***The use of alpha lipoic acid as adjuvant therapy in breast cancer patient***

***A case- control study***

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***Abstract***

***Background****: oxidative stress plays a major role in the process of carcinogenesis, this fact highlights the questions about the effect of Antioxidants in cancer therapy, ALA has gained a lot of concern in the last decade as antioxidant in many pathological conditions including cancer therapy.*

***Objectives****: This study was conducted to identify the effect of alpha lipoic acid (ALA) when used with chemotherapy in breast cancer. The study examined its effect on oxidative stress, Malondialdhyde (MDA) as oxidative stress biomarker was examined, the adverse events of chemotherapy mainly Chemotherapeutic Induced Peripheral Neuropathy (CIPN), chemotherapeutic Induced nausea and vomiting (CINV), dyslipidemia , and the effect on renal function.*

***Methodology****: This case control study was conducted in Al - Shefa Hospital in Gaza strip, women who were admitted to the oncology department and diagnosed as breast cancer patients, 15 cases were given ALA (600 mg per day for six months), and 15 control, the MDA, Creatinine and lipid profile were examined at zero time, after 3 months and after 6 months, the adverse events was examined by face to face questionnaire to the cases at zero time and after 6 months .*

 ***Results:*** *MDA was significantly decreased in first 3 months ( P- value = 0.013), after six months it was not significantly decreased (P – value = 1). Creatinine was significantly decreased after six months (P- value = 0.000). while urea was not changed significantly ,concerning to 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 stop feeling of pain, Imbalance also was not significantly improved, only 33% of cases stopped feeling of imbalance ( P – value 0.125). while 80 % of cases stopped feeling of numbness (P –value = 0.008). 83% of cases stopped feeling tingling sensation ( P – value = 0.002) , 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( .*

***Conclusion****: the use of ALA has beneficial effect on oxidative stress which has major role in the process of carcinogenesis, It has protective effect on renal functions. It has beneficial effect on the CIPN, CINV.*

**الملخص باللغة العربيه**

**ملخص الدراسه**:تلعب الاكسده دورا اساسيا في عملية السرطنه وهذا يسلط الضوء على دور مضادات الاكسده في الحد من هذا الدور وقد لاقى مركب الالفا ليبويك اسيد اهتماما كبيرا في العقد الاخير في كثير من الامراض المتعلقة بالاكسده وخاصة في مجال السرطان.

**الاهداف**: اجريت هذه الدراسه للتعرف على دور مركب الفاليبويك اسيد كعلاج مساند للعلاج الكيميائي المستخدم في سرطان الثدي. اختبرت الدراسة تأثير الفا ليبويك اسيد على الاكسده المتمثله بمستوى المالون داي الدهايد في الدم . كما تختبر الدراسه تأثير الفاليبويك اسيد على الاثار السلبيه المصاحبة للعلاج الكيميائي مثل الاعتلال العصبي المسبب بواسطة العلاج الكيميائي والاستفراغ والغثيان المسبب بواسطة العلاج الكيميائي وتختبر الدراسة ايضا تأثيره على مستوى الدهون في الدم التي ترتفع في سرطان الثدي.

**منهجية البحث:** وقد أجريت هذه الدراسة (الحالات والشواهد). وقد تم اختيار العينة من النساء الذين يدخلون إلى قسم الأورام في مستشفى آل الشفاء

 في قطاع غزة ويتم تشخيصهم على أنهم مرضى سرطان الثدي. الحالات تم اعطاؤهم الفاليبويك اسيد بواقع حبة يوميا .mg 600 وتم عمل فحص لمستوى المالون داي الدهايد كمؤشر على مستوى الاكسده عند نقطة الصفر كذلك تم فحص الكرياتينين واليوريا كمؤشر على وظائف الكلى وكذلك فحص مستوى الدهون . ثم اعيدت هذه التحاليل بعد ثلاثة اشهر وبعد ستة اشهر لكل من الحالات والشواهد . وتم عمل استبانه للحالات قبل وبعد استخدام الفاليبويك اسيد فيما يخص اعراض الاعتلال العصبي المصاحب للعلاج الكيميائي اعراض الغثيان.

