The Use of Alpha-Lipoic Acid as Adjuvant Therapy in Breast Cancer Patients: Quasi Experimental Study

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ABSTRACT

Oxidative stress plays a major role in the process of carcinogenesis. This fact highlights the questions about the effect of antioxidants in cancer therapy. Alpha-lipoic acid (ALA) has gained a lot of concern in the last decade as an antioxidant in many pathological conditions, including cancer therapy. This study was conducted to identify the effect of ALAwhen used with chemotherapy in breast cancer. The study examined its effect on oxidative stress. We examined Malondialdehyde (MDA) as an oxidative stress biomarker, chemotherapeutic Induced Peripheral Neuropathy (CIPN), chemotherapeutic Induced nausea and vomiting (CINV), dyslipidemia, and renal function. This experimental study was conducted in al-Shefa hospital in Gaza Strip among women who were admitted to the oncology department and diagnosed as breast cancer patients. Fifteen cases were given ALA, 600 mg per day for six months, and 15 control groups in which the MDA, Creatinine and lipid profile were examined at zero time. After 3 months and 6 months, the adverse events were examined by a face-to-face questionnaire to the cases at zero time. The median of MDA was significantly decreased in the first 3 months from 8.4 nmol/ ml to 5.4 nmol (P-value = 0.013). However, after six months, it was not significantly decreased. The median of MDA was 4.6 nmol/ml (P-value = 1). Creatinine was significantly decreased after six months (P-value = 0.000). In contrast, urea was not changed significantly. Concerning the lipid profile TC, LDL was elevated significantly, and HDL was not significantly improved. According to adverse events, CIPN, pain was not significantly improved; only 58% (P-value = 0.07) of the cases stopped feeling pain. Imbalance also was not significantly improved; only 33% of cases stopped feeling imbalanced (P-value 0.125), while 80 % of cases stopped feeling numb (P-value = 0.008). On the other hand, 83% of cases stopped feeling a tingling sensation (P-value = 0.002), and 81% of cases have no trouble in holding things (P-value = 0.004). No patient complained of vomiting after the use of ALA (P-value = 0.00). The use of ALA has a beneficial effect on oxidative stress, which has a major role in the process of carcinogenesis. It has a protective effect on renal functions and a beneficial effect on both CIPN, CINV.

Keywords: Oxidative Stress, Alpha Lipoic Acid, MDA.

الملخص

تلعب الأكسدة دورا أساسيا في عملية السرطنة، وهذا يسلط الضوء على دور مضادات الأكسدة في الحد من هذا الدور، وقد لاقى مركب (الالفا ليبويك أسيد) اهتماما كبيرا في العقد الآخير في كثير من الآمراض المتعلقة بالأكسدة وخاصة في مجال السرطان. أجريت هذه الدراسة في مشفى الشفاء للتعرف على دور مركب (الفاليبويك أسيد) كعلاج مساند للعلاج الكيميائي المستخدم في سرطان الثدي. اختبرت الدراسة تأثير (الفاليبويك أسيد) على الأكسدة المتمثلة بمستوى المالون (داي الدهايد) في الدم. كما تختبر الدراسة تأثير (الفاليبويك أسيد) على الآثار السلبية المصاحبة للعلاج الكيميائي، مثل: الاعتلال العصبي المسبب بوساطة العلاج الكيميائي، والاستفراغ، والغثيان المسبب بوساطة العلاج الكيميائي، وتختبر الدراسة أيضا تأثيره على مستوى الدهون في الدم التي ترتفع في سرطان الثدي. وقد أجريت هذه الدراسة (الدراسة التجريبية). وقد تم اختيار العينة من النساء الذين يدخلون إلى قسم الأورام في مستشفى الشفاء قطاع غزة، ويتم تشخيصهم على أنهم مرضى سرطان الثدي. الحالات تم اعطاؤهم (الفاليبويك أسيد) بواقع حبة يوميا (600 mg)، وتم عمل فحص لمستوى المالون (داي الدهايد) كمؤشر على مستوى الأكسدة عند نقطة الصفر، وتم فحص (الكرياتينين واليوريا) كمؤشر على وظائف الكلى، وكذلك فحص مستوى الدهون. ثم تم إعادة هذه التحاليل بعد ثلاثة أشهر، وبعد ستة أشهر لكل من الحالات والشواهد. وتم عمل استبانة للحالات قبل استخدام (الفاليبويك أسيد) وبعده فيما يخص أعراض الاعتلال العصبي المصاحب للعلاج الكيميائي أعراض الغثيان. أظهرت النتائج فيما يتعلق بمستوى الأكسدة فان قياسات (المالون داي الدهايد) تحسن في وسيطها للحالات في أول 3 أشهر (بدلاله احصائيه0،013) بينما لم يكن نزول المستوى في الوسيط في الثلاث أشهر الثانية لم يكن ذا دلاله إحصائية (دلاله إحصائية = 1) ولم يكن هناك دلاله إحصائية بالنسبة للشواهد (دلاله إحصائية =0،6). وفيما يتعلق بمستوى (اليوريا) لك يكن هناك تغير ذا دلاله إحصائية في كل من الحالات والشواهد، أمّا فيما يتعلق (الكرياتينين) فان التغير فيه كان ذا دلاله إحصائية بعد ستة أشهر (دلاله احصائي =0،003). ولم يكن هناك تحسن في مستوى الدهون، وإنما كان هناك ارتفاع ذا دلالة إحصائية في مستوى (الكوليسترول) في الحالات (دلاله إحصائية = 0،003) فان هناك ارتفاعا، ولكنه لم يكن ذا دلاله إحصائية في كل (م ن LDL)، أما فيما يخص (الكوليسترول) الخبيث لم يكن هناك ارتفاع ذا دلاله إحصائية (HDL) الحالات والشواهد. كذلك (الكوليسترول) الحميد أعراض العلاج الكيميائي تحسنت بدلاله

إحصائية ما عدا الألم، والشعور بعدم الاتزان. ولم يكن هناك تحسن في مستوى الدهون، وإنّما كان هناك ارتفاع ذا دلالة إحصائية في مستوى (الكوليسترول) في الحالات (دلاله إحصائية = 0،003)، وكان هناك ارتفاع، ولكنه لم يكن ذا دلالة إحصائية في كل (م ن LDL)، أمّا فيما يخص (الكوليسترول) الخبيث لم يكن هناك ارتفاع ذا دلاله إحصائية (HDL) الحالات والشواهد. كذلك (الكوليسترول) ا لحميد أعرنض العلاج الكيميائي جميعها تحسنت بدلاله إحصائية ما عدا الألم، والشعور بعدم الاتزان. وخلص البحث إلى أنه توجد تأثيرات ايجابيه لاستخدام (الفا ليبويك أسيد) كعلاج مساند للعلاج الكيميائي تقليل لمستوى الأكسده، سضاف إلى ذلك وجود تأثيرات ايجابية لاستخدام (الفاليبويك أسيد) على وظائف الكلى، وجد تأثيرات ايجابيه (للفا ليبويك أسيد) على الأعراض الجانبية للعلاج الكيميائي توجد تأثيرات ايجابيه للالفاليبويك اسيد على الأعراض الجانبية للعلاج الكيميائي المتمثلة بالاعتلال العصبي واعارض الغثيان والاستفراغ. وأوصى البحث لمقدمي الخدمة الصحية لإقرار استخدام (الالفاليبويك أسيد) كعلاج مساند للعلاج الكيميائي.

