Association between the Ratio of Omega-6/Omega-3 Fatty Acids Intake to Plasma Malondialdehyde Level in Patients with Knee Osteoarthritis

S R Angelia¹, N R M Manikam², A M T Lubis³, C Siagian⁴, and N Mudjihartini⁵*

¹Department of Nutrition, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya no 6, Central Jakarta 10430, Jakarta, Indonesia
²Department of Nutrition, Cipto Mangunkusumo National General Hospital, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya no 6, Central Jakarta 10430, Jakarta, Indonesia
³Department of Orthopedic Surgery, Cipto Mangunkusumo National General Hospital, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya no 6, Central Jakarta 10430, Jakarta, Indonesia
⁴Department of Orthopedic Surgery, Bhayangkara RS Sukanto Hospital, Jl. RS Polri, East Jakarta 13510, Jakarta, Indonesia
⁵Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya no 6, Central Jakarta 10430, Jakarta, Indonesia

*nunikbiokim@gmail.com

Abstract. One of several factors in the pathogenesis of osteoarthritis (OA) is the generation of oxidative stress, inducing lipid peroxidation, and producing malondialdehyde (MDA). Omega-3 fatty acids have role in inhibiting the oxidative stress, but their levels are determined by the omega-6/omega-3 ratio. This study aims to investigate the association between the ratio of omega-6/omega-3 intake and plasma MDA level in knee OA patients. This study was conducted at orthopedic clinic at Bhayangkara Hospital and Cipto Mangunkusumo Hospital, in grade 2-4 of knee OA patients, aged between 40-60 years (n=57). The 1-month-history of omega-3 and omega-6 intake was obtained by using semi-quantitative food frequency questionnaire. The omega-6/omega-3 ratio was calculated by dividing the average daily intake of total omega-6 by the average daily intake of total omega-3. The plasma MDA level was measured by Wills spectrophotometry. The median for omega-3 and omega-6 intake were 0.864 and 6.830 g/day. Thus the ratio of omega-6/omega-3 intake was 8.8:1, and the mean plasma MDA level was 0.773 nmol/mL. Through multiple linear regression test, the results found were the increase of 1 unit of omega-6/omega-3 intake ratio would increase MDA level of 0.023 nmol/mL (β =0.023, 95% CI =0.004 – 0.042, p =0.017).

Keywords: Omega-3 fatty acids, osteoarthritis, malondialdehyde

1. Introduction

Osteoarthritis (OA) is one of the chronic joint disease which is complex and multifactorial. OA causes pain thus it affects daily activity and health status of the patient.[1] Prevalence of OA is strongly affected by age. It is often found at age over 45 years and greatly increased at age over 60 years.[2] Based on World Health Organization (WHO) in 2010, OA ranks 11 from 289 chronic disability-causing diseases.[3] According to the 2013 Riset Kesehatan Dasar (Riskesdas) data, the prevalence of joint disease based on diagnosis and symptoms over the age of 15 years in Jakarta Special Capital Region was 21.8%.[4]

Many factors are involved in the pathogenesis of OA, such as age, gender, traumatic event, mechanical stress and obesity. Biochemistry reaction also occurs in the inflammatory process of OA,
one of which is excessive oxidative stress which plays an important role in inducing degeneration and apoptosis of the chondrocyte, cartilage degradation and loss of extracellular matrix volume.[5] Oxidative stress occurs due to an imbalance between free radical production and antioxidant capacity in the body. Unstable and reactive free radicals can interact with lipid in the cell membranes, causing lipid peroxidation, resulting in cell death through damage of the chondrocyte membrane. Lipid peroxidation produces malondialdehyde (MDA), which is used as a marker of oxidative stress status.[6; 7; 8; 9; 10]

Omega-3 fatty acids may also act as antioxidants and inhibit lipid peroxidation in OA.[11; 12] However, the benefits omega-3 fatty acids are influenced by omega-6 fatty acids which have a pro-inflammatory property. This is caused by the synthesized eicosanoids of omega-3 fatty acids compete with omega-6 fatty acids and have opposite functions with each other. The level of both fatty acids in the blood is determined by the intake from the food consumed, thus the ratio between the intake of omega-6/omega-3 fatty acids is important to keep its balance, with the ideal ratio is about 1:4:1. Changes in the dietary pattern of the community nowadays cause this ratio tends to increase, which can lead to inflammation and oxidative stress.[13; 14; 15] This is supported by study from Yang et al.[16] which found that MDA levels increased as the ratio of omega-6/omega-3 fatty acids increased.

