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The effect of oral administration of omega fatty acids on lipid peroxidation and oxidative stress enzymes activity in patients with gastric adenocarcinoma after chemotherapy- A double blind clinical trial study

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Abstract

***Background & Aims*:** Gastric cancer is the fourth most common cancer and the second cause of death in the world. According to the study, gastric cancer cells have a relative resistance to chemotherapy. Therefore, the purpose of this study was to evaluate the effect of oral administration of omega fatty acids PUFAs) on lipid peroxidation and oxidative stress in gastric cancer in people with gastric cancer before and after chemotherapy.

***Materials & Methods*:** This is a double-blind clinical trial. The target group was gastric cancer patients who were first identified and under chemotherapy. Among them, 30 were selected and randomly assigned to two groups. they got. In the control group, the treatment was routine with cisplatin plus placebo. In the case group, treatment with cisplatin plus supplemental capsules of Natural Factors Ultimate-Omega Factors with a dose of 1200 mg per day was 3,600 mg Three tablets of 1200 mg (for three courses) started on horizons three weeks. Three samples of stomach biopsy were taken from all patients before and after chemotherapy. Lipid peroxidation was measured by the Thiobarbituric Acid method and the activity of superoxide dismutase and glutathione peroxidase enzymes by Randox kit. To compare the results, Independent- t-test and Mann-Whitney SPSS software.

***Results*:** In the case group, the percentage of lipid peroxidation of the gastric cancer tissue after omega-unsaturated fatty acids increased significantly (P value less than 0.0001). Omega fatty acids caused a significant reduction in the activity of the enzyme superoxide dismutase in the case group was statistically lower than the control group (P value less than 0.0001)

***Conclusion*:** The results of this study indicate that the use of omega fatty acids as a complement to cisplatin for controlling gastric cancer can be helpful in increasing the amount of oxidative stress and lipid peroxidation of the gastric cancer tissue in this The study was shown.

***Keywords*:** Gastric Cancer, PUFAs, Lipids Peroxidation, Oxidative Stress

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Introduction

Gastric cancer is the fourth most common cancer and the second leading cause of death in the world. According to global estimates, 93,000 cases of cancer are diagnosed annually, with 700,000 deaths. Areas at risk for this disease are in Iran, northern and northwest of the country (1).

Many cases of gastric cancer are diagnosed when the disease is in its advanced stages and surgery has a very limited role in therapy, and systemic chemotherapy is the only effective way of treating the disease, including the drugs used to chemotherapy for adenocarcinoma The stomach is used, platinum combinations alone or by combining three drugs with fluoropyrimidine plus methacrylate or antaroxylin (2). However, the response to treatment is very different in patients with gastric adenocarcinoma, and some tumors are completely resistant to this type of chemotherapy. Hence, one of the main barriers to the success of chemotherapy is increased resistance to this type of chemotherapy (3).

According to results published in recent years in different countries, it has been determined that gastric cancer is susceptible to chemotherapy and only a few cases of tumor growth are observed (4). Also, longevity with chemotherapy has not been well established, and in many cases, resistance to chemotherapy has been observed. This drug resistance will cause a number of patients to be treated vainly and, on the other hand, will bear high costs and excessive side effects. Essential unsaturated fatty acids, including Omega, are a group of fatty acids that the body cannot make and should be fed through food (5). Improper diets by eliminating or restricting the intake of some types of fat have led our bodies to face a shortage of them.

The shortage of chemicals that cause the body to lose its vital role in various vital tasks can lead us to a variety of problems. The lack of essential fatty acids and the inappropriate ratio of omega-6 to omega-3 cause serious discomforts, such as heart attack, cancer, Insulin resistance, asthma, lupus disease, schizophrenia, depression, postpartum depression, premature ageing, vascular arrest, obesity, diabetes, arthritis and Alzheimer's disease. (6, 7) The results of the research are the product of studies in cell culture media and animal studies. Therefore, the evidence in these studies does not accurately explain the mechanism of anticancer effects of omega-3 fatty acids and cannot be generalized to clinical specimens. Therefore, our study is the first study that examines the effects of omega-fatty acids on lipid peroxidation and gastric acid oxidative stress on human specimens.

Therefore, the main purpose of this study was to investigate the effect of oral administration of omega-3 fatty acids on the amount of lipid peroxidation, activity of superoxide dismutase enzymes and glutathione peroxidase in gastric cancer patients before and after chemotherapy.