الكلمات المفتاحية: مالون داي الدهايد، مفاتيح الكلمات: الاكسده، سرطان الثدي، الالفاليبويك اسيد، ROS,CIPN,CINV.

INTRODUCTION

Cancer is a group of diseases characterized by abnormal and uncontrolled growth and the spread of cells. Anyone can develop cancer, as the risk of being diagnosed increases with age. Most cases occur in adults who are middle age or older. About 78% of all cancers are diagnosed in patients by the age of 55 years and older (Alhams et al., 2014).

Cancer is the second leading cause of death after heart disease worldwide and a major public health problem in the USA and other countries (Seigel et al., 2015).

Cancer begins when cells in a part of the body start to grow out of control. Cancer cells differ from normal cells in that cancer cells will not die. They continue to grow and form new abnormal cells (American cancer society, 2015)

"Breast cancer is a complex multifactorial disease where there is a strong interplay between genetic and environmental factors" (Martin and Weber, 2000).

Eleven million cases of cancer now occur annually worldwide, six million of them in low- and middle-income countries, with around five million deaths from cancer (Alhams et al., 2014).

Epidemiology

There are one million new cases of breast cancer worldwide each year and comprise 18% of all women malignancies, where the age-standardized incidence and mortality are the highest in the UK (McPherson et al., 2000).

One out of nine women in the UK and the USA will develop the disease in their lifetime (Abdulkareem, 2013).

Breast cancer is the most common type of cancer among women. An estimated 232,340 new cases of invasive breast cancer were diagnosed among women in the USA during 2013. Deaths are estimated at 40,030 (American cancer society, 2014).

In Palestine, breast cancer occupied the most prevalent type of cancer (31.4%, about 45% in Gaza strip), followed by colorectal (9.2%), and then trachea and bronchus (5.5%) (Al hams et al, 2014).

According to mortality, breast cancer occupies third place after colorectal (second) and lung (the first) in Gaza Strip and West Bank. Breast cancer was known to be the first leading cause of death among females (21.1%) (Al hams et al, 2014).

In Gaza strip breast cancer prevalence rate is 149.1/100000, the number of all breast cancer cases in female is 1207 which represents 31.1% of all female cancer cases. The maximum level of cases is 317 cases in the age group 55 – 64 (Palestinian Health information centre,2015).

Treatment

Local Treatment

Surgery may vary from local excision or segmental mastectomy and breast conservation for masses less than 4 cm in diameter. For simple mastectomy, the choice is taken according to the breast mass location and extent in relation to the breast size and the patient's preferences. Surgery of the axilla is by sentinel lymph node guided sampling (after dye injection) (Kumar and Clark, 2009).

Her2 therapy (Kumar and Clark, 2009).

Adjuvant systemic treatment-This is divided into four lines:

Chemotherapy

- AC/EC – doxorucin or epirupcin and cyclophsphamide
- DC- docetaxol and capicitabine
- PG-paclitaxel and Gemicitabine
- VC- vinorelbine and cabcitabine (Kumar and Clark, 2009).

A third-generation regimen with a taxane (AC-T) cyclophosphamide, adriamycin, taxane (Kumar and Clark, 2009). This regimen is the one that is used in the Gaza Strip as a protocol of therapy. It decreases the relative risk of death by 33% and increases the toxicity (Kumar and Clark, 2009).

Menopausal status does not affect the relative efficacy of chemotherapy. Since the recurrence is less after menopause, the absolute improvement in survival is lower. Toxicity may be higher, so the choice must be individualized to each patient. The combined chemotherapy and radiotherapy and tamoxifen or aromatase inhibitors halve the risk of dying of breast cancer for the appropriately selected patients (Kumar and Clark, 2009).

Apoptosis and Oxidative Stress

Apoptosis (programmed cell death) "Is genetically regulated form of cell death, it has a role in biological processes, including embryogenesis, aging, and many diseases, the molecular mechanisms involved in death signals, genetic regulation, activation of effectors have been identified" (Renehan, 2001).

The genetic basis of apoptosis implies that cell death, like any other metabolic process, can be disrupted by mutation. In fact, defect in the apoptotic pathway is now thought to contribute to a number of human diseases ranging from neurodegenerative disease to malignancy (low and lin, 2000)

Oxidative Stress Definition

"Is a state of where oxidative forces exceed the antioxidant system due to loss of balance between them" (Yoshikawa, 2002).

Effect of Oxidative Stress in the Process of Carcinogenesis

Epidemiological studies indicated that chronic oxidative stresses are strongly associated with carcinogenesis (et al,.2011).

ROS, which results from oxidative stress, can damage critical cellular macromolecules and/or modulate gene expression pathways. Oxidative damage resulting from ROS generation can participate in all stages of the cancer process. An association of ROS generation and human cancer induction has been shown. It appears that oxidative stress may both cause as well as modify the cancer process. Recently, the association between polymorphisms in oxidative DNA repair genes and antioxidant genes (single nucleotide polymorphisms) and human cancer susceptibility has been shown (Klaunig et al,.2011).

Antioxidant

"Antioxidants are responsible for the neutralizing action of these reactive species. As definition, an antioxidant is any substance that, present in low concentrations in relation to the oxidizable substrate, retards or inhibits the oxidation of such a substrate, including enzymatic and non-enzymatic compounds" (Rossi et al, 2009)

Oxidative Stress Biomarker

Oxidative stress biomarker can be defined as "predictive indicators of the development of a pathology able to detect in vivo oxidative damage." Such markers can be subdivided into pro-oxidant and antioxidant, in accordance with the affected system (Rossi et al., 2009)

Malondialdehyde (MDA).

As mentioned before, lipid peroxidation is one of the pathways of oxidation in the body. Lipid peroxidation has an end product called Malondialdehyde (MDA) which is a biomarker. MDA is a naturally occurring product of lipid peroxidation; it can also be generated during prostaglandin biosynthesis in cells (Jetawattana,2005).