**النتائج:** فيما يتعلق بمستوى الاكسده فان قياسات المالون داي الدهايد اظهرت تحسن في وسيطها للحالات في اول 3 شهور(بدلاله احصائيه0،013 بينما لم يكن نزول المستوى في الوسيط في الثلاث شهور الثانيه لم يكن ذا دلاله احصائيه (دلاله احصائيه = 1) ولم يكن هناك دلاله احصائيه بالنسبه للشواهد (دلاله احصائيه =0،6). فيما يتعلق بمسنوى اليوريا لك يكن هناك تغير ذا دلاله احصائيه في كل من الحالات والشواهد ,اما فيما يتعلق الكرياتينين فان التغير فيه كان ذا دلاله احصائيه بعد ستة اشهر (دلاله احصائي =0،003

لم يكن هناك تحسن في مستوى الدهون وانما كان هناك ارتفاع ذا دلالة احصائيه في مستوى الكوليسترول في الحالات (دلاله احصائيه = 0،003

اما فيما يخص الكوليسترول الخبيث LDL فان هناك ارتفاع ولكنه لم يكن ذا دلاله احصائيه في كل من الحالات والشواهد . كذلك الكوليسترول ا لحميدHDL لم يكن هناك ارتفاع ذا دلاله احصائيه

جميع اعراض العلاج الكيميائي تحسنت بدلاله احصائيه ما عدا الالم والشعور بعدم الاتزان .

الخدلان : 80% من الحالات لم تعد تشعر بالخدلان(دلاله احصائيه = (0.008

الشعور بالوخز : 83% من الحالات لم تعد تشعر بالوخز (دلاله احصائيه = 0.002)

عدم القدرة على حمل الاشياء: 81% من الحالات لم تعد تعاني من هذا الغرض (دلاله احصائيه = (0.004

لم يعد هناك أي من الحالات تعاني من الاستغراغ والغثيان( دلاله احصائيه = (0.00

**الخلاصة:** توجد تأثيرات ايجابيه لاستخدام الفا ليبويك اسيد كعلاج مساند للعلاج الكيميائي كمقلل لمستوى الاكسده

توجد تاثيرات ايجابية لاستخدام الفاليبويك اسيد على وظاءف الكلى

توجد تأثيرات ايجابيه للفا ليبويك اسيد على الاعراض الجانبية للعلاج الكيميائتوجد تأثيرات ايجابيه للفا ليبويك اسيد على الاعراض الجانبية للعلاج الكيميائي المتمثله بالاعتلال العصبي واعراض الغثيان والاستفراغ

التوصيات : لمقدمي الخدمة الصحيه لاقرار استخدام الالفاليبويك اسيد كعلاج مساند للعلاج الكيميائي

مفاتيح الكلمات: الاكسده، سرطان الثدي، الالفاليبويك اسيد ، مالوندايالدهايد، CIPN ،CINV، ROS

***introduction***

*Cancer is a group of disease characterized by abnormal and uncontrolled growth and spread of cells, anyone can develop cancer, as the risk of being diagnosed increases with age, most of cases occur in adults who are middle age or older, about 78% of all cancers are diagnosed in patient 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 major public health problem in*

*USA and other countries* ***(Seigel et a., 2015).***

*Cancer begins when cells in part of 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, five million deaths from cancer* ***(Alhams; et al., 2014).***

***Epidemiology***

*One million new cases of breast cancer all over the world each year and compromises 18% of all women malignancies , where the age standardized incidence and mortality is the highest in U.K* ***(McPherson, et al,. 2000).***

*One in nine woman in U.K and USA will develop the disease in their lifetime* ***(Abdulkareem, 2013).***

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

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

*In mortality (both West Bank and Gaza Strip)breast cancer occupies the third place after colorectal (second) and lung (the first), 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 prevalance 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 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, so simple mastectomy, the choice is taken according to the location and extent of the breast mass in relation to the breast size and the patient preferences. Surgery of the axilla is by sentinel lymph node guided sampling ( after dye injection)* ***(Kumar and Clark, 2009).***