Materials and Methods

This study was a double-blind clinical trial (before and after) with a target population of patients with gastric adenocarcinoma first identified and under chemotherapy. This study was a research project of the Center for Digestive and Liver Diseases Research Center of the University of Medical Sciences Tabriz, which was approved by the Ethics Committee of the Tabriz University of Medical Sciences during the license No. 199/128, dated 12/12/1392. The clinical trial was also registered at the Clinical Trials Control Center of the Ministry of Health and Medical Education under the number IRCT2014031016922N1.

For calculation of sample size according to the study type, the formula $N \geq S^2(\Sigma\alpha/2 + \Sigma\beta)/\ \delta^2$ was used. The sample volume was selected for each group of 33 samples so that the results could be generalized to the total number of people with gastric cancer.

Among the patients referring to the endoscopy clinic of Tabriz University of Medical Sciences who have been diagnosed with probable gastric cancer, 66 subjects were selected according to the goals and

conditions of this study. They were randomly selected and randomly divided into two groups of 33 We divided. After a definitive diagnosis of gastric cancer, 3 biopsy samples were removed from the tumor of the stomach, a third distal and transferred to the nitrogen tank. Patients were selected with written consent and with full knowledge of the subject. Subsequently, these patients have referred to an oncology specialist and the chemotherapy of these patients began under the supervision of this physician.

In the first two groups, the first group received 75mg / day cisplatin with a total of 75mg / m2 bodyweight every three weeks for intravenous administration without supplementation, and the second group received cis-platin treatment plus Natural Factors Ultimate-Omega Factors 1200mg tablets Supplements of omega-3, omega-6 and omega-9 fatty acids with Formulation: Fish Oil Blend 400 mg, Flaxseed Oil 400 mg, 400 mg of Borage Oil, daily 3600 mg (three tablets 1200 mg) for 3 courses (Hercules three weeks) (8). The pattern of fatty acids present in these capsules was determined by gas chromatography device made in Buck Corporation in America compared with relative standards. (Fig1), (Tab1) After this period, the endoscopy was followed up with compulsory follow-up therapy and the treatment of a tumour was followed by the gastric biopsy. Again, samples taken from patients were immediately transferred to the Nitrogen-tank.

Malondialdehyde (MDA) was measured in gastric cancer tissue by thiobarbituric acid. Measurement of the activity of superoxide dismutase and glutathione peroxidase enzyme was performed by manual kit and cholera metric of Randox 1 plant. Measurement of gastric mucosal protein was also performed by Laurie method.

Due to the independence of the studied groups, the mean of the results was calculated by SPSS software version 22 in each group, and then the distribution of the normal results was evaluated by Shapiro Wilks test.

The results of normal distribution were compared between two groups by Independent Sample t-Test and the results without the normal distribution were compared by non-parametric Mann Whitney test.

Results

Comparison of demographic data of patients in the studied groups:

In Table 2, demographic data of both groups of patients have been shown to be statistically compared and, as can be seen, the studied groups are well-matched with regard to age, sex, tumour location, tumour grade, and other information. In all cases, $p > 0.05$).

Comparison of lipid peroxidation and superoxide dismutase and glutathione peroxidase activity of gastric cancer in two groups:

As shown in Table 3, the mean for malondialdehyde (marker indicating lipid peroxidation) and the activity of superoxide dismutase and glutathione peroxidase enzymes (indicative of oxidative stress status) were compared in two groups.

Comparison of malondialdehyde level of gastric mucosal cancer tissue in two groups:

As shown in Fig. 2, malondialdehyde levels of gastric mucosal cancer tissue were compared in two groups. The level of malondialdehyde in the post-chemotherapy group with supplementation of omega-3 fatty acids increased significantly compared to the control group, ie patients with chemotherapy without supplementation, using Independent Sample T-Test It was found that the mean ± standard deviation of malondialdehyde in the case group was 15.85 ± 1.81 nmol / g and in the control group 8.86 ± 1.19 nmol / g and the p-value was less than 0.0001

Comparison of superoxide dismutase enzyme activity in gastric mucosal cancer tissue in two groups:

As shown in Figure 3, the level of enzyme activity of superoxide dismutase in the gastric mucus cancer tissue was compared in two groups. The level of activity

of this enzyme in the post-chemotherapy group with supplementation of omega-3 fatty acids compared to the control group, ie, patients with chemotherapy without supplementation showed a significant decrease. Using Independent Sample T-Test It was found that mean ± SD of the enzyme activity of superoxide dismutase in the case group was 9.44 ± 1.85 units per mg of protein (IU / mg protein) and in the control group was 17.21 ± 2.77 Per mg of protein (IU / mg protein) and the amount of p is less than 0.0001.

