trace elements [lead (Pb); cadmium (Cd); Iron (Fe); copper (Cu); manganese (Mn) and zinc (Zn)] was done by Perkin Elmer 2380 Atomic Absorption Spectrophotometer [9]. Instrument start – up and optimization were carried out as detailed in the operating manual. The source of thr flame was an air – acetylene mixture. Wavelengths were set at 228.8, 217.6, 357.9 and 213.9 nm.

Oxidative stress assays:

1. Measurement of Glutathione in blood (GSH): Glutathione was determined by the method of Beutler [9]. virtually all of the non – protein sulfhydryl of red cells is in the form of reduced glutathione (GSH). 5,5 – Dithiobis (2 – Nitrobenzoic acid) (DTNB) is a disulfide compound which is reaily reduced by sulfhydryl compounds, forming a highly coloured yellow anion. The optical density of this yellow substance is measured at 412nm.

2. Measurement of Glutathione Reductase Activity (GR): Glutathionereductase catalysis the reduction of glutathione (GSSG) in the presence of NADPH, which is oxidized to $NADPH^{+}$ the decrease in absorbance, is measured at 340nm according to the method of Goldberg& Spooner [10].

3. Measurement of Glutathione Peroxidase Activity (GSH – Px): The activity of erythrocyte glutathione peroxidase (GSH – Px) was estimated by the method of Koktanur and Jelling [12].(GSH – Px) catalyses the oxidation of reduced glutathione (GSH) to oxidized glutathione (GSSG) by hydrogen peroxide.

Statistical analysis:

Statistical analysis was based on comparing the values of control group as compared to the cancerous group. There was a statistical analysis of the entire data with the help of the present SPSS statistical package Version 16. This data was further presented as mean ± standard Deviation of Means (S.D.M). There was also a comparison exercise done between the two groups that was carried out with the help of t – test and p value was considered statistically significant if <0.05.

3. Results:

Table(1) and figure(1) demonstrated that, there was a significant differences between groups 1 and control group as regard iron, copper, zinc, lead and manganese before first and after last cycles except for cadmium there was no significant difference between both groups. The present results showed a significant increase in Iron, Lead, Manganese, Cadmium in breast cancer patient compared with control group except copper and Zinc were higher in control group. The only effect of L- carnitine on trace metals was on iron level which decrease in patients received L- carnitine otherwise no effect.

Table (2) demonstrated that, there was significant difference between studied groups as regard to glutathione, GSH-R, GSHpx differences. The significant increase of glutathione and GSH-R was observed in groups 1 and 2 respectively. On the other hand, the marked decrease of GSHpx in group 1.

This table revealed that, there was significant difference between groups 1 and 2 from one side and control group from the other side as regard to glutathione, GSH-R and GSHpx after first and last cycles. In addition, there was significant difference between groups 1 and 2 as regard the same parameters after the same cycles except for glutathione after last cycle.

Table (1): Comparison between studied groups as regard heavy metals concentration at first and last cycles of treatment

Variable		Control	Group 1	Group 2	a	b	C
Iron	first	0.08±0.03	1.97±0.78	1.91±0.72	***<0.001(S)***	***<0.001(S)***	0.73(NS)
	Last	0.11±0.02	1.90±0.74	1.28±0.59	***<0.001(S)***	***<0.001(S)***	***<0.001(S)***
Copper	first	0.79±0.07	0.42±0.31	0.43±0.29	***<0.001(S)***	***<0.001(S)***	0.82(NS)
	Last	0.79±0.08	0.44±0.31	0.43±0.23	***<0.001(S)***	***<0.001(S)***	0.86(NS)
Zinc	first	0.95±0.08	0.64±0.06	0.63±0.06	***<0.001(S)***	***<0.001(S)***	0.98(NS)
	Last	0.96±0.07	0.65±0.06	0.83±0.08	***<0.001(S)***	***<0.001(S)***	***<0.001(S)***
Lead	first	0.29±0.17	1.11±0.45	1.19±0.41	***<0.001(S)***	***<0.001(S)***	0.38(NS)
	Last	0.30±0.14	1.13±0.45	1.17±0.039	***<0.001(S)***	***<0.001(S)***	0.58(NS)
Manganese	first	0.018±0.01	0.16±0.14	0.13±0.04	***<0.001(S)***	***<0.001(S)***	0.34(NS)
	Last	0.018±0.004	0.14±0.05	0.12±0.04	***<0.001(S)***	***<0.001(S)***	0.32(NS)
Cadmium	first	0.06±0.12	0.12±0.17	0.09±0.15	0.21(NS)	0.53(NS)	0.53(NS)
	Last	0.049±0.08	0.12±0.20	0.09±0.16	***0.049(S)***	0.26(NS)	0.50(NS)