Alphalipoic acid (ALA; thioctic acid, 5-(1,2-dithiolan-3-yl) pentanoic acid). It is a naturally occurring antioxidant synthesized in small

amounts by plants and animals, including humans (Dozio et al ,2010)

Alpha-lipoic acid (ALA) plays an essential role in mitochondrial bioenergetic reactions, which has gained attention as a nutritional supplement and as a therapeutic agent. Moreover, LA conjugates with other pharmacophores represent a promising approach toward the development of multifunctional drugs (Maria Koufaki, 2014).

LA exists in the form of two enantiomers, R or S. In physiological condition, LA is present in the form of lipoate with the proton of the hydroxyl functional group substituted by remains of organic alcohol or with an inorganic ion. LA (in the form of lipoate) acts as a cofactor in reactions of aerobic metabolism. It participates in the transfers of acyl and methylamine groups. It is essential for aerobic processes of life and serves as a coenzyme in the Krebs cycle (Novotny et al, 2007).

The common use of ALA is an antioxidant used to treat many neurological disorders such as diabetic polyneuropathy and multiple sclerosis as it can cross the blood-brain barrier. It has been shown to improve endothelial function and blood flow. It accelerates glutathione synthesis, which plays a crucial role in regulating the expression of several antioxidants and anti-inflammatory genes (Choi et al., 2015).

Studies on cancerous cell-based models have suggested that the tumor-suppressive effect of ALA corresponds with apoptosis induction, which is a critical parameter impaired in cancer cells. This induction is selectively exerted in cancer and transformed cells while being less active toward non transformed cells (selvkumar and heish,2008).

However, ALA is soluble in both water and lipids. ALA is highly reactive due to the tension of the S-S-C bond in the heterocyclic disulfide circle. ALA is relatively stable as a solid, but it polymerizes when heated above its melting point (47.5 c; Novotny et al., 2007).

Pharmacological Effect of ALA

Antioxidant effect:

ALA is ROS scavenger and metal chelating the disulfide group of LPA, which can be reduced to DHLPA. Both of them have been reported to scavenge a variety of oxygen species. Additionally, the pharmacological impact of LPA/DHLPA redox couple is due to metal chelating properties (Feuerecker et al,.2012).

It has powerful lipophilic antioxidants both in vitro and in vivo. It is known to act as a scavenger of many reactive oxygen species (ROS). ALA has been proposed as a treatment for oxidative disorders of the nervous system characterized by an increase of free radicals (Ranieri et al., 2010)

Recycling Other Antioxidants

In addition to ROS scavenging, LA has also been shown to be involved in recycling other cellular antioxidants, including vitamins C and E, and glutathione (Biewenga et al., 1997).

Antitumor effect of alpha-lipoic acid exerts antitumor effect by the following mechanisms

Unlike normal cells, tumor cells survive in a specific redox environment where the elevated reactive oxygen species, which play a role in critical signaling molecules, contribute to enhance cell proliferation and suppress apoptosis. The alpha-lipoic acid was able to induce cell cycle arrest and apoptosis in different cancer cell lines (Dozio et al.,2010).

ALA inhibits glycolysis:

Cancer cells perform higher glycolysis rates, i.e., conversion of glucose to lactate instead of complete oxidation of glucose to water and CO2 for the generation of ATP (***Zhang et al.,2015).***

The presence of ALA shifts ATP production by TCA cycle towards oxidative phosphorylation instead of glycolysis in which cancer cells stick, and consequently, apoptosis is inhibited. Feuerecker examined the effect of ALA on human cells; a slight dose-dependent increase of pyruvate dehydrogenase activity was observed ***(Feuerecker et al.,2012).***

Apoptosis induced by LA was found to be mediated through the mitochondrial death pathway, which requires caspase-9 activation death ***(Moungjaroen et al.,2006).***

ALA Prevents Metastasis

In 2010, Lee et al. studied the effect of alpha-lipoic acid in the prevention of metastasis in the cell system. They hypothesized that LA inhibits

metastasis via inhibition of matrix metalloproteinase (MMP) in vitro. The enzyme is responsible for metastasis. MDA-MB-231 cells, a human breast cancer cell line, were treated with various concentrations of LA (0, 250, 500, or 1000 μmol/L) to measure metastasis, MMP activity, and mRNA expression. The viability of cells was examined by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay. The effect of LA on metastasis was evaluated using the motility, migration, and invasion assay in vitro. The activity and mRNA expression of MMP-2 and MMP-9 were measured. After LA treatment, cell motility and cell migration were significantly decreased ($P < .05$). The α-Lipoic acid also reduced cell invasion through a Matrigel-coated chamber ($P < .05$). Activities of MMP-2 and MMP-9 were decreased by LA treatment in a dose-dependent manner. The analysis confirmed the reduction in mRNA expression level of MMP-2 and MMP-9 by LA treatment. They concluded that LA treatment inhibits cancer metastasis in this cell culture model, and this inhibition is likely due to the decrease in the activity and mRNA expression levels of MMP-2 and MMP-9 caused by LA ***(Lee et al., 2010).***

Effect on Lipid

Lipid peroxidation, the oxidative deterioration of the polyunsaturated fatty acids (PUFA), leads to the formation of hydroperoxides, short-chain aldehydes, ketones and other oxygenated compounds. This process is considered responsible for the development of various diseases like cancer ***(Zulkhairi et al., 2001).***

Minimizing the Side Effects of Chemotherapy

The effect of alpha-lipoic acid also was investigated against the minimizing of the side effects of chemotherapy. In 2008, Melli et al. studied the neuroprotective effect of alpha-lipoic acid. They used an *in vitro* model of chemotherapy-induced peripheral neuropathy that is closely similar to the *in vivo* condition by exposing primary cultures of dorsal root ganglion (DRG) sensory neurons to paclitaxel, widely used and highly effective chemotherapeutic drugs. This approach allowed the investigation of the efficacy of alpha-lipoic acid in preventing axonal damage and apoptosis and the function and ultrastructural morphology of mitochondria after exposure to toxic agents and alpha-lipoic acid. Their results demonstrated that paclitaxel cause early mitochondrial impairment with loss of membrane potential and induction of autophagic vacuoles in neurons. Alpha-lipoic acid exerts neuroprotective effects against chemotherapy-induced neurotoxicity in sensory neurons. It rescues mitochondrial toxicity. These findings suggest that alpha-lipoic acid might reduce the risk of developing peripheral nerve toxicity in patients undergoing chemotherapy ***(Melli et al.,2008)***

ALA prevents the nephrotoxic effect of Adriamycin; ALA is also capable of influencing the nephrotoxicity potential of adriamycin.