Comparison of glutathione peroxidase activity of gastric mucosal cancer tissue in two groups:

As shown in Fig. 4, the level of glutathione peroxidase activity of the gastric mucus cancer tissue was compared in the two groups. The level of activity of this enzyme in the post-chemotherapy group with supplementation of omega-3 fatty acids compared to the control group, ie, patients with chemotherapy without supplementation showed a significant decrease. Using Independent Sample T-Test It was found that the mean ± standard deviation of glutathione peroxidase activity in the case group was 15.5 ± 2.05 international units per mg of protein (IU / mg protein) and in the control group was 20.10 ± 1.76 units International is per mg of protein (IU / mg protein) and the amount of p is less than 0.0001.

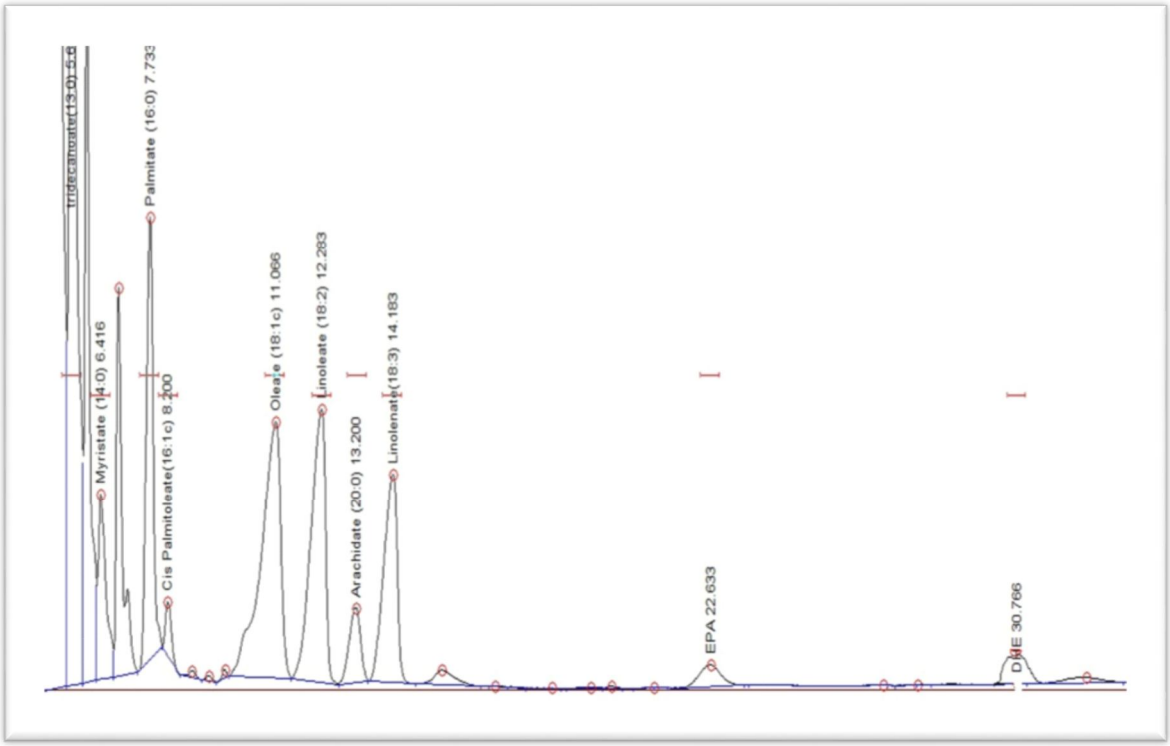

Fig 1. Chromatography shows the combination of fatty acid capsules "natural agents of the ultimate Omega Agent" to patients participating in this study. The level below the curve of each fatty acid was measured using Peaksimple 3.59 software and data was provided on a percentage scale.