NB: a = group 1 vs control group; b: group 2 versus control; c group 1 versus group 2
Group 1: patients received chemotherapy only, group 2: patients received chemotherapy and L- carnitine

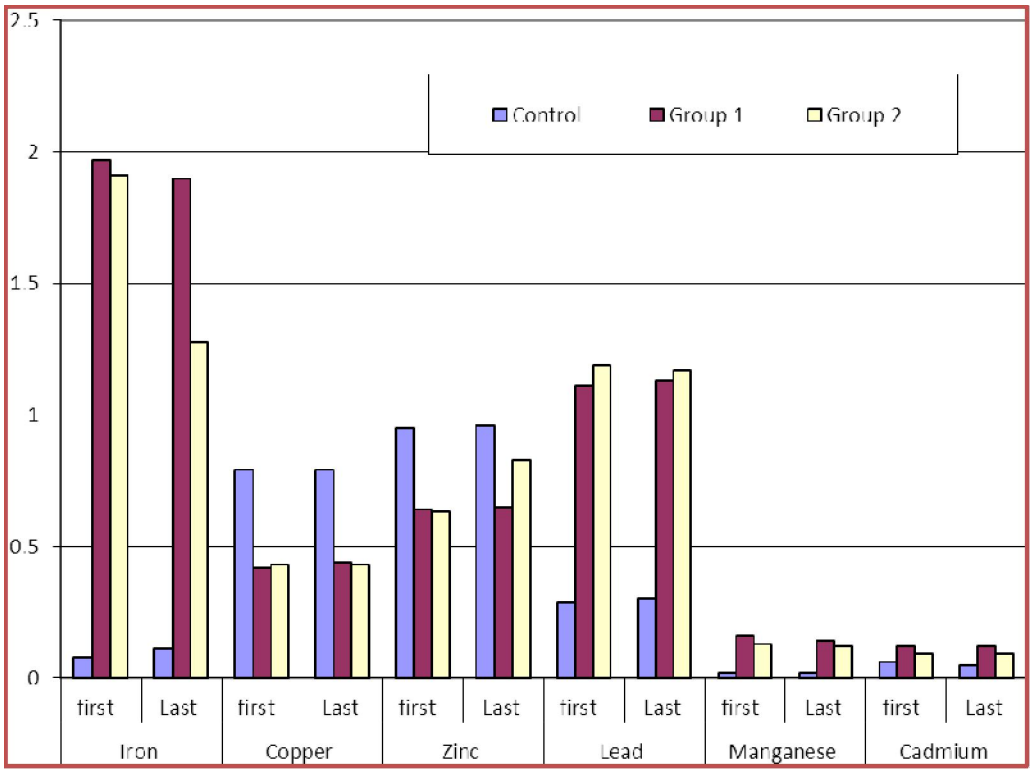

Figure (1): Comparison between studied groups as regard heavy metals at first and last cycles

Table (2): Comparison between study groups as regard difference between first and last rounds of oxidative stress markers