***Chemotherapy***

*In advanced cancer , patients who will not respond to hormonal therapy or who fail to respond endocrine therapy or who require rapid response if at risk as liver or respiratory failure chemotherapy if chosen carefully will provide good palliation this is the list of chemotherapy used:*

*AC/EC – doxorucin or epirupcin and cyclophsphamid*

*DC- docetaxol and capicitabin*

*PG-paclitaxel and gemicitabine*

*VC- vinorelbine and cabcitabine* ***(Kumar and Clark, 2009)****.*

*A third generation regmin with taxane (AC-T) cyclophsphamide, adiramycin , taxane* ***(Kumar and Clark, 2009)****. This regmin is the one which is used in Gaza strip as a protocol of therapy it decreases the relative risk of death by 33% but it also increases the toxicity* ***(Kumar and Clark, 2009)****.*

*Menopauseal status does not affects the relative efficacy of chemotherapy , since the recurrence is less after menopause , the absolute improvement in survival is lower*

***Endocrine therapy:***

*This indicated in all patients with detectable ER expression (defined as ≥1% of invasive cancer cells) irrespective of the use of chemotherapy and/or targeted therapy The choice of agent is primarily determined by the patient’s menopausal status. Other factors include differences in efficacy and side-effect profiles* ***(Senkus et al., 2015).***

*One third of breast cancer patient will have estrogen / progesterone receptors positive and will be given Tamoxifen as adjuvant therapy immediately after surgery , receotor- positive disease reduces death from breast cancer by about 25%* ***(Kumar and Clark, 2009).***

 ***Her2/cerb2 targeted therapy***

*There are recent trials on adjuvant IV trazumab with chemotherapy for 25%of patient who over expresses her 2 have all shown significant decrease in risk of mortality trazumab which is monoclonal antibody has direct toxic effect on myocardiam so left ejection fraction must be monitored before and during therapy especially if given after or with anthracyclins which has additional cardiotoxic effect* ***(Kumar and Clark, 2009).***

*Toxicity may be higher so the choice must be individualized to each patientt , the combined chemotherapy and radiotherapy and tamoxifen or aromatase inhibitors halves the risk of dying of breast cancer for appropriately selected patient* ***(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, ageing, 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 the cell death as any other metabolic process can be disrupted by mutation, in fact defect in apoptotic pathway are 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 exceeds 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* ***(Klaunig 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 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)***

***Malondialdhyde (MDA).***

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

*Alpha-lipoic acid (ALA) (thioctic acid, 5-(1,2-dithiolan-3-yl)pentanoic acid,) 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, has gained attention as nutritional supplement and as 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 an 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 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 as antioxidant used for treatment of many neurological disorders such as diabetic polyneuropathy and multiple sclerosis as it can cross Blood brain barrier. It has been shown to improve endothelial function and blood flow, and accelerate glutathione synthesis, which plays a crucial role in regulating the expression of several antioxidant and anti-inflammatory genes* ***(Choi et al., 2015)***

*studies on cancerous cell-based models have suggested that the tumor – suppressive effect of LA corresponds with apoptosis induction, a critical parameter impaired in cancer cells, and 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. LA is relatively stable as a solid but it polymerizes when heated above its melting point (47.5 oC)* ***(Novotny et al., 2007).***

***Pharmacological effect of ALA***

 ***Antioxidant effect :***

*ALA is ROS scavenger and metal chelating The disulfide group of LPA 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 a powerful lipophilic antioxidant both in vitro and in vivo It is known to act as 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)***

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*Figure(1)alpha lipoic and dihydro lipoic acid*

***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 role critical signalling molecules , contribute in enhancing cell proliferation and to suppress apoptosis, alpha-lipoic acid was able to induce cell cycle arrest and apoptosis in different cancer cell lines* ***(Dozio et al.,2010).***

