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LONG AND VERY LONG FATTY ACID FRACTIONATION IN SYSTEMIC LUPUS ERYTHEMATOSUS IN THE ACTIVE AND INACTIVE STATUS

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ABSTRACT

Objective: Flare in Systemic Lupus Erythematosus (SLE) is an exacerbation of SLE clinical features that were earlier quiescent. The disease activity changes from inactive to active with an increase of several immunological profiles; the rise of immune activity induces a metabolic shift in SLE patients. The previous study aimed to investigate the long and very long fatty acid fractions (LCFA and VLCFA) in the active and inactive statuses of SLE patients and showed there were dynamic changes in fatty acid fractions in SLE patients, compared to healthy subjects. The aim of this preliminary study is to investigate LCFA and VLCFA in the active and inactive condition of SLE patients.

Methods: Four serum samples of active and inactive statuses from the same SLE patients were used in this study. Serum LCFA and VLCFA fractions were analyzed by a 7890 Gas Chromatography (GC) System 5977 Mass Selective Detector (MSD).

Results: All of the LCFA and VLCFA fractions were increased in the active condition, compared to SLE patients in inactive, although they were statistically not different ($p>0.05$). The total fatty acid fraction was 38% higher in active condition compare to inactive. The prominent increase of fatty acid fractions was alpha-linolenic acid (inactive vs. active: 23.25±17.97 vs 48.25±38.58 µmol/l), oleic acid (1300±190.4 vs 1774±866.3 µmol/l) and myristic acid (31.25±12.76 vs 59.25±40.4 µmol/l).

Conclusion: The serum of LCFA and VLCFA fractions in SLE patients tend to increase in active conditions.

Keywords: Disease activity, Fatty acid, LCFA, SLE, VLCFA

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INTRODUCTION

Incidences of Systemic Lupus Erythematosus (SLE) have increased around the world, with race, gender, and ethnicity affecting the incidences. A review by Stojan and Petri showed Asians had a higher incidence and prevalence of SLE compared to Caucasians, but lower than African Americans [1]. In Indonesia, the prevalence of this disease is 0.5% of the total population with a tendency toward an increased number of incidences every year, and women 15–44 y of age are more likely to be affected [2]. From other previous study, which was conducted from 2008 to 2017, showed 95.6% of 813 SLE patients were female, and there was an 8.1% mortality rate [3].

SLE is known as the disease with multi-organ involvement and is characterized by a relapse-remission pattern or flare pattern. Flare in SLE is an exacerbation of SLE clinical features that were earlier quiescent. This condition is indicated active disease by an increase of several immunological profiles; inflammation is marked by an intensifying number of cytokines, chemokines and reactive oxygen species (ROS) that act as immunological activities of this disease [3, 4]. These activities induce metabolic shifts in SLE patients, including free fatty acids (FFAs) levels.

Several previous studies proposed a correlation between FFA metabolism and SLE [5, 6]. There is a dynamic change of fatty acid fractions in SLE patients compared to healthy subjects. LCFA and VLCFA, including palmitoleic, myristic and eicosenoic acids, significantly increase in SLE patients. Conversely, the level of linoleic, stearic, caproic, eicosanoic and arachidonic acids significantly decrease in SLE patients [7]. Based on saturation, fatty acid is categorized as unsaturated fatty acids (oleic, palmitoleic, eicosenoic, linoleic, stearic and arachidonic acids) and saturated fatty acids (myristic, caproic and eicosanoic acids) [8]. The plasma of patients with SLE contains low concentrations of polyunsaturated fatty acids and high levels of saturated fatty acids [9].

There was an alteration of the lipid profile between active and inactive of patients with SLE. The ratio of low-density lipoprotein cholesterol to high-density lipoprotein cholesterol significantly increased in active conditions suggesting a high risk of atherosclerosis evidence in SLE patients [10]. Further investigation is necessary to analyze fatty acid fractionation between active and inactive conditions in SLE patients.

