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Evaluation of the Relationship between Endometriosis and Omega-3 and Omega-6 Polyunsaturated Fatty Acids

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ABSTRACT

Background: Endometriosis is a common chronic inflammation causing major problems including infertility. The role of omega-3 and omega-6 fatty acids as their potential anti-inflammatory effects in endometriosis needs to be further explored. The objective of this study was to compare serum phospholipid fatty acid profile in endometriosis patients with controls, and to explore the correlation of this profile with the severity of the disease. **Methods**: Sixty-four endometriosis patients and 74 control women, in reproductive age, participated in this study. Among the endometriosis patients, 19 cases were in stage I, 27 cases in stage II, 8 cases in stage III, and 10 cases in stage IV. Each patient underwent laparoscopy. Before surgery, 5 ml of blood was obtained. After extraction of the total lipids, serum total phospholipid fraction was isolated by thin layer chromatography. Fatty acid composition of the phospholipid fraction was determined by gas chromatography and the resulted profile was compared in endometriosis patients and controls. The profile was also compared in the endometriosis group based on the severity of disease. **Results:** Stearic acid was significantly lower in the endometriosis group as compared to controls (P= 0.030). No other fatty acid compositions were significantly different between patients and controls. Serum ratio of eicosapentaenoic acid (EPA) to arachidonic acid (AA) was in reasonable correlation with the severity of endometriosis (r = 0.34, P = 0.006). **Conclusion**: According to these findings, levels of fatty acids in serum total phospholipids seem not to be a marker for endometriosis, but the EPA to AA ratio was a relevant factor indicating severity of illness. *Iran. Biomed. J. 16 (1): 38-43, 2012*

Keyword: Endometriosis, Phospholipids, Omega-3 fatty acids

INTRODUCTION

Atypical growth of endometrial tissue outside of the uterus is called endometriosis [1]. There is a benign, but locally invasive growth of endometrial cells in this disease and typically, definitive diagnosis is confirmed surgically by laparoscopy [2]. The common symptoms of this disease are dysmenorrhoea and dyspareunia that affect the quality of life, working ability, social relationships and sexual performance [3]. Endometriosis is leading to major problems, such as infertility in women during their reproductive age [4]. The prevalence rate of this disease has been reported between 12 to 45 percent in different populations [5]. According to severity, the disease is classified as minimal (stage I), mild (stage II), moderate (stage III) and severe (stage IV) [6].

Despite extensive investigations and health care costs associated with the disease, the etiology has not been clearly elucidated yet [7]. Endometriosis is considered as a multi-factorial disease and most probably a chronic inflammation in the peritoneal cavity triggers the disease [8]. Despite medical treatment such as different hormonal medication (progestins, GnRH agonists), there is no effective medicinal treatment and in advanced endometriosis, removal of the ectopic tissue by laparoscopy has been suggested [3]. Since

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this disease mostly affects women in reproductive age, diagnosis of the potential factors involved in this disease and providing effective treatment approach play important roles in enhancing health and quality of life among women.

A study on laboratory animals has shown that dietary supplementation with fish oil containing the omega-3 polyunsaturated fatty acid eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) could slow down the growth of endometriotic implants [9]. Also, several independent studies have reported pivotal role of diet in relief of pain in endometriosis. Proctor and Murphy [10] demonstrated that intake of complementary B vitamins and omega-3 fatty acids (fish oil) reduced endometriosis pain and inflammation. It has been suggested that omega-3 and omega-6 fatty acids affect biosynthesis of active prostaglandins (PGE_2) which result in relief of pelvic pain [10]. In addition, Sesti *et al.* [11] have shown a role for supplementation with vitamins and omega-3 fatty acid in reducing pelvic pain and improving quality of life in endometriosis patients stage III-IV.

In a prospective cohort study, Missmer *et al.* [12] have demonstrated that long-term intake of omega-3 fatty acids results in low risk of endometriosis, conversely long-term consumption of trans-unsaturated fatty acids are related with high risk of the disease. Several studies have revealed that the composition of fatty acids in serum phospholipids may be affected mostly by diet rather than metabolic factors. In a recent study, serum phospholipid fatty acids has been emphasized as a suitable biomarker for determining level and type of these results from diet intake [13].