1. *ALA inhibit glycolysis:*

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

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

*c-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 prevention of metastasis in cell system, their hypothesis was 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). 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. analysis confirmed the reduction in mRNA expression level of MMP-2 and MMP-9 by LA treatment. they conclude that in this cell culture model, LA treatment inhibits cancer metastasis, 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 alphalipoic acid they used an in vitro model of chemotherapy induced peripheral neuropathy that 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 investigating the efficacy of alpha-lipoic acid in preventing axonal damage and apoptosis and the function and ultra structural 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 the 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)***

*a-ALA prevents nephrotoxic effect of Adiramycin, ALA is also capable of influencing the nephrotoxixity potential of adiramycin.*

*c-It can help minimize CINV by its antioxidant effect and so its benefit effect on other cells that is harmed by chemotherapy , Mantovani et al examined the effect of antioxidant 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***

***3.1. Materials and Methods***

***3.1.1.Materials***

1. *Thiobarbeturic acid Kit for measurement of malondaildhyde which purchased from {Biodiagnosteic company – Egypt}, and preserved in refrigerator in 2-8 C°*
2. *Alpha lipoic acid (Neurogaurd ® 600 mg ) donated from the { 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}*

***3.1.2. Study Design***

*The study conducted is retrospective case control study , the sample was chosen from study population who are women diagnosed with breast cancer and admit to the oncology department in Al- heaf hospital, the sample was chosen according to specific criteria*

*Non pregnant , Non lactating, Non metastatic cases who are at the beginning of the chemotherapy, the choice was under the supervision of the head of oncology department.*

*The patient are all on AC-T protocol*

*The patient were given informed consent*

*serum sample was collected from patient when admitted for chemotherapy*

***Study group (cases)***

*patients were given information about the alpha lipoic acid and given the amount of drug enough for one month and given the instruction for use ( one tablet of 600 mg ) which was donated from the Advanced company*

*blood sample were collected after the first chemotherapy and before the second dose*

***Control group***

*no drug intervention but the blood samples were collected, the samples collected in serum tubes on vaccum without pushing the sample through the needle to avoid hemolysis , all samples were transmitted immedialtely to the laboratory.*

 ***Methods***

1. *the Kit of MDA is thiobarbeturic acid which is chlorometric ,thiobarbeturic acid reacts with MDA in acidic media at temperature of 95 ͦ C for 30 min to form thiobarbituric acid reactive*

***Serum MDA =*** $\frac{A Sample}{A standard }×10 nmol/ml$

1. *the blood samples were mixed and the test tubes were covered with glass beads , heated in boiling water bath for 30 min , cooled then mixed and the absorbance was read at 534nm , the*
2. *Analysis of serum lipids Total cholesterol, LDL, HDL were analysed every three months.*
3. *Urea and creatinine were also analysed*

*All tests were performed in Al Nebrass Laboratory which is registered by ministry of health.*

*Abstract sheet was performed to compare between the complain of CIPN pre and post ALA , and to compare between number of vomiting per day during chemotherapy.*

*The face to face questionnaire( Yes or No) was performed asking patient about the side effect related to chemotherapy.Chemotherapeutic Induced peripheral Neuropathy before and after treatment with ALA Imbalance, Pain, Tingling ,Numbness, Trouble in Holding or picking things Chemotherapeutic induced Nausea and Vomitting (CINV)*

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

***3.1.4. Statistical analysis***

*Patients were given symbols*

*Cases were given letters from (A1 – A15)*

*Controls were given letters from (B1 – B15)*

*Descriptive analysis were performed for study data , data were entered to SPSS program.*

 *Alphalipoic acid was given to 15 breast cancer patient who were chosen to be the cases , drug (Neurogaurd) was given month by month (30) tablets for each patient and the compliance of the patients was measured individually to ensure taking the drug.*

*Blood serum of 30 newly diagnosed breast cancer and have been analysed , MDA, CBC, UREA , CREAT , Cholesterol , HDL, LDL have been measured.*

* *A repeated measures ANOVA with a Greenhouse-Geisser correction was used for continous data*
* *Wilcoxon rank test was done for number of vomiting pre and post treatment with ALA.*
* *QI square (McNemar test) was used to measure 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 hypothized to be statistically significant on 0.05*

