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ORIGINAL RESEARCH REPORT

Omega 3 supplementation and NF-kB levels in children with acute lymphoblastic leukemia (ALL) receiving induction phase chemotherapy

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ABSTRACT

Background: Several evidence showed that omega 3 fatty acid inhibit NF-kB at any type of cancers. But the effects of omega 3 supplementation to the NF-kB level on children with ALL is still limited. **Purpose:** Analyse omega 3 fatty acids supplementation at NF-kB level on children with ALL receiving induction phase of chemotherapy and the outcomes of chemotherapy. **Methods**: an open label randomized pre- and post-test control group design clinical study, conducted at October 2014 until March 2015 at inpatient unit of Haematology Oncology ward Dr. Soetomo Distric Hospital Surabaya. Subjects were divided into 2 groups: treatment group receiving omega 3 softgel 1000 mg orally once a day at the first day of the induction phase were conducted until the last day of the induction phase; and control group. NF-kB were measured before (day 0) and after induction phase (day 42) of chemotherapy, using Human NF-kB (Elabscience®) in ng/ml and analysed statistically using paired sample t test and independent sample t-test using SPSS ver 21 (IBM, US). **Results**: A total of 22 subjects were included in this study, divided into 11 as the treatment group and 11 as the control group. NF-kB level were reduced at both group; 3.10 ± 4.308 ng/ml to 0.76 ± 0.762 ng/ml at the treatment group and 8.20 ± 11.130 ng/ml to $1.748 \pm$ SD 3.072 ng/ml. There was no significant difference of NF-kB levels before and after the induction phase after omega 3 supplementation at the treatment group (p=0.065). **Conclusion:** There is no significant difference of NF-kB level between the subjects receiving omega 3 supplementation and the subjects who did not had omega 3 supplementation, and there is no relationship between NF-kB levels with chemotherapy outcomes.

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Highlights

1. NF-kB regulating cellular apoptosis and growth processes in tumorigenesis.
2. Activated NF-kβ complexes exacerbate the tumorigenesis in acute lymphoblastic leukemia (ALL).
3. Omega 3 fatty acid inhibit NF-kB at any type of cancers

BACKGROUND

It is well known that the activation of NF-kB signalling pathways lead to cascade of inflammatory responses (Allam-Ndoul *et al.*, 2016), which plays an important role in inflammatory, stress response and tumor cell resistance to apoptosis. NF-kB is activated by various number of stimuli, such as cytokines, mitogens, bacteria or chemotherapeutic agents (Fahrmann, 2013). NF-kβ was activated in various solid tumors and hematological malignancy leading to dysregulation of inflammatory state. It was found that pro-inflammatory cytokine were higher in children with acute lymphoblastic leukemia (ALL) than normal children, including tumor necrosis factor α (TNF- α), interleukin-6 (IL-6), IL-8, monocyte chemoattractant protein-1 (MCP-1), IL-10, T helper 1 (interferon-γ and IL-12), IL-1β, IL-2, IL-4, IL-13, and IL-17, while the transforming growth factor β (TGFβ) were low (Pérez-Figueroa *et al.*, 2016). The activation this pathways will cause the progression of lymphomas and leukemias rapidly (Gilmore, 1999).

Omega 3 fatty acids contain anti-inflammatory properties with a therapeutic efficacy to alleviate the pro-inflammatory markers such as resolvins, maresins and protectins (Skarke *et al.*, 2015), although the molecular mechanisms is still unclear. DHA was sensitive toward peroxidation, yielding bioactive lipids (Musiek *et al.*, 2008) with beneficial effects. The study investigating omega 3 supplementation at the culture medium of THP1 macrophage cells with the concentration of 50 µM and 10 µM, EPA + DHA at 50 µM showed there were a reduction in the expression of genes related to NF-kβ pathways (MAPK, AKT1 and NFKB) (Allam-Ndoul *et al.*, 2016). Several study conducted in human showed that omega 3 supplementation ameliorate and prevent colorectal cancer (Lee *et al.*, 2017), breast cancer (Fabian, Kimler and Hursting, 2015) and prostate cancer (Gu *et al.*, 2013), but the evidence of omega 3 supplementation in children with ALL is still limited.