Hence, in the present study, for the first time in clinical level and independent of the other factors, serum total phospholipid fatty acid profile as biomarkers of diet intake have been evaluated between endometriosis patients and control subjects. Alternatively, this study was aimed to explore potential role of omega-3 and omega-6 fatty acids as preventive agents with comparing serum phospholipid fatty acid profile in endometriosis patients and controls. Moreover, to find out the therapeutic effects of omega-3 and omega-6 fatty acids in this disease, correlation between fatty acid level in serum phospholipid fraction and severity of the disease has been determined. We hypothesized that omega-6 would positively and omega-3 fatty acids would inversely associate with endometriosis.

MATERIALS AND METHODS

Materials. HPLC grade methanol, chloroform, HPLC grade benzene, acetyl chloride, thin layer chromatography (TLC) plate, acetic acid, diethyl ether and hexane were purchased from Merck Company (Germany). Antioxidant, internal standard powder (13:0) and fatty acid standard as methyl esters (14:0, 16:0, 16:1, 18:0, 18:1, 18:2, 18:3, 20:4, 20:5 and 22:6) were obtained from Sigma-Aldrich Company (USA). Capillary column with high polarity, solid phase: 70% cyanopropyl colysilphenylene-siloxane, film thickness: 25 µm, length: 60 m and internal diameter: 0.25 mm was obtained from Teknokroma Company (Spain).

Patient selection and study design. Women undergoing laparoscopy and laparotomy at Alzahra Hospital (Tabriz University of Medical Sciences, Tabriz, Iran) and Sarem Hospital (Tehran, Iran) were selected for this cross-sectional study. The study was approved by the Ethic Committee of Avicenna Research Center (Evin, Tehran, Iran). Informed consent was obtained from all women. Endometriosis patients and control group were between 18 to 42 years old (mean age 30.57 ± 5.04 vs. 30.57 ± 5.71, respectively). Mean BMI in Patients and control group was 26.08 ± 3.98 vs. 26.39 ± 4.52, respectively. All participants had regular menstrual cycles and were chosen from both luteal and proliferative phases. Women were excluded from the study if they had received anti-inflammatory drugs during last three months prior to surgery or if they had any diseases, such as endometritis, gastrointestinal or urological disease with pelvic pain, liver or endocrine autoimmune disease, previous endometriosis or neoplastic disorders and chronic inflammatory diseases in pelvic, uterus, ovaries. Sixty four women in endometriosis patient group were diagnosed based on the results of the laparoscopy. The clinical stages of the patients were diagnosed according to the revised American Fertility Society classification [14]. Among the endometriosis patients, 19 cases were in stage I, 27 cases in stage II, 8 cases in stage III, and 10 cases in stage IV. Seventy four women underwent laparoscopy without endometriosis and those were included in the control group. They were diagnosed as uterine myoma, dermoid cyst, serous cyst, paraovarian cyst or mucinous cyst. The phase of menstrual cycle and the disease diagnoses were histologically confirmed. Before laparoscopy, peripheral fasting blood samples were obtained and subjected to centrifuged at 800 ×g for 5 minutes. The resulting sera were stored at -70°C until fatty acid measurement.

Phospholipid extraction method. The first step was the extraction of the serum total lipids, which was performed based on Bligh and Dyer protocol [15]. The second step was the purification of the total phospholipids that was carried out using TLC technique [16]. In brief, extracts from the first step were evaporated by nitrogen gas and then the

Table 1. Comparison of serum total phospholipid fatty acid composition in control group and endometriosis patients.

Fatty Acids (%)	Control (n = 74) mean ± SD	Endometriosis (n = 64) mean ± SD	*P* value
14:0 (myristic acid)	0.26 ± 0.11	0.29 ± 0.21	0.240
16:0 (palmitic acid)	48.53 ± 7.43	49.14 ± 5.28	0.570
16:1(palmitoleic acid)	0.39 ± 0.18	0.34 ± 0.20	0.150
18:0 (stearic acid)	13.35 ± 2.60	12.46 ± 2.29	0.030*
18:1 n-9 (oleic acid)	6.28 ± 1.72	6.13 ± 1.31	0.570
18:2 n-6 (linoleic acid)	19.66 ± 3.59	20.31 ± 3.19	0.260
18:3 n-3 (α-linolenic acid)	0.35 ± 0.14	0.37 ± 0.19	0.440
20:4 n-6 (AA)	7.23 ± 2.31	6.93 ± 1.92	0.410
20:5 n-3 (eicosapentaenoic acid)	0.36 ± 0.24	0.35 ± 0.35	0.801
22:6 n-3 (docosahexaenoic acid)	0.70 ± 0.75	0.79 ± 0.82	0.500
SFA	62.14 ± 6.32	61.89 ± 4.57	0.780
MUFA	6.66 ± 1.73	6.47 ± 1.33	0.460
Omega-3 fatty acids	1.41 ± 0.79	1.51 ± 1.03	0.520
Omega-6 fatty acids	26.89 ± 4.48	27.25 ± 3.41	0.600
SFA/UFA	1.84 ± 0.39	1.80 ± 0.33	0.450
Omega-3/Omega-6	0.05 ± 0.02	0.05 ± 0.03	0.570
EPA/AA	0.05 ± 0.04	0.05 ± 0.05	0.740