***Results***

 ***MDA For Healthy Individuals (Negative control)***

*MDA has been measured for 10 healthy individuals, the mean of MDA was about 2.66nmol/ml*

*The healthy individuals were choosen with no other diseases to avoid any reason for elevated values of MDA*

 ***MDA for cases***

*The MDA for cases was measured at zero time and after 3 months and after 6 months, the median value of MDA was 8.4 nmol/ ml . after three months of the use of ALA , the median declined to 5.4 nmol/ ml with P- value 0.013*

*Then after 3 months (six months from the beginning of the study). the median value became 4.6 nmol/ml with P- value 1 , the significance across time reveals that the first three months was significant while the second three months was not significant* *P value: 0.013*

 *Table (1): measurement of MDA in cases at zero, after 3 months and after 6 months*

|  |  |  |
| --- | --- | --- |
|  | *median* | *Standard deviasion* |
| *At zero* | *8.4* | *3.1995* |
| *After 3 months* | *5.4* | *2.3789* |
| *After 6 months* | *4.6* | *3.1806* |

 *Figure (2)median of MDA versus time*

***MDA for controls***

*The MDA was measured at zero time for controls , the median was 7.1 , after three months was 7 , after six months , no change on the median of MDA, still the same after six months*

*P- value : 0.6*

*Table(2): measurement of MDA in controls at zero, after 3 months and after 6 months*

|  |  |  |
| --- | --- | --- |
|  | *mean* | *Standard deviation* |
| *At zero*  | *7.1* | *3.3833* |
| *After 3months* | *7* | *3.2295* |
| *After 6 months* | *7.1* | *4.3582* |

*Figure (3):median of MDA of controls versus time*

|  |  |  |
| --- | --- | --- |
|  | *median* | *Standard deviasion* |
| *Urea at zero time* | *29.000* | *7.6874* |
| *Urea after 3months* | *30.000* | *8.4797* |
| *Urea**after 6 months* | *31.00* | *8.908* |

 ***Urea for cases***

*Table(3): measurement of urea for cases at zero time, after 3months and after 6 months*

*the urea was not significantly affected with the use of ALA*

*P- value 0.548*

*Figure (4): mean of urea of cases versus time*

***Urea for control***

*Urea of controls was measured for urea at zero , three and six months , the median was 26 mg/dl , after three months the median was 32mg/dl , after six months it was 30 mg/dl*

*Table (4): Table(3): measurement of urea for controls at zero time, after 3months and after 6 months*

|  |  |  |
| --- | --- | --- |
| *Time* | *Median**mg/dl* | *Standard deviation* |
| *Urea at zero time* | *26* | *11.0005* |
| *Urea after 3 months* | *32* | *7.6923* |
| *Urea after 6 months* | *30* | *9.306* |

*P- value: 0.212*

*Figure (4): mean of urea of 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 creatinie was not significantly declined with P- value 1, after six months the dedian of creatine declined significantly to 0.67 mg/ dl with P-value 0.03*

|  |  |  |
| --- | --- | --- |
|  | *median* | *Standard deviation*  |
| *Creatanine at zero time* | *0.86* | *0.15989* |
| *Creatinine after 3 months* | *0.74* | *0.29014* |
| *Creatinine after 6 months* | *0.67* | *0.10487* |

 *Table (5) measurement of creatinine in cases at zero time, After three months and after six months*

*P- value: 0.03*

 *Figure (6): median of creatinine of cases versus time*

*.*

* 1. ***Creatinine for controls***

*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 , 0.8 after six months*

*P- value: 0.525*

 *Table (6) :measurement of creatinine for controls at zero time, after 3months and after 6 months*

|  |  |  |
| --- | --- | --- |
| *Time* | *Median**mg/dl* | *Standard deviation*  |
| *Creatanine at zero time* | *0.77* | *0.16164* |
| *Creatinine after 3 months* | *0.8* | *0.14192* |
| *Creatinine after 6 months* | *0.8* | *0.12182* |