It can help minimize CINV using its antioxidant effect; therefore, it has a beneficial effect on other cells that are harmed by chemotherapy. Mantovani et al. examined the effect of antioxidants on side effects of chemotherapy as fatigue, nausea, and vomiting. All patients were given as basic treatment polyphenols plus antioxidant agents α-lipoic acid, carbocysteine, and vitamins A, C, and E, all orally. After 2 years, no severe side effects, including nausea and vomiting, were observed (***Mantovani et al.,2008).***

METHODOLOGY

- **Materials and Methods**
  - **Materials**
    1) Thiobarbituric acid Kit for measurement of malondialdehyde which was purchased from Biodiagnosteic company – Egypt, and preserved in the refrigerator at 2-8°C.
    2) Alpha-lipoic acid (Neurogaurd ® 600 mg) donated from the Advanced company- Gaza.

    - Biochemical Parameters:
      1- Total Cholesterol TC.
      2- Low-density Lipoprotein LDL
      3- High-density Lipoprotein HDL
      4- Serum urea level
      5- Serum creatinine levels
      6- Lipid peroxide {measured as MDA}
  - **Study Design**

The study conducted a randomized clinical trial study on a population of women diagnosed with breast cancer and admitted to the oncology

department in al-Shefa hospital. The sample was chosen according to specific criteria.

Cases: 15 patients

Eligibility:

Non-pregnant
Non-lactating
Non-metastatic
Under (Adriamycin, cyclophosphamide, Taxol) A CT protocol.

All women were at the beginning of their chemotherapy; the choice was made under the supervision of the head of the oncology department.

The patients were given informed consent.

A serum sample was collected from the patient when admitted for chemotherapy after three months and six months.

The case group was given alpha-lipoic acid, 600 mg once daily for six months.

Control group

Eligibility criteria

Non-pregnant
Non-lactating
Non-metastatic patients
No drug intervention

Blood samples were collected in serum tubes on vacuum without pushing the sample through the needle to avoid hemolysis. All samples were transmitted immediately to the laboratory.

- **Methods**

1- The Kit of MDA is thiobarbituric acid which is chlorometric. Thiobarbituric acid reacts with MDA in acidic media at a temperature of 95 ◦C for 30 min to form thiobarbituric acid reactive

$$\text{Serum MDA} = \frac{\boldsymbol{A\ Sample}}{\boldsymbol{A\ standard}} \times \mathbf{10}\ \boldsymbol{nmol/ml}$$

The blood samples were mixed, and the test tubes, which were covered with glass beads, heated in boiling water bath for 30 min, cooled, then mixed, and the absorbance was read at 534nm.

2- Analysis of serum lipids total cholesterol, LDL and HDL, was analyzed every three months.

3- Urea and creatinine were also analyzed.

All tests were performed in al-Nebrass Laboratory, which is registered by the Ministry of Health.

An abstract sheet was performed to compare the complaint of CIPN pre- and post-medication and compare the number of vomiting per day during chemotherapy.

The face-to-face questionnaire (Yes or No) was performed, asking patients about the side effect of chemotherapy.

Chemotherapeutic Induced Peripheral Neuropathy before and after treatment was performed to measure ALA imbalance, pain, tingling, numbness, and trouble in holding or picking things.

Chemotherapeutic Induced Nausea and Vomiting (CINV):

Patients were asked about the number of vomiting per day before and after treatment with ALA.

- **Statistical Analysis**

Patients were given symbols.

Cases were given letters from (A1 – A15).

Controls were given letters from (B1 – B15).

Descriptive analyses were performed for study data; data were entered into the SPSS program.

Alpha-lipoic acid was given to 15 breast cancer patients who were chosen to be the cases. Neurogaurd drug was given month by month, 30 tablets for each patient, and the patients' compliance was measured individually to ensure taking the drug.

The blood serum of 30 newly diagnosed breast cancer has been analyzed, and MDA, CBC, UREA, CREAT, Cholesterol, HDL, LDL have been measured.

- A repeated-measures ANOVA with a Greenhouse-Geisser correction was used for continuous data.
- Wilcoxon rank test was done for the number of vomiting pre- and post-treatment with ALA.
- QI square (McNemar test) was used to measure the significance of pain, imbalance, numbness, tingling, and trouble in picking or holding things before and after the end of six months treatment.
- The median was taken, and P-value was hypothesized to be statistically significant at 0.05.

RESULTS

MDA For Healthy Individuals

MDA has been measured for 10 healthy individuals to be sure that the normal blood level of MDA is the same as mentioned in previous studies, the mean of MDA was about 2.66nmol/ml.

The healthy individuals were chosen with no other diseases to avoid any elevation in MDA value.

MDA for cases

The MDA for cases was measured at zero time, after 3 months and 6 months; the median was chosen for study values because the sample is small.

The median value of MDA was 8.4 nmol/ ml. After three months of ALA use, the median declined to 5.4 nmol/ ml with a P-value of 0.013.

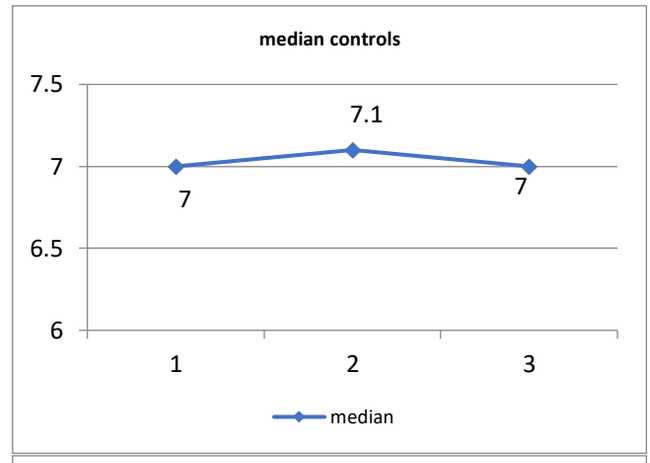
Then after 3 months (six months from the beginning of the study), the median value became 4.6 nmol/ml with a P-value of 1. The significance across time reveals that the first three months were significant while the second three months were not significant.

MDA for controls

The MDA was measured at zero time for controls; the median was 7.1. After three months, it was 7; after six months, no change in MDA median was noticed. It was still the same after six months.