*$P<0.05$; SFA, saturated fatty acids; MUFA, mono unsaturated fatty acids; SFA/UFA, saturated fatty acids to unsaturated fatty acids; EPA/AA, eicosapentaenoic acid to arachidonic acid

concentrated total lipid samples were loaded on TLC plate. After trans-sterification of the phospholipids, fatty acid methyl esters were separated on a gas chromatograph (Buck Scientific model 610, USA) [17]. The relative amount of each fatty acid was stated as the percentage of total area on chromatograms. The analyses for the fatty acids were also carried out according to different fatty acid classes: saturated fatty acids (SFA: 14:0, 16:0 and 18:0), Unsaturated fatty acids (UFA: 16:1, 18:1, 18: 2, 18:3, 20:4, 20:5 and 22:6), mono unsaturated fatty acids (MUFA: 16:1 and 18:1), omega-3 fatty acids (18:3, 20:5 and 22:6) and omega-6 fatty acids (18:2 and 20:4).

Statistical analysis. Data are presented as mean ± SD. Statistical significance was tested using Student's un-paired *t*-test or analysis of variance (ANOVA) as appropriate. Data regarding categorized characteristics were analysed by Fisher's exact test and Chi-square. To assess correlation between fatty acid patterns and severity of endometriosis, correlation bivariate test was performed. For all tests, $P<0.05$ was considered statistically significant. Data were analyzed using SPSS software version 15.

RESULTS

There was no significant difference in omega-3/omega-6, saturated/unsaturated, and EPA/AA (arachidonic acid) ratios between proliferative and luteal phases of menstrual cycle in patients and control groups separately (data not shown).

There were no significant differences in the level of serum total phospholipid fatty acids (except stearic acid, $P = 0.030$), SFA, MUFA, omega-3, omega-6, omega-3/omega-6 ratio, EPA/AA ratio and SFA/UFA ratio between endometriosis patients and control group (Table 1). Among various stages of endometriosis patients and control groups, no significant differences in the level of the above mentioned factors were observed (Table 2). Also, no significant difference in the levels of the fatty acids between the endometriosis patients in stages I and II as the first and the second subgroups and the patients in stages III and IV as third subgroup were observed (data not shown). Moreover, there were not any significant differences between endometriosis patients in stages I and II (early stages) as the first subgroup and endometriosis patients in stages III and IV (late stages) as the second subgroup and control group in terms of the level of fatty acids according to different fatty acid classes (Table 3).

There was no significant correlation between omega-3, omega-6, omega-3/omega-6 ratio, saturated/ unsaturated ratio with severity of the disease (data not shown). However, only EPA/AA ratio showed a direct correlation with severity of the disease. (correlation coefficient = 0.34, $P = 0.006$).

DISCUSSION

Apart from stearic acid, results did not reveal any meaningful differences when considering the level of serum total phospholipid fatty acids, SFA, MUFA, omega-6, omega-3, omega-3/omega-6, EPA/AA, and SFA/UFA among endometriosis patients and our control subjects. Stearic acid level was remarkably

Table 2. Comparison of serum total phospholipid fatty acid composition in control group and four different stages of endometriosis patients.