MATERIALS AND METHODS

Subjects

This was a cross-sectional study. The participants were four patients who were referred to the rheumatology clinic, our hospital, in 2018. Participants were selected because they had periodically experienced inactive and active disease activity. Patients fulfilled at least four classification criteria as defined in 1997 by the American College of Rheumatology [11]. Disease activity was assessed with the SLEDAI-2K, for which ≥ 4 is defined as an active disease and<4 as inactive [12]. The patient's histories were reviewed and demographic and SLE-related data were collected. All patients provided fully informed consent to participate. This study was approved by the ethical committee of our university.

Fatty acid quantification

Fasting venous blood samples were collected in ethylene diamine-tetra-acetic acid (EDTA) or sera tubes and immediately centrifuged at 4 °C, 1500 rpm for 15 min. The samples were then divided into aliquots and stored at-80 °C until further examination. Four serum samples of active and inactive disease activity from the same SLE patients were used in this study. Serum LCFA and VLCFA were analyzed in the xxx Laboratory by a 7890 GC System 5977 MSD.

One hundred ul serum was prepared by adding 150 ul methanol (Merck, Germany); then 300 ul diluent, which contains chloroform

(Merck, Germany) and non-decanoate, C19:0 (Supelco) as the internal standard, was added. The serum mixture was then centrifuged at 2500 g for five minutes and the layer of chloroform, which contains analyte supernatant, was transferred to different tubes. The chloroform phase evaporated with nitrogen to dryness. The fatty acid resolved with n-hexane (Merck, Germany), which contains Tetramethyl Ammonium Hydroxide (TMAH) in methanol (Sigma), and incubated two hours for esterification, after which it was transferred to GC vials.

Fatty acid calibration curve was prepared by diluting the stock of FAME Mix, C4-C24 (Cat. 18919-1AMP, Lot. LC16765V, Supelco, Bellefonte, PA, USA) with n-Hexane (Merck, Germany) and 1.5 N Hydrochloric Acid (Merck, Germany). Stock of FAME Mix standard consists of Myristic (C14:0), Palmitic (C16:0), Palmitoleic (C16:1 w7), Stearic (C18:0), Oleic (C18:1 w9), Linoleic (C18:2 w6), y-Linolenic (C18:3 w6), a-Linolenic (C18:3 w3), AA (C20:4 w6), DGLA (C20:3 w6), EPA (C20:5 w3) and DHA (C22:6 w3). The stock of the FAME standard was serial-diluted to nine levels of calibration and transferred to GC vials. The concentration for each fatty acid varied according to the certificate of analysis. Nonadecanoat, C19:0 (Supelco) was used as an internal standard and added to every level of the calibration standard.

Standard and sample were injected into the 7890 GC System 5977 MSD (Agilent Technologies, USA) with Electron Impact (EI) source. Fatty acids were separated through a HP88 column (30 m x 0.25 mm x 0.25 um film thickness). GC oven temperature was set to increase from 50 °C to 180 °C with a 7.07 °C increase-rate-per-minute and 180 °C to 230 °C with a 7.07 °C increase-rate-per-minute. MSD was set in SIM mode. Fatty acid concentration was quantified by constructing 9 levels of the calibration curve with the internal standard calculation [13-15].

Statistical analysis

Statistical analyses were performed using the GraphPad statistical package. Variables were summarized using the mean±SD. Normal distribution was assessed with the Kolmogorov-Smirnov test. The *p*-value was calculated using the paired t-test for normal distribution and the Wilcoxon test for skewed data. Two-tailed *p*-values<0.05 were considered statistically significant.

RESULTS

The mean current age of the patients with SLE was 33±6.98 y. The mean age at the first visit was 28.75±7.14 y, while the mean age at diagnosis was 20.25±5.19 y. Two patients were overweight, and no patients were obese; musculoskeletal and renal abnormalities were found in most of the patients. This study also showed a trend of higher SBP and DBP in active condition, even though it was statistically insignificant (p>0.05).

