The Effect of High Poly Unsaturated Fatty Acid (PUFA) Dietary Supplementation on Inflammatory Status of Patients with Advanced Cervical Cancer on Radiation Treatment

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ABSTRAK

Tujuan: *untuk mengetahui pengaruh pemberian suplementasi nutrisi tinggi PUFA pada status inflamasi pasien kanker serviks stadium lanjut.* ***Metode:*** *uji klinis acak tersamar ganda pada pasien kanker serviks stadium lanjut yang sedang menjalani terapi radiasi eksterna di Departemen Radioterapi RS. Cipto Mangunkusumo, Jakarta, periode April 2013-April 2014. Status inflamasi dinilai dari kadar prostaglandin* E_2 *(*PGE_2*) serum melalui metode Elisa. Suplementasi nutrisi mengandung isokalori dan isoprotein, serta mengandung PUFA dengan rasio asam lemak (AL) n-6 : n-3 = 1,27 : 1 dan tanpa PUFA. Data dianalisis dengan Uji statistik Saphiro-Wilk, T test tidak berpasangan dan Mann-Whitney.* ***Hasil:*** *secara statistik tidak didapatkan perbedaan bermakna kadar* PGE_2 *antara kelompok perlakuan dan kontrol (p = 0,127), tetapi secara klinis didapatkan perbedaan yang nyata pada kadar* PGE_2*, yaitu kelompok perlakuan mengalami penurunan sebesar 8,9%, sedangkan kelompok kontrol mengalami kenaikan sebesar 28,1%.* ***Kesimpulan:*** *pemberian suplementasi nutrisi diperkaya PUFA mampu menurunkan status inflamasi pada pasien kanker serviks stadium lanjut. Penurunan kadar* PGE_2 *akan menurunkan ketahanan hidup sel kanker, sehingga pemberian nutrisi diperkaya PUFA dengan rasio AL n-6 : n-3 = 1,27 :1 bersamaan terapi radiasi dapat meningkatkan respon tumor terhadap radiasi.*

Kata kunci: *kanker serviks, asam lemak n-3 dan n-6 , kadar* PGE_2*.*

ABSTRACT

Aim: *to identify the effect of high-PUFA dietary supplementation on inflammatory status of patients with advanced cervical cancer.* ***Methods:*** *a randomized double-blind clinical trial was conducted in patients with advanced cervical cancer who had undergone external radiation therapy at Department of Radiotherapy, Cipto Mangunkusumo Hospital, Jakarta, between April 2013 and April 2014. The inflammatory status was evaluated based on serum prostaglandin* E_2 *(*PGE_2*) levels using ELISA method. The dietary supplementation was isocaloric, isoprotein and contained PUFA with a ratio of ω-6: ω-3 fatty acid = 1.27:1 and supplementation without PUFA. Data were analyzed with statistical tests, including Shapiro-Wilk test, independent T-test and Mann–Whitney test.* ***Results:*** *there was statistically no significant difference on* PGE_2 *level between* *treatment and control groups (p=0.127). However, there was clinically significant difference, in which the treatment group had reduced PGE_2 level by 8.9%; while the control group had increased level by 28.1%.* ***Conclusion:*** *dietary supplementation enriched with PUFA can reduce inflammatory status in patients with advanced cervical cancer. Reduced PGE_2 level will lower the survival of cancer cells; therefore dietary supplementation enriched with PUFA with a ratio of ω-6 : ω-3 fatty acid = 1.27 : 1 along with radiation therapy may improve tumor response to radiation.*

Key words: *cervical cancer, ω-3 PUFA, ω-6 PUFA, PGE2 level.*

INTRODUCTION

Approximately 75% patients with cervical cancer turn to hospital at advanced stage (>IIB stage). The five-year survival rate for advanced cervical cancer is about 0-40%.[1] The main treatment for this cancer is radiation or chemoradiation therapy. A combined treatment with chemotherapy apparently does not give a better treatment response within the 3-month follow up; moreover, it even causes more severe side effects than radiation therapy alone.[2]