*Figure (7): median of creatinine of controls versus time*

***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 0.003*

|  |  |  |
| --- | --- | --- |
|  | *median* | *Standard deviation* |
| *TC at zero time* | *177* | *23.850* |
| *TC after 3months* | *210* | *49.585* |
| *TC after 6 months* | *203.2* | *51.498* |

*Table (9): measurement of TC for cases at zero time, after 3months and after 6 months*

*Figure (8): median of total cholesterol of cases versus time*

***Total cholesterol for controls***

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

*Table(10): measurement of TC for controls at zero time, after 3months and after 6 months*

|  |  |  |
| --- | --- | --- |
|  | *median* | *Standard deviasion* |
| *TC at zero time* | *177* | *42.028* |
| *TC after 3months* | *204* | *59.258* |
| *TC after 6 months* | *222* | *58.232* |

*Figure (11): median of total cholesterol of controls versus time*

***4.10. Low density lipoprotein (LDL) for cases***

 *It was measured at zero time , after three months and after 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*

*Table(11): measurement of LDL for casesat zero time, after 3months and after 6 months*

|  |  |  |
| --- | --- | --- |
|  | *Median* | *Standard Deviation* |
| *LDL at zero time* | *118* | *16.2794* |
| *LDL after 3 months* | *119* | *40.193* |
| *LDL after 6 months*  | *133* | *39.736* |

 *P- value : 0.191*

 *Fig (12) median of LDL for cases versus time*

***Low density lipoprotein(LDL) for controls***

*For controls the median at zero time was 120 mg/dl, after three months was 118 mg/dl, after six months was 128 mg /dl (not significant)*

 *P- value : 0.267*

*Table (12):measurement of LDL of controls at zero time , after 3 months, after 6 months*

|  |  |  |
| --- | --- | --- |
|  | *Mean*  | *Standard deviasion* |
| *LDL at zero time* | *120* | *35.6103* |
| *LDL after 3 months* | *118* | *45.348* |
| *LDL after 6 months*  | *128* | *50.880* |

*Figure (13): median of LDL of controls versus time*

***High density lipoprotein (HDL) for cases***

*Table (13):**Measurement of HDL of cases at zero time , after 3 months, after 6 months*

|  |  |  |
| --- | --- | --- |
| *Time* | *Median**mg/dl* | *Standard deviation* |
| *HDL at zero time* | *38* | *8.968* |
| *HDL after 3 months*  | *44* | *15.346* |
| *HDL after 6 months*  | *47* | *12.489* |

*Figure (14): median of HDL of cases versus time*

***High density lipoprotein (HDL) for controls***

 ***Table (14): measurement of HDL of controls at zero time , after 3 months, after 6 months***

|  |  |  |
| --- | --- | --- |
|  | *median* | *Standard diviasion* |
| *HDL at zero time* | *54* | *18.4378* |
| *HDL after 3 months*  | *49* | *13.074* |
| *HDL after 6 months*  | *55* | *15.543* |

*For controls, also no significant change on HDL at zero time , after three months and after six months with P- value 0.78*

*Figure (26) mean of HDL of controls versus time*

***Measurement of vomiting pre and post treatment with alphalipoic acid***

*The mean of number of vomiting before treatment with ALA was 5.5 times per day , 10 of 15 cases had no longer vomiting while 5 still not affected with P-value 0.005*

*Figure (27) diagram demonstrating mean of CINV before and after ALA*

*Figure (28): diagram of number of cases complaining of CIPN before and after treatment with alpha lpoic 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 last two decades has revealed the mechanism by which continued oxidative stress can lead to chronic inflammation, which in turn could mediate most chronic diseases including cancer* ***(Reuter et al., 2010).***

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

***For serum measured parameters:***

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

*Alphalipoic acid is an antioxidant that is widely investigated in recent studies in cancer therapy because of its beneficial effect in tumor cells, and in peripheral neuropathy , some studies used MDA as an indicator to 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 , The aim of his study was 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 used also 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 ≤ 8.6 and those are ≥ 8.6* ***(Diazapetrin et al.,2014)***