Tab 1. The percentage of fatty acids in the supplementary capsules to the patients in the case group

Fatty acid	Myristate (14:0)	Palmitate (16:0)	Cis-palmitoleate (16:1c)	cis-oleate (18:1c)	cis-linoleate (18:2c)	Arachidate (20:0)	α-linolenate(18:3)	EPA	DHA
Percent(%)	3.44	17.20	1.65	28.71	22.76	4.83	16.04	2.67	2.90

Table 2. Demographic data of patients in the studied groups

Groups Clinical and Pathologic Factors	Control (n=17)	Case (n=17)	p-Value
Age(Years)(means±SD)	67.5±11.21	71.25±9.81	0.235
Gender			
Male (n=19)	9	10	0.695
Female (n=15)	8	7	
Tumor Size			
<4 cm (n=16)	7	9	0.759
>4 cm (n=18)	10	8	
Tumor Primary Location			
Upper (n=11)	5	6	0.714
Median (n=13)	6	7	0.790
Lower (n=10)	6	4	0.452
Stage Classification of Malignant Tumors (TNM)			
I (n=7)			
II (n=11)	4	3	
II (n=9)	6	5	0.089
IV (n=7)	4	5	
	3	4	
Systolic Blood Pressure (mmHg)	131.1±9.2	128.8±10.2	0.235
Diastolic Blood Pressure (mmHg)	85.1±7.1	79.2±7.9	0.985
Cigarette Smoking			
Current Smoking (n=12)	7	7	1
Non-Smoker (n=11)	6	5	0.714
Ex-Smoker (n=11)	4	5	0.697
Fasting Blood Sugar (mg/dl)	98.54±15.25	102.85±18.65	0.235
Cholesterol (mg/dl)	148.98±21.56	151.25±25.65	0.125
Triglyceride (mg/dl)	87.25±18.25	78.25±15.65	0.256
History of Family			
Yes (n=19)	10	9	0.73
No (n=15)	7	8	

Table 3. Information on the level of MDA and antioxidant activity of gastric cancer in the studied groups

Investigated Factors (Groups)	Mean ± standard deviation	confidence interval 95%	Test coefficient(F*)	P value
Malondialdehyde(control group)(nmol/g)	8.68±1.19	From -8.24 to -6.09	-13.60	<0.0001
Malondialdehyde(case group)(nmol/g)	15.85±1.81	From -8.24 to -6.09		
Superoxid dismutase enzyme(control group)(IU/mg protein)	17.21±2.77	From 6.12 to 9.41	3.646	<0.0001
Superoxid dismutase enzyme(case group)(IU/mg protein	9.44±1.85	From 6.12 to 9.41		
Glutathione peroxidase enzyme(control group)(IU/mg protein)	20.10±7.76	From 3.66 to 6.42	0.990	<0.0001
Glutathione peroxidase enzyme(case group)(IU/mg protein)	15.05±2.15	From 3.66 to 6.42		

* Independent Sample t-Test (Independent Sample T-Test). The mean of all factors was compared with the Independent Sample T-Test.