2. Experimental Methods
This study used cross sectional design and conducted in Orthopedic outpatient clinic of Cipto Mangunkusumo Hospital, and Bhayangkara RS. Sukanto Hospital Jakarta. Data collection was conducted from April to May 2018. Grade 2-4 knee OA patients, aged 40-60 years, who met the inclusion criteria and signed the informed consent form were included in this study with the consecutive sampling method. Subjects with a history of knee trauma, tumor, infection, congenital disease, have underwent knee joint replacement surgery, other chronic inflammatory diseases, as well as in treatments which could interfere with the absorption and metabolism of fats were removed from this study. Characteristics of the study subjects consisted of age, gender, nutritional status, and physical activity. Characteristics of the subjects were also seen based on the food intake which includes omega-3 fatty acids, omega-6 fatty acid, the ratio of omega-6/omega-3 fatty acid intake and plasma MDA levels.

2.1. Assessment of dietary fatty acid intake
Methods of assessing the intake of omega-3 fatty acids and omega-6 fatty acids during the last 1-month were performed using semi-quantitative FFQ questionnaires. The questionnaire included the types of dietary sources of omega-3 fatty acids such as alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), as well as dietary sources of omega-6 fatty acids such as linoleic acid (LA) and arachidonic acid (AA).[17] The ratio of omega-6/omega-3 fatty acids was calculated by dividing the average daily intake of total omega-6 fatty acids, with an average daily intake of total omega-3 fatty acids.

2.2. Plasma malondialdehyde analysis
Laboratory examination was done to determine plasma MDA level by using Wills spectrophotometry method. Blood samples were sent to a standardized laboratory which is Biochemistry Laboratory of Faculty of Medicine Universitas Indonesia for plasma MDA level examination.

2.3. Statistical analysis
The data was then processed using the program statistical package for social science (SPSS) version 20. To find the variables which is a potential confounder to plasma MDA level, Spearman or Pearson correlation test with significance of p <0.25 was used. The ratio of omega-6/omega-3 fatty acids intake and potential confounder variable to plasma MDA level were analyzed by linear regression test, with significance limit of p <0.05.

3. Result and Discussion
3.1. Subject characteristics
The mean age of the subjects in this study was 50 years. Age is a major risk factor for the occurrence of OA. There is an increased of incidence rate with age, due to joint damage through various mechanisms
caused by aging. This is in accordance with the research result which shows that the average age of the subjects is about 50 years.[2; 18] Most of the subjects (88%) was female. Several studies have shown that women are more susceptible to knee OA, and it was reported to have more obvious symptoms than men. This is due to many factors such as anatomical differences, cartilage volume differences and hormonal changes. Hormonal changes play a very important role in women especially when reaching menopause.[19]

Table 1. Characteristics of the subjects

| Variable                         | Result (n= 57)                           |
|----------------------------------|-----------------------------------------|
| Age (years)                      | 50.88 ± 6.19*                           |
| Gender, n(%)                     |                                         |
| Male                             | 7 (12.3)                                |
| Female                           | 50 (87.7)                               |
| Body mass index (kg/m$^2$)       | 29.16 (22.5-44.3)**                     |
| Nutritional status, n(%)         |                                         |
| Normal, n(%)                     | 4 (7)                                   |
| Overweight, n(%)                 | 2 (3.5)                                 |
| Obesity grade I, n(%)            | 24 (42.1)                               |
| Obesity grade II, n(%)           | 27 (47.4)                               |
| Physical activity, n(%)          |                                         |
| Mild                             | 14 (24.6)                               |
| Moderate                         | 12 (21.1)                               |
| High                             | 31 (54.4)                               |

*mean ± standard deviation; **median (minimum-maximum)

Nearly all subjects in the study (89.5%) were in the obesity category according to the Asia-Pacific criteria and 47% were in the obesity 2, with the highest subject BMI reaching 44.3 kg/m$^2$. Obesity has been known to be a major risk factor for OA, due to excess mechanical loads, especially in load-bearing joints such as knee and hip joints. An increase in BMI can cause a decrease in the volume and thickness of the cartilage, thus the risk of OA may increase by 36% every 2 units of BMI unit increase.[20; 21; 22] Inflammation in joints causing OA could be triggered by metabolic abnormalities which play a major role in its pathogenesis and it is called meta-inflammation. Meta-inflammation causes oxidative stress leading to cell dysfunction, including chondrocytes of the joint cartilage which may result in apoptosis and cell death.[20] In this study most of the subjects (54.4%) had high physical activity in accordance with the Physical Activity Index (IAF) according to Baecke. Physical activity is an important risk factor for the occurrence of OA, because the mechanical and repeated pressures occurs in joints could cause cartilage joints damage.[23]