Some in vivo and in vitro studies have provided evidences that ω-3 fatty acid (FA) has pro-apoptosis, anti-inflammatory, anti-proliferative and anti-angiogenesis effects.[3-5] Biological effects of ω-3 FA is often associated with the competitive inhibitory effect on eicosanoid products produced by ω-6 FA. Eicosanoids derived from ω-6 FA, linoleic acid (LA) or arachidonic acid (AA), have pro-inflammatory and tumor angiogenesis properties; while eicosanoids originated from ω-3 FA, i.e. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have anti-inflammatory properties and does not stimulate angiogenesis.[3,4] The molecular mechanism of anti-tumor effect of ω-3 FA has various pathways including the COX-2-PGE_2 pathway. Many studies on cancer have demonstrated increased COX-2 expression both in pre-cancer lesion and invasive cancer. Increased COX-2 expression and increased production of eicosanoids such as PGE_2 will activate various inflammatory factors and growth factors, which will bring bad effects on treatment response.[6,7]

Modern eating habits nowadays together with increased industrial techniques for production of vegetable oils and animal fats have caused high ω-6 FA intake and deficiency of ω-3 FA intake. The ratio of ω-6 : ω-3 FA intake now in western countries is about 10-25 : 1.[8-10] The food sources of ω-6 FA are vegetable oils such as corn oil, safflower, soybean oils and animal meats. Most of ω-3 fatty acids are found in fish oils in the form of EPA and DHA; while α-linolenic acid (ALA) is found in vegetable oils such as canola, flaxseed, linseed soybean oil and green vegetables.[9,10] Daily requirements of ω-3 fatty acid for patients with cancer has not been confirmed, however, the recommendation is about 1.6-1.1 g for a healthy adult.[11] Based on clinical trials, we know that in order to against cancer growth, an intake with ratio of ω-6 : ω-3 fatty acid as much as 1 : 1 or 2 : 1 is necessary.[3,4]

Our study was aimed to investigate whether a dietary supplementation enriched with high-ω-3 fatty acid intake together with radiation therapy can reduce the inflammatory status in patients with advanced cervical cancer, therefore, it is expected that it may enhance tumor response to radiation therapy.

METHODS

A randomized double-blind clinical trial was conducted in patients with advanced cervical cancer of stage II-IIIB squamous cell carcinoma and who had only received radiation therapy at Department of Radiotherapy, Cipto Mangunkusumo Hospital. Data collection had been performed since April 2013 until the required sample size had been completed. The inclusion criteria were: patients aged ≥18 years, with Karnofsky Index of ≥60%, with BMI of ≥16 kg/m^2, who could and were willing to drink milk and willing to sign the informed consent form. The exclusion criteria were those with liver dysfunction (SGOT >38 U/L, SGPT >41 U/L), renal dysfunction (ureum serum level >50 mg/dL, creatinine serum level >1.4 mg/dL), metabolic disorder (random blood glucose level ≥200 mg/dL) and fat malabsorption. The samples were collected using consecutive sampling and randomization was performed using sealed envelopes. The subjects were randomized according to the number on the envelopes into 2 groups, i.e. the treatment and control groups. Dietary supplementation was provided in the form of milk with A and B labeling. The products were isocaloric and isoprotein, but one of them was enriched with PUFA. Products containing PUFA were concealed by the manufacturer. The laboratory work-up measuring serum PGE_2 level was performed before and after a complete treatment of external radiation. The measurement was done using ELISA method. The subjects were considered drop out when they did not follow study protocol or if there was a delay of radiation treatment in 7 consecutive days or if they took the dietary supplementation of <80%. The analysis of dietary intake during the treatment was conducted using food-recall and food-record method. In order to control the compliance of the subjects during the study, the milk supplementation should be taken 3 times, i.e. before and after radiation treatment, which were taken at the site of the study, and the third was taken at home (the milk package should be returned and the subjects should fill in the diary of dietary supplementation). The study protocol has been approved by Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia - Cipto Mangunkusumo Hospital.