OBJECTIVE

We conducted study to analyse the effect of omega 3 supplementation to NF-kβ level in children with ALL receiving induction phase of chemotherapy.

MATERIAL AND METHOD

The study was an open label randomized pre and post-test control group design clinical study, conducted at October 2014 until March 2015 at inpatient unit of Hematology Oncology ward Dr. Soetomo Distric Hospital Surabaya. Subjects were divided into 2 groups: treatment group receiving omega 3 softgel 1000 mg orally once a day at the first day of the induction phase until the last day of the induction phase; and control group receiving placebo.

Samples

Number of samples were calculated below

$$N_{total} = \frac{(z\frac{1}{2}\alpha + z\beta)^2 . \pi^2 .}{(\delta_2)^2} \longrightarrow N_{total} = \frac{(1.96+1.282)^2 . 1}{1} = 11$$

$\beta = 0{,}20 \rightarrow z\beta = 1{,}282$

$\pi^2 / \delta_{2} = 1$ (paired samples)

The minimum number of samples for each group was 11 subjects, so the total samples were 22 subjects. The samples were taken with consecutive sampling (every subject suitable with inclusion criteria was taken as a samples), and grouped with randomisation.

Blood samples

NF-kB were measured before (day 0) and after induction phase of chemotherapy by taking out vena blood 3 ml for centrifuge at 3,000 rpm for 15 minutes to get the blood serum. NF-kB level were measured using Human NF-kβ (Elabscience®) in ng/ml.

Statistical analysis

The result of NF-kβ level were analysed statistically using paired t test or Wilcoxon U Test, depend on the distribution and test of normality (Shapiro Wilk) using SPSS ver 21 (IBM, US).

Ethical Consideration

The research was considered to be ethically appropriate by the Health Research Ethics Committee of RSUD Dr. Soetomo Surabaya with ethical eligibility certificate number 151/Panke.KKE/II/2015.

RESULTS

A total of 22 subjects were concluded in this study, divided into 11 subjects as treatment group, and 11 subjects as control group with male:female ratio were 2:1, the data was distributed homogenously and normally.

Table 1. Subject's characteristics (n=22)

Subject's characteristics	Intervention group (n=11) Mean ± SD	Control (n=11) Mean ± SD	P value
Sex, n(%)			0.180[1]
- Male	6 (54.5)	9 (81.8%)	
- Female	5 (45.5%)	2 (18.2)	
Average age	66.36 (45.63)	43.6 (30.92)	0.178[2]
Nutritional status, n(%)			0.455[1]
- Good nutritional status	5 (45.5)	8 (72.7%)	
- Wasted	3 (27.3)	3 (27.3)	
- Severely wasted	2 (18.2)	0	
- Obesity	1 (9)	0	
ALL diagnosis, n(%)			0.147[1]
- ALL-L1	11 (100)	9 (81.8)	
- ALL-L2	0	2 (18.2)	
Hemoglobin (g/dl)	9.7 (2.02)	9.9 (3.64)	0.875[2]
Leucocyte (/mm³)	5,200 (2,100-27,100)	5,200 (1,400-57,000)	1.000[2]
Thrombocyte (/mm³)	150,000	150,000	0.609[2]

[1]Fischers exact; [2]independent sample T-test

NF-κB level were reduced after the induction phase whether at the treatment group (deviation was -2.340 ± SD 3.862 ng/ml), and control group (deviation was -6.457 ± SD 10.315 ng/ml). Even the deviation of the control group was greater than the treatment group, but there was no significant difference in both groups, as summarized in **Table 2.**

Table 2. Difference of NF-κB level before and after induction phase between treatment and control group (n=22)

Induction phase	Average of NF-κB level (SD) at the treatment group	Average of NF-κB level (SD) at the control group	p value
Before (ng/ml)	3.10 (4.308)	8.20 (11.130)	0.172
After (ng/ml)	0.76 (0.762)	1.748 (3.072)	0.662
Deviation (ng/ml)	-2.340 (3.862)	-6.457 (10.315)	0.237