Table 1 Measurement of MDA in Cases and Controls at Zero, After 3 Months and After 6 Months

MDA median	Cases	p-value	Controls	Standard Deviasion cases	Standard deviasion controls	p-value
Zero	8.4		7	3.1995	3.3833	
3 months	5.4	0.013	7.1	2.3789	3.2295	0.6
Six months	4.6		7	3.1806	4.3582	



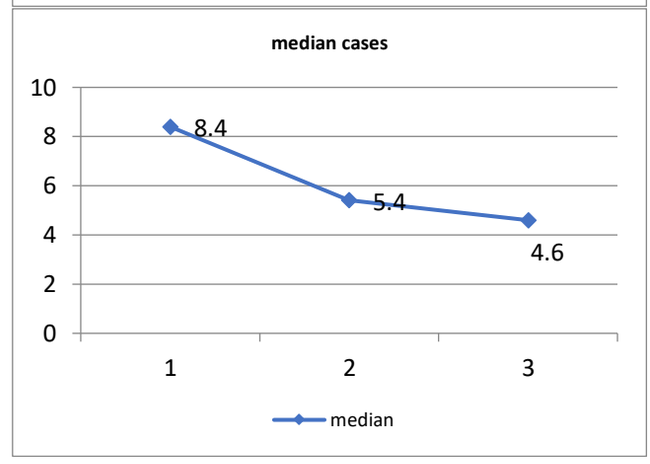


Figure 1 Median of MDA of Cases and Controls vs Time

Urea for Cases

The urea for cases was measured at zero, after three months and after six months, the median after three months was 29 mg/dl, after three months was 30 mg/dl, and after six months was 31 mg/dl. The urea was not significantly affected by the use of ALA.

Urea for Control

Urea for controls was measured for urea at zero, three, and six months. At Zero, the median was 26 mg/dl; after three months, the median was 32mg/dl, and after six months, it was 30 mg/dl.

Table 2 Measurement of Urea for Cases and Controls at Zero Time, After 3 Months and 6 Months

Urea	cases	p-value	Controls	St. Dev.cases	St.dev controls	p-value
Zero time	29		26	7.6874	11.0005	
3 months	30	0.548	30	8.4797	7.6923	0.212
6 months	31		32	8.908	9.306	

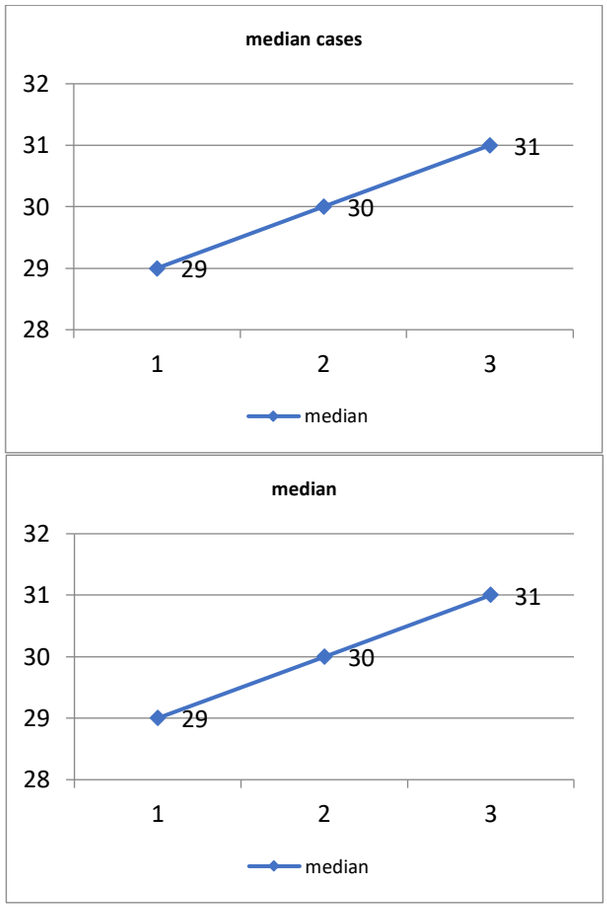


Figure 2 Median of Urea of Cases and Controls vs Time

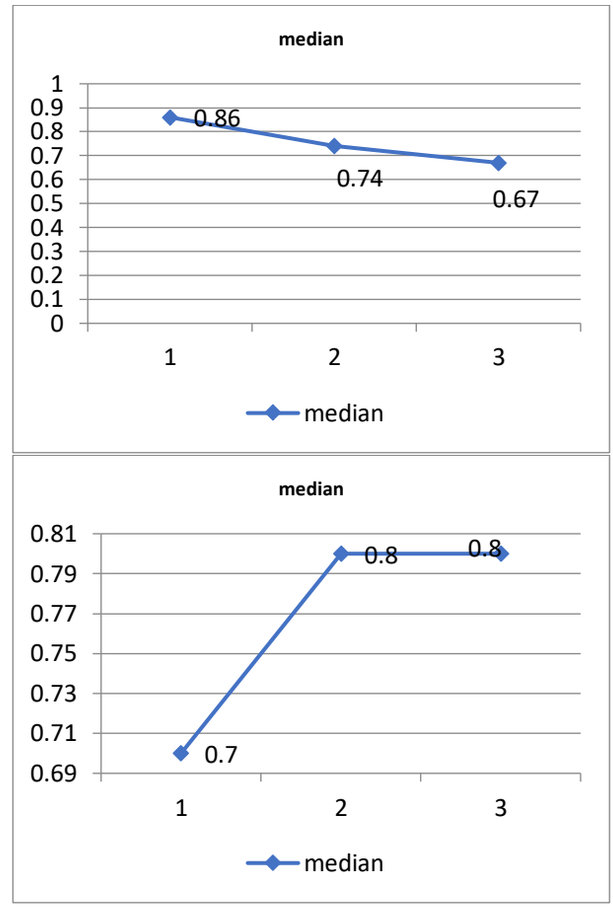


Figure 3 Median of Creatinine of Cases and Controls vs Time

Creatinine for Cases

Creatinine for cases was measured at zero, after three months, and after six months. The median at zero time was 0.86 mg/dl; after three months, the creatinine was not significantly declined with P-value at 1; and after six months, the median of creatine declined significantly to 0.67 mg/dl with P-value at 0.03.

Creatinine for controls was measured at zero, after three months and after six months. The median was 0.77 mg/dl at zero time; after three months, 0.8, and 0.8 after six months.

Table 3 Measurement of Creatinine in Cases and Controls at Zero Time, After Three Months and Six Months

Creatinine	cases	p-value	Controls	St. Dev.cases	St.dev controls	p-value
Zero time	0.86		0.77	0.15898	0.16164	
3 months	0.74	0.03	0.8	0.29014	0.14192	0.525
6 months	0.67		0.8	0.10487	0.12182	

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Total Cholesterol TC for Cases

The total cholesterol was measured for cases at zero time, after three months, and after six months. The median of cholesterol was significantly elevated after six months, with P-value at 0.003.

Total Cholesterol for Controls

Total cholesterol for controls was measured at zero time, after three months, and six months. The median at zero time was 177 mg/dl; after three months, it elevated significantly to 204 mg /dl.

Low-Density Lipoprotein (LDL) for Cases

It was measured at zero time, after three months, and six months. The median was 118 mg/dl; after three months, it elevated 119 mg /dl, and after six months, it elevated non significantly to 133mg /dl

Low-Density Lipoprotein (LDL) for Controls

For controls, the median at zero time was 120 mg/dl; after three months, it was 118 mg/dl,

and after six months, it was 128 mg /dl (not significant).