Statistical Analysis

Data were edited, coded and processed using a computer program of Statistical Program for Social Science (SPSS) version 11.5. Univariate and bivariate analysis were performed. Normality of data distribution was tested using Saphiro-Wilk test. If the data distribution was normal (p>0.05), we used mean value and standard deviation. If the distribution was not normal (p<0.05), we used median and range. In the analysis, the difference of PGE_2 level between the treatment group and control group was evaluated; if the data of the treatment and control group had normal distribution, unpaired t-test was utilized; while if one of or both data had abnormal distribution, we used Mann-Whitney test.

RESULTS

The study lasted for 12 months, i.e. between April 2013 and April 2014. Based on the inclusion and exclusion criteria, there were 45 patients with advanced cervical cancer, who was at stage IIB – IIIB and had received radiation treatment. At the end of study, there were 31 subjects who had completed the study protocol; while 14 subjects were considered drop out. There were 16 subjects who had received product A in the treatment group; while 15 subjects who had received product B were included in the control group.

The distribution of subjects before treatment (**Table 1**) did not show significant difference between the treatment and control group. Most subjects aged ≥45 years. The nutritional status of study subjects, which was based on BMI according to WHO classification for Asia Pacific population, was still in normal category (normal BMI: 18.5-22.9 kg/m^2); some of them were even overweight. Based on the stage of illness, there were 21 subjects (68%) who were at the stage of IIIB.

Table 1. Subject distribution based on age, nutritional status and disease stage

	Treatment group (n=16)	Control group (n=15)	p value
Age (Years)			
- <45 years (n)	7	4	0.320a
- ≥45 years (n)	9	11	
BMI			
- Underweight (n)	2	-	
- Normoweight (n)	7	8	1.000a
- Overweight (n)	7	7	
Stage according to FIGO classification			
- IIB (n)	6	4	0.704a
- IIIB (n)	10	11	

[a] Uji Chi Square

The dietary intake of study subjects during their external radiation treatment was evaluated using the diary which was written by the patients themselves. We found a significant difference in terms of ω-3 FA intake and the intake ratio of ω-6 : ω-3 FA, i.e. the treatment group had higher ω-3 FA intake compared to the control group.

We found reduced PGE_2 level by 8.9% in the treatment group; while the control group had increased level by 28.1%; however, there was statistically no significant difference (**Table 3**). A value of NNT (number needed to treat) of 11.11, which means that we need to treat 11 patients with interventional product/PUFA in order to reduce the PGE_2 level.

Table 2. Dietary intake of ω-6 and ω-3 FA and Intake Ratio of ω-6 : ω-3 FA during Treatment

	Treatment Group (n = 16)	Control Group (n = 15)	P value
Dietary intake of ω-6 FA (g)	2.07 (0.65-7.68)	2.18 (0.68-3.53)	0.520a
Dietary intake of ω-3 FA (g)	2.72 ± 0.31	0.51 ± 0.16	<0.001b
Intake ratio of ω-6 : ω-3 FA	0.83 (0.33-2.53)	3.60 (1.94-9.58)	<0.001a

[a] Mann Whitney Test; [b] Independent T-Test
ω-3 FA = ω-3 fatty acid; ω-6 FA = ω-6 fatty acid

Table 3. Altered serum PGE_2 level before and after treatment

	Treatment Group (n = 16)	Control Group (n = 15)	P value
PGE2 level (pg/ml)			
- Before radiation	804±803.8	545.1 (219-1454.1)	0.682[a]
- After radiation	507.3±187.1	435,9 (220.3-4623.9)	0.800[a]
Altered PGE2 level (pg/ml)	81.4 (-2624.7-213)	23,1 (-667.2-3169.8)	0.151[a]
Altered PGE2 level (%)	8.9±42.9	28.1±80.2	0.127[b]