There was no significant difference of NF-κB before and after the induction phase in the treatment group (p=0.072) and control group (p=0.065). There were 9 of 11 subjects (81.8%) with remission outcome after underwent the induction phase in the treatment group, while 8 of 11 subjects (72.7%) with remission in the control group, but there was no significant difference of the induction phase outcomes between two groups (p=1.000).
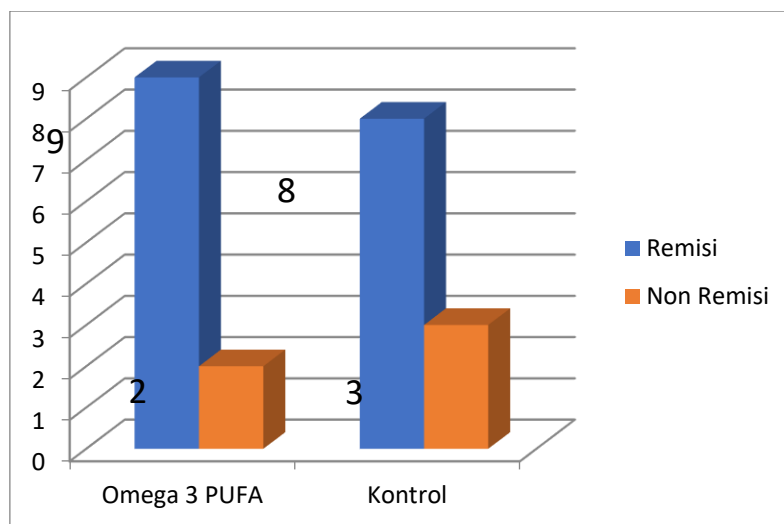
Figure 1. Induction phase outcomes at the treatment and control group

There was no significant difference between the reduction of NF-κB level (before and after induction phases) to the chemotherapy outcomes in treatment group (p=0.469) and control group (p-0.801). Logistic regression showed no relationship between the reduction of NF-κB level and chemotherapy outcomes in both group (p=0.988), as shown in Table 3.

Table 3. Change of NF-kB level and the outcomes of induction phase in both group (n=22)

Groups	Change of NF-kB level Average (SD) (ng/ml)		p value
	Remission	Non-remission	
Treatment	-1.915 (3.879)	-4.25 (4.454)	0.469
Control	-4.867 (7.612)	-3.643 (4.020)	0.801

DISCUSSION

The male subjects were more prevalent than female subjects, which similar with other study, the male/female ratio was 1.11:1 (Li *et al.*, 2008). Even in Malay children, ALL was more prevalent in male (62.74%) (Zaid *et al.*, 2012). The reason why ALL more prevalent in male is still unexplained until now. The average age of ALL diagnosis was 65,08 months or 5.4 years old, which also similar with the study in Malay, which noted the age of 4-6 years old (37.2%). Hossain et al stated that the age when ALL first diagnosis is important as it affected the survival rate the sufferers (Hossain, Xie and McCahan, 2014).

Wasted was still prevalent in ALL sufferers, 37.5%, good nutrition was 58.3%, which was in line with other study, 53.1% and 57%. Nutritional status is an important parameter for the prognostic and outcome of the medical treatment (Viana *et al.*, 1994; Antillon *et al.*, 2013; Rogers, 2014). Wasted was correlated with immune deficiency, with the consequence of infection incidence and haematologic toxicity (Rogers, 2014). However, previous study found that no significant difference of remission outcomes and mortality between wasted and goon nutritional status in ALL (Lobato-Mendizábal, Ruiz-Argüelles and Marín-López, 1989).

Anaemia was suffered by 54.5%, leucopenia 40.9%, and thrombocytopenia 68.2% of the subjects, which also in line with other study, anaemic subjects was 62.2%, leucopenia 36.6%, and thrombocytopenia 96.83% (Widiaskara *et al.*, 2016). Pui and Evans. (2006) also found similar result, with the proportion of leucopenia and thrombocytopenia was 20% and 75%. This showed that bone marrow has been replaced by leukemic lymphoblast cells (Pui and Evans, 2006).