Difference (First-last)	Group 1	Group 2	Control group	F	p
Glutathione	0.86±0.21	0.35±0.06	-0.027±0.037	288.93	***<0.001(S)***
GSH-R	1.53±0.42	2.46±0.43	-0.076±0.12	281.76	***<0.001(S)***
GSHpx	-6.28±3.16	0.69±0.54	-0.095±0.23	126.81	***<0.001(S)***

Table (3): Comparison between studied groups as regard to oxidative stress markers at first and last cycles

Variable	Control	Group 1	Group 2	a	b	c
Glutathione first	0.91±0.08	2.03±0.24	1.97±0.25	***<0.001(S)***	***<0.001(S)***	0.27(NS)
Glutathione last	0.94±0.09	1.16±0.24	1.61±0.22	***<0.001(S)***	***<0.001(S)***	***<0.001****
GSH-R first	5.68±0.35	9.49±0.54	9.22±0.59	***<0.001(S)***	***<0.001(S)***	***0.047(S)***
GSH-R last	5.76±0.33	7.95±0.39	6.76±0.70	***<0.001(S)***	***<0.001(S)***	***<0.001(S)***
GSH-px first	14.49±0.48	19.31±2.97	20.60±1.91	***<0.001(S)***	***<0.001(S)***	*0.028(S)*
GSH-px last	14.58±0.48	25.59±2.22	19.90±1.92	***<0.001(S)***	***<0.001(S)***	***<0.001(S)***

NB: a = group 1 vs control group; b: group 2 versus control; c group 1 versus group 2

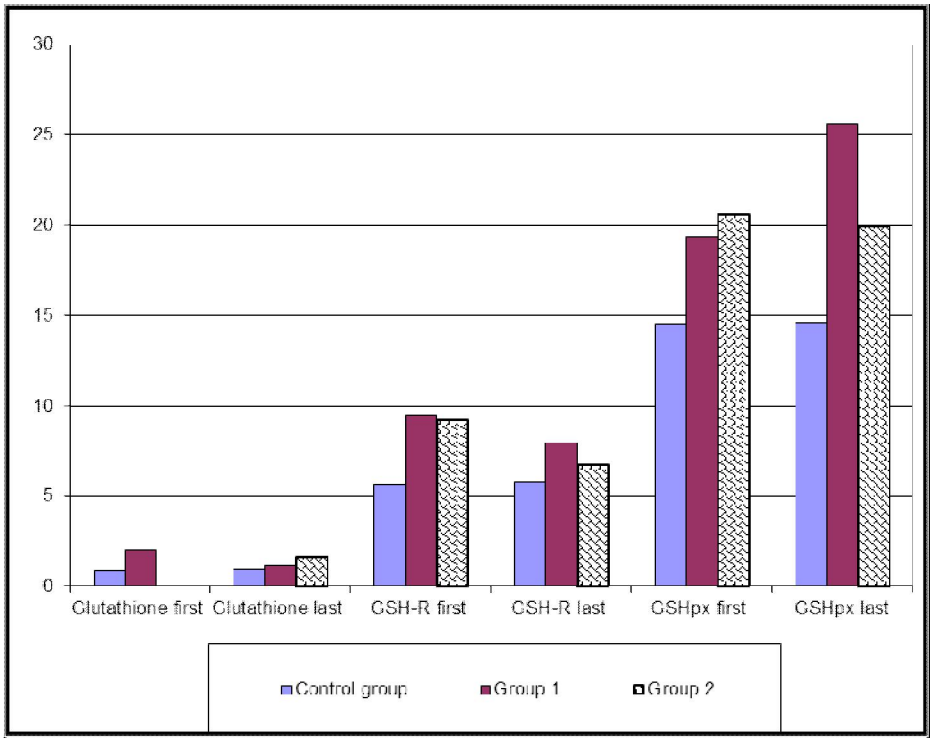

Figure (2): Comparison between studied groups as regard to oxidative stress markers at first and last cycles

4. Discussion:

Trace elements and toxic heavy metals have critical roles in cancer biology. A large number of epidermiological studies indicate a close association between heavy metals and development of breast cancer such as lead (Pb), Copper (Cu), zinc (Zn), Cadmium (Cd), Iron (Fe) and Manganese (Mn), which are found naturally in the environment [11].