Fatty Acids (%)	C (n = 74) mean ± SD	S_I (n = 19) mean ± SD	S_{II} (n = 27) mean ± SD	S_{III} (n = 8) mean ± SD	S_{IV} (n = 10) mean ± SD	*P* value
14:0 (myristic acid)	0.26 ± 0.11	0.30 ± 0.15	0.31 ± 0.30	0.22 ± 0.10	0.31 ± 0.17	0.44
16:0 (palmitic acid)	48.53 ± 7.43	48.88 ± 4.04	50.60 ± 5.20	50.88 ± 5.23	47.45 ± 5.02	0.50
16:1 (palmitoleic acid)	0.39 ± 0.18	0.34 ± 0.20	0.35 ± 0.18	0.37 ± 0.24	0.34 ± 0.29	0.85
18:0 (stearic acid)	13.35 ± 2.62	12.76 ± 1.08	12.25 ± 2.63	11.64 ± 1.70	13.06 ± 1.91	0.14
18:1 n-9 (oleic acid)	6.28 ± 1.72	6.05 ± 1.24	6.20 ± 1.50	6.55 ± 1.42	5.91 ± 1.15	0.90
18:2 n-6 (linoleic acid)	19.66 ± 3.60	19.68 ± 2.65	20.69 ± 3.57	20.88 ± 2.63	19.24 ± 2.10	0.57
18:3 n-3 (α-Linolenic acid)	0.35 ± 0.14	0.37 ± 0.16	0.38 ± 0.21	0.34 ± 0.14	0.41 ± 0.27	0.79
20:4 n-6 (AA)	7.23 ± 2.31	7.44 ± 1.87	6.14 ± 1.25	6.06 ± 1.50	7.31 ± 1.80	0.07
20:5 n-3 (eicosapentaenoic acid)	0.36 ± 0.24	0.23 ± 0.10	0.38 ± 0.50	0.30 ± 0.25	0.42 ± 0.25	0.40
22:6 n-3 (docosahexaenoic acid)	0.70 ± 0.75	0.83 ± 0.66	0.48 ± 0.52	0.52 ± 0.61	1.07 ± 1.17	0.20
SFA	62.14 ± 6.32	61.94 ± 3.89	63.16 ± 4.35	62.74 ± 4.61	60.27 ± 5.23	0.82
MUFA	6.66 ± 1.73	6.39 ± 1.30	6.55 ± 1.53	6.92 ± 1.32	6.25 ± 1.14	0.87
omega-3 fatty acids	1.41 ± 0.79	1.43 ± 0.71	1.24 ± 0.93	1.17 ± 0.61	1.90 ± 1.44	0.31
omega-6 fatty acids	26.89 ± 4.84	27.11 ± 2.90	26.83 ± 3.45	26.95 ± 3.50	26.56 ± 3.64	1.00
SFA/UFA	1.84 ± 0.39	1.80 ± 0.29	1.86 ± 0.35	1.83 ± 0.39	1.77 ± 0.25	0.96
omega-3/omega-6	0.05 ± 0.02	0.05 ± 0.02	0.04 ± 0.03	0.04 ± 0.02	0.07 ± 0.05	0.11
EPA/AA	0.05 ± 0.04	0.03 ± 0.01	0.06 ± 0.07	0.05 ± 0.04	0.06 ± 0.03	0.20

C, control group; S, stage of endometriosis; SFA, saturated fatty acids; MUFA, mono unsaturated fatty acids; SFA/UFA, saturated fatty acids to unsaturated fatty acids; EPA/AA, eicosapentaenoic acid to arachidonic acid

higher in the control group in comparison to endometriosis patients. The beneficial aspects regarding omega-3 consumption and its inverse relation to endometriosis development have been shown [12]. Also, a positive association between endo-metriosis and Trans-unsaturated fatty acid consumption has been reported [12]. Hence, an important role of diet (life style modifiable factor) and incidence of endometriosis has been suggested [12, 18]. These reports contrast a study conducted by Parazzini *et al.* [19] where no significant association between consumption of fish and a risk for endometriosis was found. An *in vitro* study showed omega-3 administration reduces survival of endometrial cells in culture medium, whereas omega-6 had no role in this regard [20]. Moreover, some studies have shown a significant inhibitory role of omega-3 fatty acids such as EPA and DHA on inflammation [21-22]. As inflammation is suggested as an inducer of endometriosis [8], the role of these fatty acids as their potentially anti-inflammatory effect in endometriosis needs to be further explored. However, this survey of serum phospholipid fatty acids and endometriosis found no support in the potential role of omega-3 fatty acids as preventive agents.