NF-κB level can be measured in blood serum was 0-100 ng/ml. Omega-3 supplementation did not differ the NF-κB level before and after the intervention, which contrast with other study, in which found that omega-3 suppressed cell proliferation and inducted cell apoptosis in breast cancer via NF-κB signal

transduction pathway (Schley *et al.*, 2005), the similar result also seen in colon cancer, in which omega-3 suppressed the expression of pro-inflammation of NF-κB gene (Narayanan, Narayanan and Reddy, 2001; Narayanan *et al.*, 2004). The effect of pro-inflammatory response by omaga-3 was seen in a cell culture study using macrophage cell, which showed that omega-3 inactivate the transduction signal of NF-κB via Ikβ complex formation (Novak *et al.*, 2003). Kordes 2000, found that 93% of LLA cells uniquely increase the p50-p65 p50 homodimer of NF-κB activation, which then strengthen tumorigenesis process (Karin *et al.*, 2002; Aggarwal, 2004; Kim, Hawke and Baldwin, 2006). The effect of NF-κB activation increase increase the resistance of cancer cells and protect cancer cells from chemotherapy (Kordes *et al.*, 2000), which lead to failure during treatment (Aggarwal, 2004). NF-κB was activated constantly at the haematological malignancy and solid tumor to support tumorigenesis. NF-κB is also activated by chemotherapeutic agents and radiation that leads to chemoresistance and radio-resistance.

A study conducted in adult with chronic ALL showed that omega-3 decrease NF-κB activity after giving omega 3 PUFA at a dose of 7.2 grams per day. The study conducted by Zaid et al. (2012), with omega-3 dose of 1.200 mg/day showed body weight increment, improve appetite and upper arm muscle diameter, however this study did not evaluate the effect of omega 3 PUFA on NF-κB levels (Zaid *et al.*, 2012). The dose used in this study was based on recent studies concluded that supplementation of omega 3 PUFA between 0.5-1g/day or slightly higher, does not seem to induce cytotoxic or pro-carcinogenic oxidative stress in normal tissue (Serini *et al.*, 2011), and there is no exact dose of omega 3 PUFA established as a therapeutic dose in children with ALL. Researchers suspect that the 1000mg dose given has not reached the optimal dose which shows a suppressive effect on NF-κβ.

In this study, of all subjects who received chemotherapy, 77.3% experienced remission and 22.7% experienced non-remission after completing the induction phase. These results are similar as other study, 20-30% of patients with ALL experiencing failure with intensive anti-cancer therapy (Nguyen *et al.*, 2008). The failure of induction phase chemotherapy or relapse after a short remission could occur (Holleman *et al.*, 2004). NF-κB had no relation with the outcome of chemotherapy or remission, which in contrast with other study, NF-κB expression made the medication did not optimal, due to chemoresistance in breast cancer patients (Montagut *et al.*, 2006). The effectiveness of omega-3 depends on the medication given to the patients, and most of them could stimulate the expression of NF-κB (Montagut *et al.*, 2006), and several tumor cells could be increase NF-κB expression due to radiation and chemotherapy medication; and increase the resistance of apoptosis (Arlt *et al.*, 2003).

Strengths and Limitation

As far as the researcher's observation, there is no dose of omega 3 PUFA that has been determined as a therapeutic dose in children with ALL. In this study, researchers did not conduct an initial test to determine the dose of omega 3 PUFA which had a suppressive effect on NF-κβ in inducing apoptosis.

CONCLUSION

There is no significant difference of NF-kB level between the subjects receiving omega 3 supplementation and the subjects who did not had omega 3 supplementation, and there is no relationship between NF-kB levels with chemotherapy outcomes.

Author Contribution

Mangihut Rumiris Manulang: subject's preparation, data gathering and analysis, drafting, and approval for the manuscript's publication; I Dewa Gde Ugrasena : Supervising the study, drafting; Mia Ratwita Andarsini: analisis the data, proof read; Maria C. Shanty Larasati : Editing.

Ethical Consideration

Subjects used as samples of this study must obtain written consent from parents or guardians. The informed consent form can be seen in the attachment. Research ethical eligibility was issued by the Health Research Ethics Committee of RSUD Dr. Soetomo Surabaya with ethical eligibility certificate number 151/Panke.KKE/II/2015.

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