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

*In contrast to my study where the MDA value has been not affected by the initial value or the stage of breast cancer the value of MDA and so 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 analysed MDA in different stages , he concluded that the severity of of oxidative stress in different stages is similar to some extent* ***(Zarini et al., 2016).***

***(Sharif et al****.,* ***2009)*** *also examined the effect of the use of antioxidant, The pretreatment mean serum MDA levels of head and neck malignancy patients showed marked and significant increase, The pretreatment mean serum MDA levels of head and neck malignancy patients showed marked and significant increase, The post treated mean serum MDA of Group I (those who has lower MDA values) patients showed statistically significant lower value.*

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

*This ensure the idea that the MDA value as an indicator to lipid peroxidation. Some studies indicated that MDA will be raised during chemotherapy but in my study the value of MDA was not affected during chemotherapy in control group.*

*In cases alphalipoic acid decreased the median of MDA, so expecting decrease in carcinogenesis and better prognosis with cancer therapy .*

*Cupta et al,. examined the oxidative stress in breast cancer patient and lipid profile , he 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, significant increase in total cholerstrol and LDL in breast cancer patient compared to control group* ***(Cupta et al.,2012).***

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

***5.2. According to complications of the use of chemo therapy***

*Involvement of Reactive oxygen species ROS produced during chemptherapy in damaging effects on the mitochonderia of kidny and so the renal function is well documented in cancer patient , Malarkodi implicated that the use of alphalipoic acid in adriamycin induced peroxidative damages in rat kidney, 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 protection effect of alphalipoic acid, significant decrease in creatinine level was observed. The first three months revealed no significant effect of alpahlipoic acid on kidney function but in the second three months it was significant, with P value 0.03 which means it is important to give alphalipoic acid for six months. But no effect 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 woman lipid profile is affected during chemotherapy by the effect of ROS so we observed that breast cancer women have 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 the mean of LDL and TC 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.*

*In present study No effect of using ALA on lipid profile as expected, Total cholesterol significantly elevated so did LDL, no significant amelioration on HDL level, this results highlights questions about the correlation between lipid profile and life style in Gaza strip in which dyslipidemia is highly prevalent.*

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

***5.2.1. 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 agent, Identification of these mechanisms might be helpful in identifying newer biomarkers for the CIPN and thus increases the chances of getting improved therapeutic strategies* ***(Areti et al.,2014).***

*The symptoms of chemotherapeutic induced peripheral neuropathy (CIPN) which were mostly seen during taxol therapy . these symptoms were ; pain , burning, tingling sensation, imbalance and trouble in holding or picking with hands.*

*All these symptoms were examined through a direct questionnaire to the cases before and after using the alphalipoic acid , the number of patient suffering from pain and imbalance were not significatly 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 from pain after treatment with alphalipoic 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 still complaining from imbalance and so no effect of alphalipoic acid on the balance of the patient, the p- value was <0.05.*

*Numbness was highly significant with P- value >0.05 , 80% of patient have no numbness after six months of alphalipoic acid treatment.*

*Tingling sensation is very upsetting symptom for patient receiving chemotherapy , the results revealed significant decrease in the percentage of patient after treatment with alphalipoic acid was 83% with P – value> 0.05.*

*Patients also usually complain from trouble in picking things from the floor or even holding things by their hands , this symptom was significantly decreased with P- value was > 0.05. 81 % of cases had no longer suffering from trouble of holding or picking things.*

 *In agreement with my study Melli et al examined the neuroprotection effect of alphalipoic acid in vitro with the use of taxol which exert neurotoxicity through by hyperstabilizing microtubules cross-linking and consequently altering axonal transport and growth*  ***(melli et al, 2008).***

*Neuroprotection effect of alphalipoic acid through investigation of MDA of spinal cord tissue, the content of MDA was examined by Toklu et al, the content of MDA in spinal cord was significantly elevated in control rats indicating the presence of enhanced lipid peroxidatioin ,the treatment of alphalipoic acid 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,. revwied the CIPN during chemothotherapy due to high oxidative stress in and suggested MDA as an indicator of oxidative stress biomarkerdue to high oxidative stress induced by chemotherapeutic agent , he suggested MDA as an indicator to oxidative stress* ***(Areti et al.,2012).***