[a] Mann Whitney Test; [b] Unpaired T-test
PGE_2 = Prostaglandin E_2

DISCUSSION

Polyunsaturated fatty acid (PUFA) is an essential fatty acid, which cannot be synthesized by the body and must be obtained from food sources. There are two types of essential fatty acids (FA), i.e. ω-6 and ω-3 FA. In daily intake, the major food sources for ω-6 FA are in the form of linoleic acid (LA) and arachidonic acid; while the ω-3 FA is obtained in the form of α-linolenic acid (ALA), EPA and DHA. Those fatty acids are structural components of phospholipid in membrane cell, which have important function to maintain the membrane fluidity, signaling and interaction between cells. Moreover, the fatty acids are precursors for producing eicosanoids, i.e. prostaglandin (PG), thromboxane (TX) dan leukotriene (LT) with the help of Cyclooxygenase (COX) and Lipoxygenase (LOX) enzymes.[12] Eicosanoids are hormone-like substances which are originated from oxygenation of essential fatty acids containing 20 carbon chains, i.e. the AA (ω-6 FA) and EPA (ω-3 FA). Eicosanoids also have roles in inflammatory response, controlling immune response, platelet aggregation, smooth muscle contraction, cell growth and differentiation. A balanced ratio of ω-6 and ω-3 intake of 1-4 : 1 is essential to maintain homeostasis as well as normal growth and development.[10,12]

In addition to reducing PGE_2 through its competition with AA as the substrate of COX enzyme, ω-3 FA also can inhibit signals for PGE_2 receptor on cell membrane; thus, it reduces inflammatory signal. An in vivo study conducted by Ruan and So[13] provided evidences that fish oil (particularly DHA) can significantly inhibit PGE_2 signal by inhibiting the binding of PGE_2 with EP_1 receptor and it is also assumed that it is also applied for other PGE_2 receptors, i.e. the EP_2, EP_3 and EP_4.

Theoretically, reduced PGE_2 level will lower the survival of cancer cells; while in contrast, increased PGE_2 will also increase the survival. Prostaglandin E_2 and its receptors have important role in cancer development, proliferation, apoptosis, angiogenesis, immunosuppression, tumor invasion and metastases.[6] The magnitude of prostaglandin effect depends on its concentration in the body. The effect of ω-6 : ω-3 level ratio on the composition of fatty acids in the plasma and prostanoid synthesis has been studied by Amira et al.[14] in an in vivo study, which demonstrated that a dietary intake with fatty acid ratio of ω-6:

ω-3 = 1 : 1 may result in a higher ω-3 FA level ($p<0.05$), a lower FA ratio of ω-6 : n-e ($p<0.05$) and lower level of AA ($p<0.05$) compared to the ratio of 6:1 or 30:1; moreover, they also found significant increase of plasma PGE_2 level in the group with a ratio of 30 : 1.

It indicates that the ratio of FA intake ω-6 : ω-3 affects the profile of plasma fatty acid, as well as the development of $PGF_2\alpha$ and PGE_2. Kobayashi at al.[8] and Calviello et al.[15] also demonstrated that a dietary intake with FA ratio ω-6 : ω-3 = 1:1 might reduce the expression of COX-2 and PGE_2 level. The reduced PGE_2 level was then followed by reduced proliferation and increased apoptosis of cancer cells which were impregnated in rats.

Our study showed that high-PUFA dietary supplementation with ω-6 : ω-3 FA ratio of 1.27: 1 during treatment using external radiation can reduce PGE_2 level as much as 8.9 ± 42.9% in the treatment group; while in the control group which did not receive PUFA, there was an increased PGE_2 of 28.1 ± 80.2% although statistically the difference was not significant.

CONCLUSION

Dietary supplementation of ω-3 FA in patients with advanced cervical cancer may help to reduce the inflammation. It is suggested that patients with cancer should increase their high ω-3 FA dietary intake or having a dietary supplementation enriched with ω-3 FA; therefore, the reduced inflammatory status may enhance tumor response to radiation therapy.

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