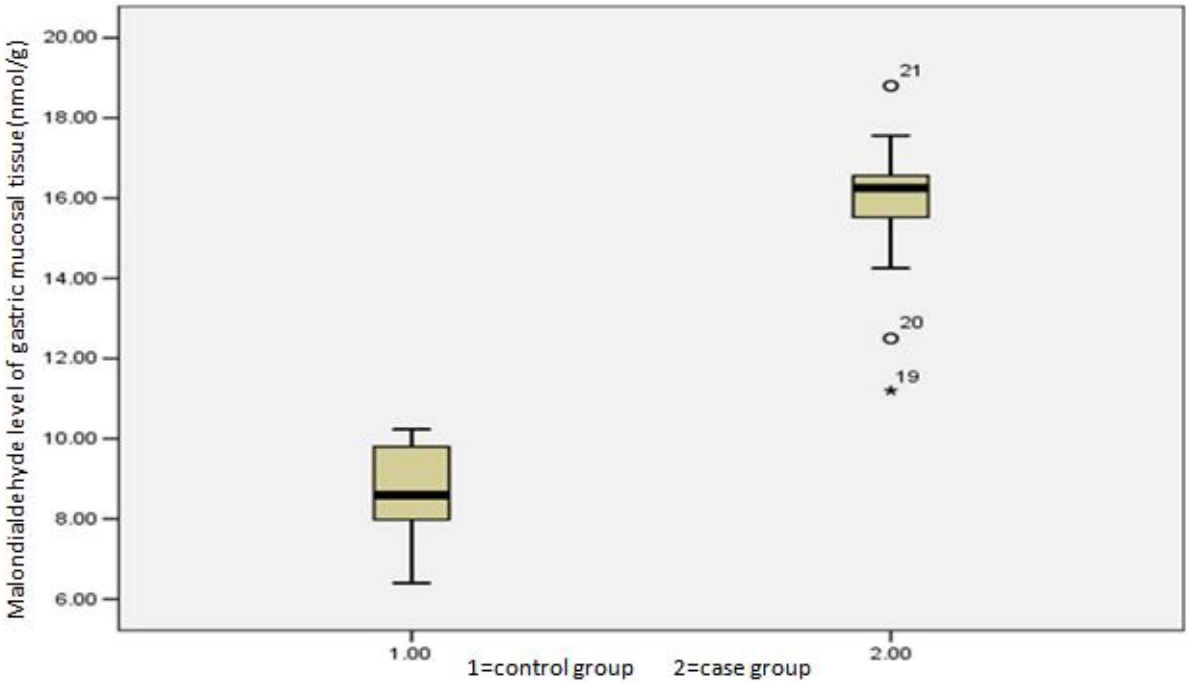

Fig 2. Box plot curve for comparing the mean malondialdehyde level of gastric cancer in two groups studied

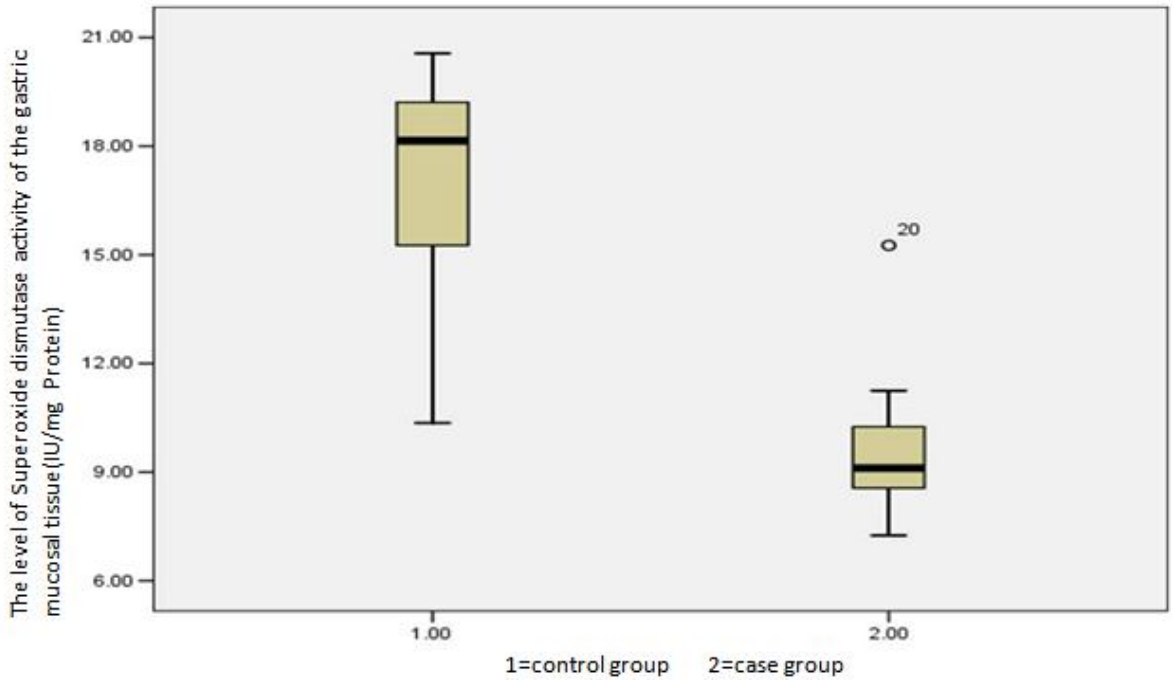

Fig 3. Box Plot curve for comparing the mean of enzyme activity of superoxide dismutase in gastric cancer tissue in two groups.