In the current study, the concentration of six heavy metals (lead, copper, zinc, cadmium, iron and manganese) were estimated in the blood samples of patients with breast cancer; it was apparent that a significant elevation of lead, copper, zinc, cadmium, iron and manganese concentrations was detected in blood of women having malignant breast tumors in comparison to control group. The present results are run in parallel with those obtained by Romanowicz – Makowska [12].

Metals can be carcinogenic in various forms including free ions, metals complexes, or particles as well as soluble metal compounds. In general, metal carcinogenicity and genotoxicity are based on three main mechanisms, namely, oxidative stress, DNA repair modulation, and disturbances of signal transduction pathways [13]. Interestingly, some trace metals are claimed to be carcinogenic and capable of including a toxic effect through the formation of ROS and acting as cofactors in the oxidative damage of biological macromolecules and DNA. However, their exact role in carcinogenesis is still unclear [14].

Martin, analyzed blood of women with malignant and benign breast tumors. The blood lead was

significantly higher in malignant cases than in those of control. Most observed mechanisms of lead carcinogenicity involved direct DNA damage oxidative DNA damage through ROS generation, endocrine disrupters, or inhabitation of DNA synthesis or repair [15].

The increased levels of Cd in cancer patients blood samples and their suggested role in tumor development could be related to their disruption of the oxidative balance, production of oxidative DNA damage and inhibition of DNA repair. Additionally, they functions also as an important class of endocrine disrupters [16]. Cadmium effects cell proliferation, differentiation, apoptosis and activation of transcription and translation factors [17]. Moreever, the demand for increased blood supply for a growing tumor provides a basis for the accumulation of many elements [18].

Iron and Zinc were involved in breast tumorigenesis and their suggested rolein tumor development could be related to their action as enzymatic co – factors involved in carcinogenesis. In addition, Zinc belongs to the group of oxidant metals causing disruption of the oxidative balance. Iron can also promote carcinogenesis by causing tissue damage as it acts as a catalyst in the conversion of hydrogen peroxide to free radical ions that attack cellular membranes, breaks DNA strands, inactive enzymes and initiate lipid peroxidation [19].

In the present study, a significant increase in iron levels in breast cancer group of patient versus control group ($p < 0.001$) was detected. Adding of L – carnitine to breast cancer patients leads to decreasing iron level Distruption of metal ion homeostasis may lead to oxidative stress, astate where increase formation of ROS overwhelms body antioxidant protdction and subsequently induced DNA damage, Lipid peroxidation, protein modification and carcinogenesis[19].

The increase levels of trace elements and metals (Pb, Cd, Cr, Fe, Mn and Zn) in cancerous patients in comparison to control highlight the role for these trace elements in the initation, promotion and progression of breast cancer. It seems likely, that the increase levels of these elements could lead to formation of free radicals or other reactive oxygen species inducing oxidative stress which affects adversely DNA and there by causing breast cancer.

Adding of L – carnitine to breast cancer patient receiving chemotherapy may have a positive impact in the form of decreasing oxidative stress process, this notice from preserving accepted level of Glutathione in group 2 and increase glutathione reductase and glutathione peroxidation after L – carnitine administration in comparisonto group 1.

It is recommended to use trace elements and antioxidant activity as biomarkers for breast cancer and its progression and also prevention of this disease.

Conclusion:

The increase levels of trace elements and metals (Pb, Cd, Cr, Fe, Mn and Zn)in cancerous patients in comparison to healthy persons highlight the role for these trace elements in breast cancer. Adding of L – carnitine to breast cancer patient receiving chemotherapy may have a beneficial effect through its anti- oxidative stress activity and decreasing iron level. we may recommend using trace elements and antioxidant activity as biomarkers for breast cancer and its progression and also prevention of this disease.

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