3.2. Dietary intakes of subjects

The median of omega-3 fatty acids total intake of the subjects was 0.864 g/day, which only 76.7% of the total recommended based on Recommended Dietary Allowance (RDA). The total intake of omega-6 fatty acids of the study subjects was 6.830 g/day, which was about 59% against the recommended value. Although the intake of omega-3 and omega-6 fatty acids in the subjects was still lacking, the average omega-6/omega-3 fatty acids intake ratio in the study subjects remained high, at 8.81, which was higher than the recommended ratio. There are several studies that state that the ideal ratio of omega-6/omega-3 fatty acid intake to prevent non-communicable diseases is 1-4:1.[15] Experimental animal studies have shown that the optimal ratio of omega-6/omega-3 fatty acids can prevent the progressivity of OA.[16]

Table 2. Dietary intakes of the subjects (n =57)

| Variable                          | Result (n =57) |
|-----------------------------------|----------------|
| Omega-3 fatty acids intake (g/day)|                |
3.3. Plasma malondialdehyde level

This study found that the mean plasma MDA concentration in the research subjects was 0.773 ± 0.199 nmol/mL, with the highest MDA levels of 1.217 nmol/mL. A study by Suantawee et al.[24] using plasma to measure MDA, obtained a plasma MDA level of 1.3 nmol/mL in subjects with knee OA. The result was significantly higher (p <0.005) than in healthy subjects of 0.5 nmol/mL. This shows that the study subjects experienced oxidative stress which resulted in lipid peroxidation which was characterized by increased plasma MDA.

3.4. Association between the ratio of omega-6/omega-3 fatty acid intake to plasma MDA level of the subjects

Pearson or Spearman correlation test was performed to determine the potential confounder variables which could affect plasma MDA levels in knee OA patients. Based on the analysis, age, BMI and physical activity score are potential confounder which can influence plasma MDA level (Table 3). These variables were included in the multiple linear regression model.

| Variable                        | Plasma MDA level |
|---------------------------------|------------------|
|                                 | r                |
| Age                             | -0.156           |
| BMI                             | 0.222            |
| Physical activity score         | -0.169           |
| P                               | 0.246*           |
|                                 | 0.097*           |
|                                 | 0.209*           |

P : Pearson correlation test; S : Spearman correlation test; *: potential confounder (p <0.25)

The results of the linear regression analysis after controlling the confounding factors (age, BMI, and physical activity score) on MDA levels showed that there was a correlation between the ratio of omega-6/omega-3 fatty acids intake to plasma MDA levels in knee OA patients. An increase in the intake ratio of omega-6/omega-3 fatty acids by 1 unit will increase plasma MDA levels by 0.023 (with the range between 0.004-0.04) units and this is statistically significant (p <0.05).

The intake of fatty acids has an effect on the inflammatory process and the cells involved in it. High AA levels and very low levels of EPA and DHA were found in the composition of cell membranes involved in inflammation, such as lymphocytes, macrophages and neutrophils from experimental animals that were given a high-fat diet in the form of plant oils that were high in AA content.

| Variable                        | B         | SE     | β        | CI        |
|---------------------------------|-----------|--------|----------|-----------|
|                                 | B         | SE     | β        | CI        |
|                                 | B         | SE     | β        | CI        |
|                                 | B         | SE     | β        | CI        |

*: significant if p <0.05
The composition of the phospholipid membrane in human subjects with the Western diet type (high intake of omega-6 fatty acids), contains around 15-20% AA, 0.5-1% EPA and 2-3% DHA, and when given a high omega-3 fatty acids diet, the content of EPA+DHA in the cell’s phospholipid membrane has increased. In this study, the diet pattern of the subject is still dominated by omega-6 fatty acid, so that it can cause an increase in AA levels in cell’s phospholipid membranes, thus can lead to increased production of pro-inflammatory eicosanoids. Low intake of omega-3 fatty acids can also reduce levels of EPA and DHA in the phospholipid membrane, causing activation of NFkB which leads to increased levels of pro-inflammatory cytokines, inducing oxidative stress, which play a role in the pathogenesis of OA.[25]

| Variable                        | B  | 95% CI      | SE B | β   | p-value |
|---------------------------------|----|-------------|------|-----|---------|
| Ratio of omega-6/omega-3 fatty acids intake | 0.023 | 0.004-0.04 | 0.009 | 0.314 | 0.017*  |

4. Conclusions
Based on the research result, it is concluded that high ratio of omega-6/omega-3 fatty acids intake can increase plasma MDA levels in patients with knee OA. Subsequent education is required on the importance of increasing the consumption of dietary sources of omega-3 fatty acids, in order to achieve an optimal ratio between the intake of omega-6 and omega-3 fatty acids.

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