High-Density Lipoprotein (HDL) for Controls

For controls also, there were no significant changes on HDL at zero time, after three months and six months with a P-value of 0.78.

Table 4 Measurement of TC for Cases and Controls at Zero Time, After 3 Months and 6 Months

	Cases						Controls				
	TC.	St dev	LDL	St dev	HDL	St dev	TC	St dev	LDL	St dev	HDL
Zero time	177	23.850	118	16.28	38	8.97	107	42.02	120	35.6	54
3 months	210	49.585	119	40.2	44	15,3	204	59.25	118	45.3	49
6 months	203	51.498	133	39.7	47	12.48	222	58.23	128	50.88	55
p-value	0.003		0.191		0.266		0.00		0.267		0.78

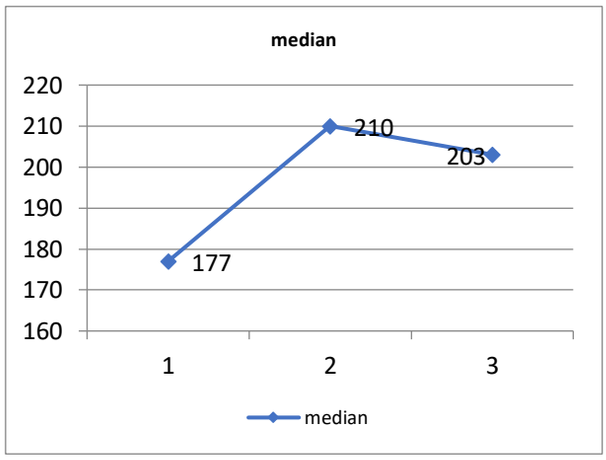

Figure 4 Median of Total Cholesterol of Cases vs. Time

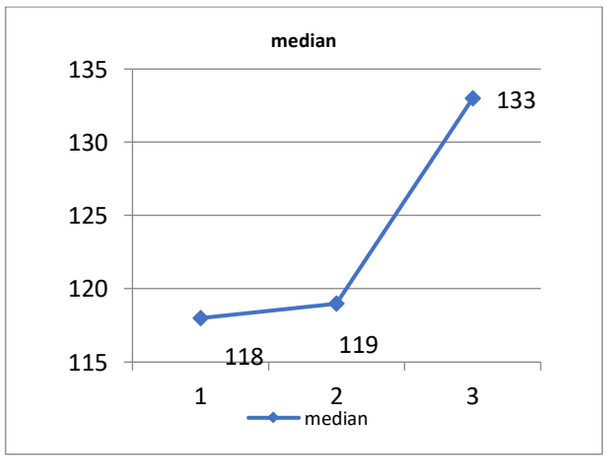

Figure 5 Median of LDL for Cases vs. Time

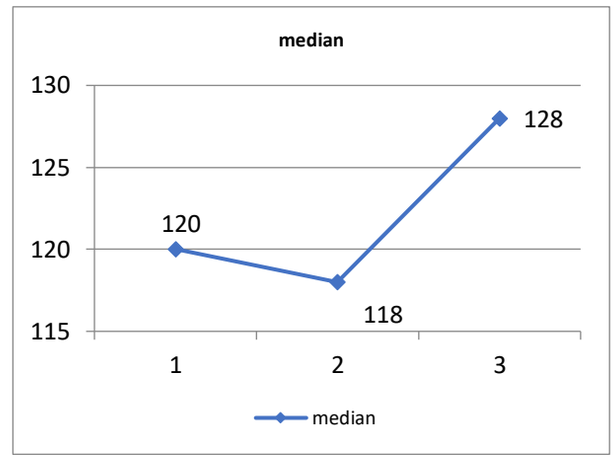

Figure 6 Median of LDL of Controls vs. Time

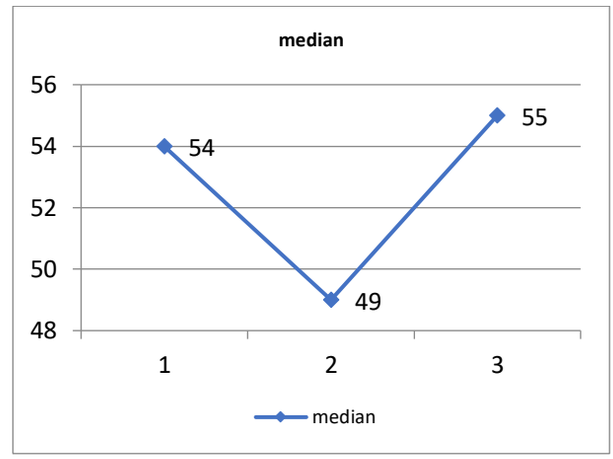

Figure 7 Median of HDL of Controls vs. Time

Measurement of Vomiting Pre and Post Treatment with Alpha-lipoic Acid

The mean of the number of vomiting before treatment with ALA was 5.5 times per day; 10 out of 15 cases had no longer vomiting problems, while 5 were still not affected with P-value at 0.005.

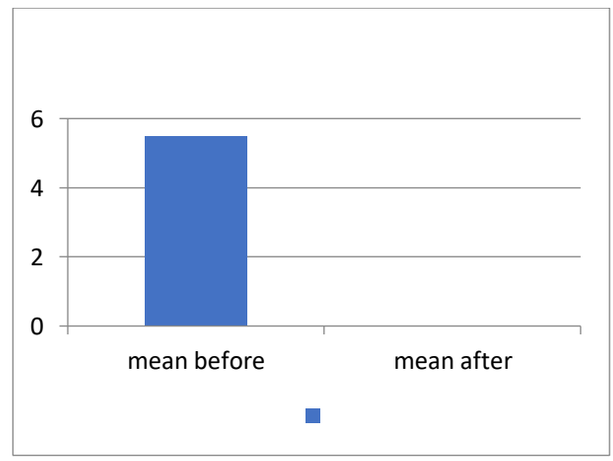

Figure 8 Diagram Demonstrating the Mean of CINV Before and After ALA

Table 5 Demonstrating the Percentage of Cases that Are no Longer Complaining of CIPN

	pain	burning	tingling	Trouble in holding things	numbness	balance
Decrease in Percentage of cases complaining After ALA	58 %	69%	83%	81%	80%	33%
Significance level	0.07	0.004	0.002	0.004	0.008	0.125