Covens *et al.* [9] identified that fish oil supplementation including EPA and DHA in surgically induced endometriosis in rabbits remarkably reduced the size of endometriotic lesion after 8 weeks. These fatty acids were able to inhibit the development of endometriosis in this animal model. Also, Yano *et al.* [23] studied the role of dietary supplementation with pure EPA on endometriosis in the same animal model and confirmed the positive potential role of EPA in the treatment of infertile endometriosis patients.

Table 3. Comparison of serum total phospholipid fatty acid composition in control group and endometriosis patients according to early and late stages.

Fatty acids (%)	C (n = 74) mean ± SD	$S_{(I+II)}$ (n = 46) mean ± SD	$S_{(III + IV)}$ (n = 18) mean ± SD	*P* value
SFA	62.14 ± 6.32	62.65 ± 4.16	61.67 ± 4.92	0.79
MUFA	6.66 ± 1.73	6.48 ± 1.43	6.55 ± 1.23	0.83
omega-3 fatty acids	1.41 ± 0.79	1.32 ± 0.84	1.57 ± 1.17	0.57
omega-6 fatty acids	26.89 ± 4.48	26.95 ± 3.20	26.73 ± 3.48	0.98
SFA/UFA	1.84 ± 0.39	1.84 ± 0.32	1.80 ± 0.31	0.92
omega-3/omega-6	0.05 ± 0.02	0.05 ± 0.03	0.06 ± 0.04	0.35
EPA/AA	0.05 ± 0.04	0.05 ± 0.06	0.05 ± 0.03	0.83

C, control group; $S_{(I+II)}$, early stages of endometriosis; $S_{(III + IV)}$, late stages of endometriosis; SFA, saturated fatty acid; MUFA, mono unsaturated fatty acids; SFA/UFA, saturated fatty acids to unsaturated fatty acids; EPA/AA, eicosapentaenoic acid to arachidonic acid.

Moreover, Netsu *et al.* [24] found that the rats with EPA in their diet for six weeks had a remarkable increase in the ratio of omega-3 to omega-6 in addition to a significant decrease in the thickness of endometriotic tissue. It has been suggested that EPA supplementation might be useful as a valid strategy or at least an adjuvant for treatment of endometriosis. Except for EPA/AA (inflammation-related phospholipid fatty acids) ratio that revealed a direct significant correlation with the severity of the disease, although in the direction opposite to those assumed, our results did not show any significant correlation between the level and the class of fatty acids in serum total phospholipids and the severity of the disease. The most striking aspect of our findings is that EPA/AA was not in the direction assumed. We assumed that the EPA would be correlated with reductions in severity of disease, while the AA would increase the severity. EPA is hypothesized to reduce disease severity through their anti-inflammatory and immunomodulatory effects [25]. EPA is the most important component of omega-3 and AA, an omega-6 fatty acid and plays an important role in biological systems. AA has a substrate role for production of certain mediators such as PGE_2 and leukotriene (LTB_4). PGE_2 and LTB_4 are initiators for endometriosis and pain [24]. On the other hand, EPA plays a role in biosynthesis of LTB_5 and PGE_3 which have less inflammatory effect compared with PGE_2 and LTB_4 [24]. EPA is a competitive inhibitor in conversion of AA to LTB_4 and PGE_2 [26]. Irrespective of study design, our results were in agreement, in part, with the *in vitro* experiments by Gazvani *et al.* [20] that showed a high ratio of omega-3 to omega-6 in endometrial cell culture from endometriosis patients induce higher concentrations of IL-8 productions in cell supernatant. IL-8 as a pro-inflammatory and angiogenic cytokine has a significant role in endometriosis [27].

In our study, the existence or absence of the endometriosis in the patients examined was verified by two methods: macroscopically (by laparoscopy) and microscopically (by histological examination/ pathology reports) to strengthen the study by reducing the risk of misdiagnosis. The difference between our results and the other studies may be due to the difference in type of control subjects. Also, fatty acids were considered as a percentage rather than a concentration [28] when stated as a proportion, a positive association with one fatty acid may result in a falsely inverse association with another [29]. The present study was also small in scale. It was claimed that serum phospholipid fatty acid profile appears not to be a suitable pattern to illustrate fatty acid regimen in long term.

Overally, the results showed that the components and the types of the fatty acids in serum total phospholipids seem not to be a marker for endometriosis, but the EPA/AA ratio is a relevant factor to indicate severity of illness.

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