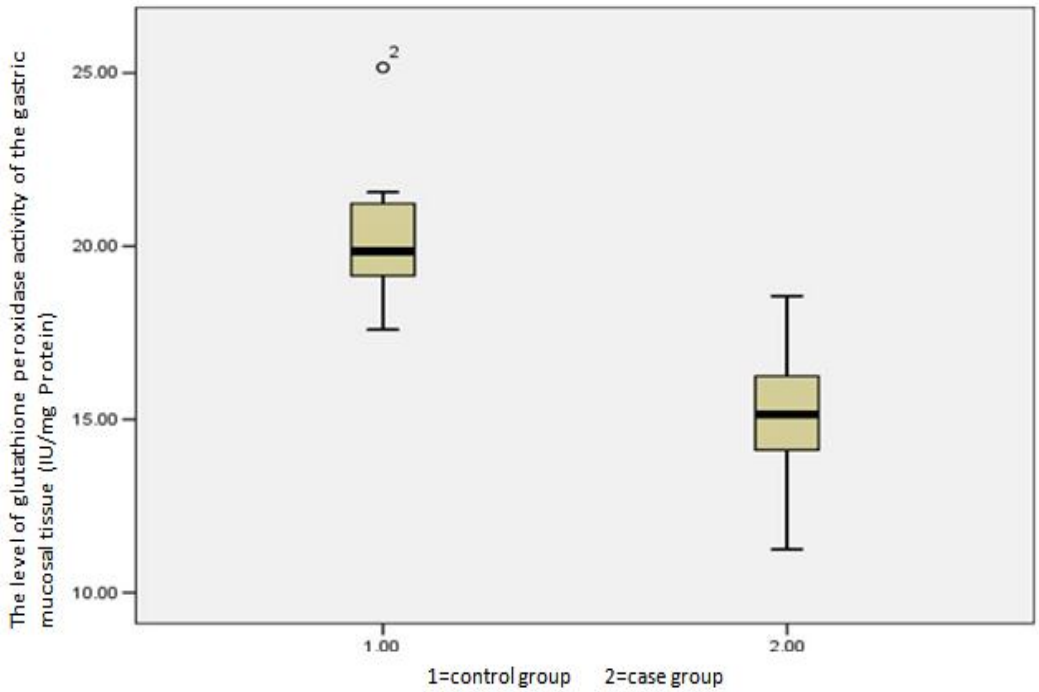

Fig 4. Box plot plot on the comparison of the mean level of glutathione peroxidase activity of the gastric cancer tissue in the two groups studie

Discussion

Chemotherapy is one of the standard therapies for the treatment of many cancers (9). While most of these therapies are not capable of inducing cell death or apoptosis in cancer cells, it is often associated with recurrence of cancer and ultimately leading to the death

of the patient (7). Gastric cancer treatment is also one of the major challenges in medical science (10). The purpose of this study was to evaluate the effect of oral administration of omega-3, 6 and 9 fatty acids on the oxidative stress of gastric mucosal tissue in patients with gastric adenocarcinoma under chemotherapy.

A study by Dai et al (11), Begin et al., (8) Das (12) showed that unsaturated fatty acids increase the peroxidation of lipids in cancer cells and, as a result of the accumulation of toxic products caused by peroxidation of lipids Ultimately, it causes cancer cells to die. In a Das study (13) IP et al. (14) showed that omega-unsaturated fatty acids decrease the antioxidant defence system and subsequently increase the reactive oxygen species in the cancerous cell.

In a study by Ramesh et al. (15) Ramesh and Das (16), Claire feld Wald (17) Duwie et al (18) and his colleagues comba in 2010 (19) The anticancer mechanisms of omega-3 fatty acids have been shown to inhibit the conversion of fatty acids to eicosanoids in cancer cells. The 1997 Cumar Dos study (20) showed that the anticancer mechanisms of omega fatty acids alter the nature and character of the cell membrane. Berkein et al. (21), Le et al. (22), showed that the anticancer mechanisms of omega-unsaturated fatty acids are associated with the induction of reticulum endoplasmic system stress in cancer cells. Coresta et al. (23) showed that the anticancer mechanisms of unsaturated fatty acids are associated with excessive hemostasis of ionised calcium in cancer cells. Shirota et al. (24), Liu et al(25), Mengaz and colleagues in 2006 (26), Narayan et al. (27) Showed that the anticancer mechanisms of omega-unsaturated fatty acids, ultimately, increase the cell death rate in cancer cells.