*Vomiting is a great problem in treatment with chemotherapy, this study examined the effect of alphalipoic acid on vomiting by asking patient directly about the number of vomiting before and after treatment with alpha lipoic acid, decrease in the number of vomiting per day after treatment with P value< 0.05 , similar to my study findings* ***Mantovani et al*** *examined the effect of antioxidant 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).***

***Conclusion***

*From the results we have we can conclude the beneficial effect of alphalipoic acid as antioxidant in breast cancer patient and this was obvious from number of factors that were significant.*

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

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

*Chemotherapeutic agent induce ROS and so increasing the oxidative stress during therapy , this ROS will not only affect the cancer cells , on the contrary it will affect the normal cells and so induce the side effects reported from chemotherapy and will affect the QOL of the patient.*

*Treatment with Alphalipoic acid significantly decrease the median of MDA by 3.8 nmol/ml and attain it to about the normal range. Which means decrease in the oxidative stress in breast cancer patient and so decrease in carcinogenesis and also restrict the ability of metastasis which is the major problem with cancer patient, although the significance was in the first three months, but giving alphalipoic acid for six months can attain the MDA to about the normal level or at least restrict the elevation resulting from chemotherapy , we cannot forget that the effect of the patient compliance which must be taken into consideration and may be affect the results.*

 *According to Kaplan Meier scale for survival decreasing the median of MDA to about 4 will increase the survival rate of the cases used alphalipoic acid and further investigations have to be done in order to prove this .*

*Alphalipoic acid has beneficial effect on creatinine, so protecting effect on kidney function as expected from the effect of antioxidants when used concomitantly with chemotherapy , breast cancer protocol in Gaza strip include Adiramycin which has harmful effect on renal function , the significance was observed in the second three months which means that if we want the protective effect of alphalipoic acid on renal function we have to give alphalipoic acid for six months.*

*Symptoms of CIPN upsets patient and affect their QOL, patients are weak, feeling severe pain, unable to do anything by himself, unable to eat or sleep because of the neuropathy .*

*Alphalipoic acid has 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 in holding and picking things ) all are significantly ameliorated by use of alphalipoic acid . so giving alpha lipoic acid for six months has beneficial effect for chemotherapy induced peripheral neuropathy and help patient to overcome the harmful period (the course of chemotherapy) and contribute to improve quality of life for these patients specially in breast cancer patients who are women and are mothers of children and play great duties in community.*

*No effect of alphalipoic acid on lipid profile of breast cancer patients who usually have elevated levels of total cholesterol, LDL, HDL and no beneficial effect from using alphalipoic acid for hypercholesteremia.*

*chemotherapeutic induced nausea and vomiting which restrict patient's ability to eat and leads to weekness and severe gastric pain, ALA helped patients to overcome this problem and decrease their comlaining from nausea and vomiting ,so patient complaining from vomiting during chemotherapy can take it as adjuvant to other antiemetic drugs.*

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

***Recommendations***

*We recommend Ministry of health to add Alpha lipoic acid to the regmin of breast cancer patient to mimic the cytotoxic effects of chemotherapy and to ameliorate the side effects of chemotherapy.*

*Physician should be aware of the importance of antioxidant in the nutrient of breast cancer patient and the supplement of synthetic antioxidant specially ALA the regenerator of other antioxidant.*

*Further studies have to be done in this field to see the feedback of decreasing the MDA in cancer patient 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.*

*More facilities should be done for researcher in order to do his research.*

*I ask companies that import the ALA to reduce the prize of the product so poor patient can by it.*

*IV of ALA can be more effect than oral and so we recommend to prescribe IV product*

*More attention has to be given for ALA from physician to prescribe it to minimize the suffer of cancer both as preventive of metastasis and as ameliorating to the side effects of chemotherapy*

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