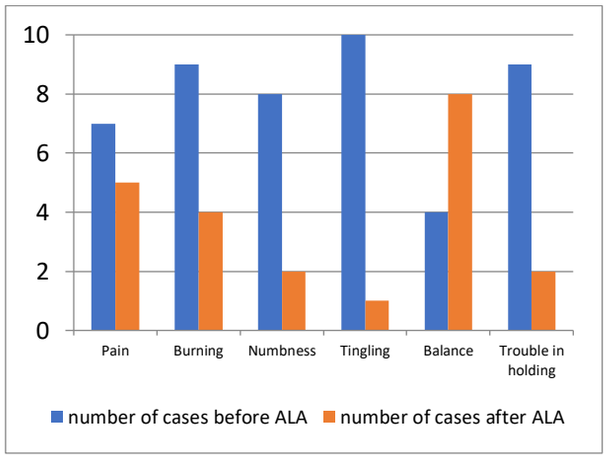

Figure 9 Diagram of Number of Cases Complaining of CIPN Before and After Treatment with Alpha-Lipoic Acid

DISCUSSION

Breast cancer is one of the global public health problems. It is the third most common cancer leading to the death of women worldwide ***(Tupurani et al.,2013).***

Extensive research during the last two decades revealed the mechanism by which continued oxidative stress can lead to chronic inflammation and mediate most chronic diseases, including cancer ***(Reuter et al., 2010).***

In cancer treatment, there is a consequent reduction of the antioxidant defense system. Also, a direct attack of ROS produced during chemotherapy treatment causes oxidative damage in cellular structures ***(Rossi et al.,2009).***

Serum Measured Parameters:

Malondialdehyde (MDA) is a widely used oxidative stress biomarker. It is used to measure the oxidative stress in a cancer patient because it tells us how much carcinogenesis are there in those patients. It was used in different types of cancers as a biomarker for oxidative stress ***(Sharif et al.,2009).***

Alpha-lipoic acid is an antioxidant widely investigated in recent studies in cancer therapy because of its beneficial effect on tumor cells and peripheral neuropathy. Some studies used MDA as an indicator of the effect of alpha-lipoic acid.

Several areas of investigation have implicated that MDA levels in breast cancer women are elevated because of high oxidative stress ***(Gonec et al.,2001).***

Junior et al. examined the MDA in breast cancer women. His study aimed to evaluate the oxidative parameters of erythrocytes and genotoxicity in leukocytes of patients with breast cancer. His study involved a total of 56 individuals, including 28 patients exposed to chemotherapy by the AC protocol (Adriamycin 60 mg/m2and cyclophosphamide 600 mg/m 2) and 28 patients not exposed to chemotherapy. Results showed that the oxidative was increased, represented by MDA elevation compared to controls ***(Junior et al.,2015).***

The value of MDA was also used by Diazapetrin et al. as an indicator for patient survival. He used Kaplan- Meier survival estimates for this purpose in which he divided the values of MDA to $\leq$ 8.6 and those are $\geq$ 8.6 ***(Diazapetrin et al.,2014).***

Sharif et al. (2009) examined the level of MDA in different types of malignancies and found that the value of MDA increased with advanced stages.

In this study, MDA value has not been affected by breast cancer, and so the value of MDA has not related to stage.

Similar to my findings, Zarini et al. (2016) examined oxidant/antioxidant status in breast cancer patients in different stages. He analyzed MDA in different stages and concluded that the severity of oxidative stress in different stages is similar to some extent.

Shariff et al. (2009) also examined the effect of the use of antioxidants. The pretreatment means serum MDA levels of head and neck malignancy patients showed a marked and significant increase. The pretreatment mean serum MDA levels of head and neck malignancy patients showed a marked and significant increase. The post-treated mean serum MDA of Group I (those with lower MDA values) showed a statistically significant lower value.

The study results agreed with these studies, as the median of MDA was significantly decreased after three months of antioxidant (alpha-lipoic acid) from 8.4 to 4.6), with a P-value of 0.013. The median of MDA after 6 months was also not significantly decreased, but it mostly became close to the normal level of MDA(2-4) mmol/l in healthy individuals, while the median of control remained 7.1.

These findings stressed the idea that the MDA value as an indicator of lipid peroxidation. Some studies indicated that MDA would be raised during chemotherapy, but MDA's value was not affected during chemotherapy in the control group in my study.

In some cases, alpha-lipoic acid decreased the median of MDA, so expecting a decrease in carcinogenesis and a better prognosis with cancer therapy.

Cupta et al. examined the oxidative stress in breast cancer patients and lipid profile. The study stated that Reactive oxygen species (ROS) such as hydrogen peroxide, superoxide anions, and hydroxyl radicals are capable of abstracting a hydrogen atom from polyunsaturated fatty acids in membrane lipids to initiate lipid peroxidation.; a significant increase in total cholesterol and LDL in breast cancer patients compared to control group ***(Cupta et al.,2012).***

Data of present investigation revealed a significant increase in the level of cholesterol levels.

Complications of the Use of Chemotherapy

The involvement of reactive oxygen species (ROS) produced during chemotherapy has damaging effects on the mitochondria of the kidney. So the renal function is well documented in cancer patients. Malarkodi implicated that the use of alpha-lipoic acid in adriamycin-induced peroxidative damages in rat kidneys. The study has highlighted the beneficial effects of lipoic acid pretreatment in reversing the damages caused by adriamycin ***(Malarkodi et al.,2003).***

In this study, creatinine and urea were examined to investigate the protective effect of alpha-lipoic acid; significant decrease in creatinine level was observed. The first three months revealed no significant effect of alpha-lipoic acid on kidney function, but in the second three months, it was significant, with P value at 0.03, which means it is important to give alpha-lipoic acid for six months. However, there were no effects on urea.

Cisplatin-induced decreases in renal function, measured by blood urea nitrogen, serum creatinine level, and renal tubular injury scores, were attenuated by α-LA treatment ***(Kang et al.,2009).***

In breast cancer women, the lipid profile is affected during chemotherapy by ROS's effect, so we observed that breast cancer women have a significant increase in total cholesterol, LDL, and decrease in HDL. Cupta et al. examined total cholesterol, LDL, and HDL in breast cancer women and found a significant increase in LDL and TC mean but not HDL. This agreed with my study, which revealed that cholesterol was significantly raised in all breast cancer patients in both cases and controls.

The present study shows no effect of using ALA on lipid profile as expected. The total cholesterol was significantly elevated so was the LDL. There was no significant amelioration on HDL level; this result highlights questions related to the correlation between lipid profile and lifestyle in Gaza Strip in which dyslipidemia is highly prevalent.

In contrast to my study, Zulkhairi et al. investigated the effect of alpha-lipoic acid on lipid profile. The analysis revealed that the level of TC was significantly reduced in most of the treatment groups compared to control.