Also, in a study by Nassar et al(28), Chilick et al. (29), Liang et al. (30), showed that omega-3 and omega-6 fatty acids had a toxic effect on cancer cells They cause the oxidative stress and decrease the growth of cancer cells. The results of this study are quite consistent with the results of this study. Most often, the results of the studies and the mechanisms mentioned above are in cell culture media and laboratory animal studies. For this reason, the evidence in these studies does not accurately describe the mechanism of the anti-cancer effects of omega-3 fatty acids and can be generalized to the sample Clinical and results of our study are not. Therefore, we can say that our study is the first study to examine the effects of omega-3 fatty acids on human samples.

As the results of our study, omega-3, 6 and 9 fatty acids supplementation with cisplatin in patients with gastric adenocarcinoma has been shown to increase lipid peroxidation and increase the activity of superoxide dismutase and glutathione peroxidase enzymes in It is clearly shown in Figures 2, 3 and 4 of this article that the omega-3 fatty acids may be able to increase by increasing the amount of stomach cancer Oxidative stress, cell death in gastric cancer cells Activate more than once that chemotherapy is done with cisplatin alone. Omega fatty acids can increase the level of oxidative stress in stomach tissue in patients with gastric adenocarcinoma under chemotherapy. An obscure point in this study is whether the toxic effects of omega-2 fatty acids on healthy cells are the same.

In a study by Dai et al (11), Begin et al (8) and Das et al(12), it has been suggested that the toxic effects of these fatty acids are selectively It is only on cancer cells and has no effect on very healthy cells or very little, and if the dosage of these fatty acids is greater than 40 µg per ml per 105 cells, it can also be toxic to healthy cells. Therefore, according to the results of these studies, we chose the dosage for selected patients in this very moderate (ie 3600 mg/day or better 1200 mg/day for each fatty acid), so only the effects Observe the toxicity of cancer cells. This is not unconstrained, and one of the drawbacks of our study is that this study should be done on the healthy stomach tissue that should be considered in later studies of this issue.

Conclusion

According to the results of this study and various studies, it seems that the effect of oral administration of omega-3, 6, and 9 fatty acids, along with common chemotherapy drugs used to control gastric cancer, may be helpful in increasing the amount of oxidative stress This study showed that it could be a hopeful tip for reducing drug resistance in patients with gastric adenocarcinoma.

References

1. Cancer today [Internet]. [cited 2019 Jan 27]. Available from: http://gco.iarc.fr/today/home
2. Dicken BJ, Bigam DL, Cass C, Mackey JR, Joy AA, Hamilton SM. Gastric adenocarcinoma: review and considerations for future directions. Ann Surg 2005;241(1):27–39.
3. Fan T-J, Han L-H, Cong R-S, Liang J. Caspase family proteases and apoptosis. Acta Biochim Biophys Sin (Shanghai) 2005;37(11):719–27.
4. Reed J.C. Mechanisms of apoptosis. Am J Pathol 2000; 157(5): 1415-30.
5. Wiktorowska-Owczarek AM, Berezinska J.Z. Nowak, PUFAs: structures, metabolism and functions. Adv Clin Exp Med 2015; 24(6): 931-41.
6. Papackova Z, Cahova M. Fatty acid signaling: the new function of intracellular lipases. Int J Mol Sci 2015;16(2):3831–55.
7. Bang Y-J, Kim Y-W, Yang H-K, Chung HC, Park Y-K, Lee KH, et al. Adjuvant capecitabine and oxaliplatin for gastric cancer after D2 gastrectomy (CLASSIC): a phase 3 open-label, randomised controlled trial. Lancet 2012;379(9813):315–21.
8. Bégin ME, Das UN, Ells G, Horrobin DF. Selective killing of human cancer cells by polyunsaturated fatty acids. Prostaglandins Leukot Med 1985;19(2):177–86.
9. Li J, Qin S, Xu J, Guo W, Xiong J, Bai Y, et al. Apatinib for chemotherapy-refractory advanced metastatic gastric cancer: results from a randomized, placebo-controlled, parallel-arm, phase II trial. J Clin Oncol 2013;31(26):3219–25.
10. Kim HS, Kim HJ, Kim SY, Kim TY, Lee KW, Baek SK, et al. Second-line chemotherapy versus supportive cancer treatment in advanced gastric cancer: a meta-analysis. Ann Oncol 2013;24(11):2850–4.
11. Dai J, Shen J, Pan W, Shen S, Das UN. Effects of polyunsaturated fatty acids on the growth of gastric cancer cells in vitro. Lipids Health Dis 2013;12:71.
12. Das UN. Tumoricidal action of cis-unsaturated fatty acids and their relationship to free radicals and lipid peroxidation. Cancer Lett 1991;56(3):235–43.
13. Das UN, Prasad VV, Reddy DR. Local application of gamma-linolenic acid in the treatment of human gliomas. Cancer Lett 1995;94(2):147–55.
14. Ip C. Controversial issues of dietary fat and experimental mammary carcinogenesis. Prev Med 1993;22(5):728–37.
15. Ramesh G, Das UN, Koratkar R, Padma M, Sagar PS. Effect of essential fatty acids on tumor cells. Nutrition 1992;8(5):343–7.
16. Ramesh G, Das U. Effect of cis-unsaturated fatty acids on Meth-A ascitic tumour cells in vitro and in vivo. Cancer Letters 1998; 123(2): 207-14.
17. Klurfeld DM, Bull AW. Fatty acids and colon cancer in experimental models. Am J Clin Nutr 1997;66(6 Suppl):1530S-1538S.
18. Das UN, Ramadevi G, Rao KP, Rao MS. Prostaglandins and their precursors can modify genetic damage-induced by gamma-radiation and benzo(a)pyrene. Prostaglandins 1985;29(6):911–20..
19. Comba A, Maestri DM, Berra MA, Garcia CP, Das UN, Eynard AR, et al. Effect of ω-3 and ω-9 fatty acid rich oils on lipoxygenases and cyclooxygenases enzymes and on the growth of a mammary adenocarcinoma model. Lipids Health Dis 2010;9:112.
20. Sravan Kumar G, Das UN. Cytotoxic action of alpha-linolenic and eicosapentaenoic acids on myeloma cells in vitro. Prostaglandins Leukot Essent Fatty Acids 1997;56(4):285–93.
21. Berquin IM, Edwards IJ, Kridel SJ, Chen YQ. Polyunsaturated fatty acid metabolism in prostate cancer. Cancer Metastasis Rev 2011;30(3–4):295–309.