Symptoms of CIPN

Several prospective experimental studies in animal models suggested that mitochondrial dysfunction is associated with chemotherapy and axonal mitotoxicity contributes to neuropathic symptoms produced by various chemotherapeutic agents. Identification of these mechanisms might help identify newer biomarkers for the CIPN and thus increase the chances of getting improved therapeutic strategies ***(Areti et al.,2014).***

The symptoms of chemotherapeutic-induced peripheral neuropathy (CIPN) were mostly seen during taxol therapy. These symptoms were; pain,

burning, tingling sensation, imbalance, and trouble holding or picking with hands.

All these symptoms were examined through a direct questionnaire to the cases before and after using the alpha-lipoic acid. The number of patients suffering from pain and imbalance was not significantly decreased, while other symptoms were significantly decreased. The study showed significant palliation to symptoms of CIPN.

Pain was not significant; the percentage of cases complaining of pain after treatment with alpha-lipoic acid was 58%. The P-value was> 0.05. It might be significant if the sample was larger.

The imbalance was not significant even if the sample was larger. Patients would still be complaining from imbalance, so there was no effect of alpha-lipoic acid on the balance of the patient, where p- value was > 0.05.

Numbness was highly significant with P-value <0.05; 80% of patients have no numbness after six months of alpha-lipoic acid treatment.

Tingling sensation is a very upsetting symptom for patients receiving chemotherapy. The results revealed a significant decrease in the percentage of patients after treatment with alpha-lipoic acid, which was 83% with P-value < 0.05.

Patients also usually complain of trouble picking things from the floor or even holding things with their hands. This symptom was significantly decreased with P-value of < 0.05. 81% of cases were no longer suffering from trouble holding or picking things.

In agreement with my study, Melli et al. examined the neuroprotection effect of alpha-lipoic acid in vitro using taxol, which exerts neurotoxicity through hyper stabilizing microtubules cross-linking and consequently altering axonal transport and growth ***(melli et al. 2008).***

Neuroprotection effect of alpha-lipoic acid through investigation of MDA of spinal cord tissue, in which the content of MDA was examined by Toklu et al. The content of MDA in the spinal cord was significantly elevated in control rats indicating the presence of enhanced lipid peroxidation in the treatment of alpha-lipoic acid, which completely prevented the elevation of MDA ***(Toklu et al., 2010).***

The results of these studies which agree with my study results give a potentially promising area of neuroprotective drug discovery for CIPN.

Areti et al. reviewed the CIPN during chemotherapy due to high oxidative stress and suggested MDA as an indicator of oxidative stress biomarker due to high oxidative stress induced by a chemotherapeutic agent. He suggested MDA as an indicator of oxidative stress ***(Areti et al.,2012).***

Vomiting is a significant problem in the treatment with chemotherapy. This study examined the effect of alpha-lipoic acid on vomiting by asking patients directly about the number of vomiting before and after treatment with alpha-lipoic acid. It showed a decrease in the number of vomiting per day after treatment with a P value> 0.05. Similar to my study, the findings of Mantovani et al. examined the effect of antioxidants on the side effects of chemotherapy as fatigue, nausea, and vomiting. All patients were given as basic treatment polyphenols plus antioxidant agents α-lipoic acid, carbocysteine, and vitamins A, C, and E, all orally. After 2 years, no severe side effects, like nausea and vomiting, were observed ***(Mantovani et al.,2008).***

CONCLUSION

The results concluded the beneficial effect of alpha-lipoic acid as an antioxidant in breast cancer patients. This was obvious from the number of factors that significantly affected the patient.

There is an elevated level of MDA, the biomarker of lipid peroxide, in breast cancer patients compared to the normal healthy individual, supporting the idea that cancer patients have an imbalance between oxidant/antioxidant and so high oxidative stress.

The oxidative stress plays a great role in the pathogenesis of cancer and will also increase the chance of metastasis to other organs.

Chemotherapeutic agent induces ROS increasing the oxidative stress during therapy. This ROS will not only affect the cancer cells; on the contrary, it will affect the normal cells inducing the side effects reported from chemotherapy and will affect the QOL of the patient.

Treatment with alpha-lipoic acid significantly decreases the median of MDA by 3.8 nmol/ml and attain it to about the normal range. This leads to a decrease in oxidative stress in breast cancer patients and a decrease in carcinogenesis. It

will also restrict the ability of metastasis which is the major problem with cancer patient. Although the significance was in the first three months, giving alpha-lipoic acid for six months can attain the MDA to about the normal level or restrict the elevation resulting from chemotherapy. We cannot forget that the patient compliance effect must be taken into consideration and may affect the results.

Alpha-lipoic acid has a beneficial effect on creatinine, so protecting the effect on kidney function as expected from antioxidants when used concomitantly with chemotherapy. Breast cancer protocol in Gaza Strip includes Adriamycin which has a harmful effect on renal function. The significance was observed in the second three months, which means that if we want the protective effect of alpha-lipoic acid on the renal function, we have to give alpha-lipoic acid for six months.

Symptoms of CIPN disturbed patients and affected their QOL. Patients are weak, feeling severe pain, unable to do anything by themselves, unable to eat or sleep because of the neuropathy.

Alpha-lipoic acid has a neuroprotective effect. Only in pain and imbalance alpha-lipoic acid has no significant effect on the number of cases complaining. Other symptoms (burning sensation, tingling sensation, numbness, and trouble holding and picking things) are all significantly ameliorated using alpha-lipoic acid. Thus, giving alpha-lipoic acid for six months has a beneficial effect for chemotherapy-induced peripheral neuropathy and helps patients overcome the harmful period (the course of chemotherapy) and contribute to improving quality of life for these patients, especially breast cancer patients who are women and are mothers of children and play important roles in the community.

There was no effect of alpha-lipoic acid on the lipid profile of breast cancer patients who usually have elevated levels of total cholesterol, LDL, HDL, and no beneficial effect from using alpha-lipoic acid for hypercholesteremia.

Chemotherapeutic-induced nausea and vomiting restrict the patient's ability to eat and lead to weakness and severe gastric pain. ALA helped patients to overcome this problem and decrease their complaints of nausea and vomiting. Patients complaining of vomiting during chemotherapy can take it as an adjuvant to other antiemetic drugs.

All effects of ALA come from its potent antioxidant effect, which is represented by its great role as a neuroprotective, antiemetic, and preventive effect of metastasis.

RECOMMENDATIONS

We recommend that the Ministry of Health add alpha-lipoic acid to the breast cancer patient's regimen to mimic the cytotoxic effects of chemotherapy and ameliorate the side effects of chemotherapy.

Physicians should be aware of the importance of antioxidants in the nutrient of breast cancer patients and the supplement of synthetic antioxidants especially ALA, the regenerator of other antioxidants.

Further studies have to be done in this field to see the feedback of decreasing the MDA in cancer patients and how it will be reflected on the prognosis of the disease.

More attention should be given to the scientific research in cancer therapy in Gaza Strip. IV of ALA can be more effective than oral, and so we recommend prescribing IV product.

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