22. Lu X, Yu H, Ma Q, Shen S, Das UN. Linoleic acid suppresses colorectal cancer cell growth by inducing oxidant stress and mitochondrial dysfunction. Lipids Health Dis 2010;9:106.
23. Corsetto PA, Montorfano G, Zava S, Jovenitti IE, Cremona A, Berra B, et al. Effects of n-3 PUFAs on breast cancer cells through their incorporation in plasma membrane. Lipids Health Dis 2011;10:73.
24. Shirota T, Haji S, Yamasaki M, Iwasaki T, Hidaka T, Takeyama Y, et al. Apoptosis in human pancreatic cancer cells induced by eicosapentaenoic acid. Nutrition 2005;21(10):1010–7.
25. Liu W-H, Chang L-S. Arachidonic acid induces Fas and FasL upregulation in human leukemia U937 cells via Ca2+/ROS-mediated suppression of ERK/c-Fos pathway and activation of p38 MAPK/ATF-2 pathway. Toxicol Lett 2009;191(2–3):140–8.
26. Monjazeb AM, High KP, Connoy A, Hart LS, Koumenis C, Chilton FH. Arachidonic acid-induced gene expression in colon cancer cells. Carcinogenesis 2006;27(10):1950–60.
27. Narayanan BA, Narayanan NK, Reddy BS. Docosahexaenoic acid regulated genes and transcription factors inducing apoptosis in human colon cancer cells. Int J Oncol 2001;19(6):1255–62.
28. Nassar BA, Das UN, Huang YS, Ells G, Horrobin DF. The effect of chemical hepatocarcinogenesis on liver phospholipid composition in rats fed N-6 and N-3 fatty acid-supplemented diets. Proc Soc Exp Biol Med 1992;199(3):365–8.
29. Celik GE, Erkekol FO, Misirligil Z, Melli M. Lipoxin A4 levels in asthma: relation with disease severity and aspirin sensitivity. Clin Exp Allergy 2007;37(10):1494–501.
30. Liang G, Pu Y, Yin L, Liu R, Ye B, Su Y, et al. Influence of different sizes of titanium dioxide nanoparticles on hepatic and renal functions in rats with correlation to oxidative stress. J Toxicol Environ Health Part A 2009;72(11–12):740–5.