Table 1: Characteristics of the patients in an inactive and active state of our hospital

	Patient SLE (n=4)	Inactive	Active	*p*-value
Current age, yo, mean±SD	33±6.98			
Age when diagnosed, yo, mean±SD	20.25±5.19			
Age when the first visit, yo, mean±SD	28.75±7.14			
Disease Duration, year, mean±SD	12.75±6.13			
BMI, kg/m^2, n (%)				
Normal (18.5–24.9)	2 (50%)			
Overweight (25.0–29.9)	2 (50%)			
Clinical Symptomatology, n (%)				
Mucocutaneous	1 (25)			
Musculoskeletal	3 (75)			
Renal	3 (75)			
Hematology	1 (25)			
Ocular	1 (25)			
SBP, mm/Hg, mean±SD		125±16.51	142.7±23.86	0.29
DBP, mm/Hg, mean±SD		77.5±10.6	89.33±16.77	0.34
ESR, mm/hour, mean±SD		48.75±26.4	43±16.27	0.77

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; yo= years old. N=54 patients. The *p*-value was calculated using the Paired t-test. This test for comparing the values of the patients in active and inactive conditions.

Table 2: Generally, LCFA and VLCFA increased in SLE patient in the active state

No	Free fatty acid	Patient SLE state		*p*-value
		Inactive	Active	
1	Alpha-Linolenic Acid (ALA) (µmol/l)	23.5±17.97	48.25±38.58	0.41
2	Eicosapentaenoic Acid (EPA)(µmol/l)	17.25±12.58	27.75±7.63	0.63
3	Docosahexaenoic Acid (DHA)(µmol/l)	158±106.10	234.25±83.51	0.44
4	Omega 3(µmol/l)	199±132.87	300.75±126.82	0.46
5	Linoleic Acid (µmol/l)	1680±569.11	2294.5±823.72	0.63
6	Gamma-Linoleic Acid (GLA)(µmol/l)	20.25±12.01	27.25±15.20	0.61
7	Dihomo-gamma-linoleic Acid (DGLA)(µmol/l)	74±50.99	92±12.19	0.58
8	Arachidonic Acid (AA)(µmol/l)	328.75±217.19	363±31.96	0.75
9	Omega 6 (µmol/l)	2103.75±812.08	2777±842.54	0.47
10	Oleic Acid (µmol/l)	1300±190.38	1773.75±866.35	0.4
11	Myristic Acid (µmol/l)	31.25±12.76	59.25±40.41	0.27
12	Palmitic Acid (µmol/l)	1355.25±317.53	1658.5±465.04	0.48
13	Stearic Acid (µmol/l)	420.5±32.07	475.25±120.75	0.47
14	Saturated (µmol/l)	1806.75±351.51	2193.25±617.39	0
15	Palmitoleic Acid (µmol/l)	345.5±80.59	477.25±239.17	0.43
16	Oleic Acid (µmol/l)	1300±190.38	1773.75±866.35	0.4
17	Monounsaturated (µmol/l)	1645.75±247.23	2251.25±1100.46	0.4
18	Polyunsaturated (µmol/l)	2302.75±942.75	3087.75±929.09	0.46
19	Total Fatty Acid (µmol/l)	5755.25±1477.52	7531.75±2622.24	0.44
20	Omega 6/Omega 3	20.975±22.25	9.5±3.46	0.63
21	AA/EPA	21.75±6.70	13.5±3.70	0.14
22	Omega 3 Index	3.25±2.22	4.25±1.26	0.42

Data presented as mean±SD, n=4. The *p*-value was calculated using the paired t-test for normal distribution and Wilcoxon test for skewed data. These tests for comparing values of the patients in an active and inactive condition.

Table 2 shows an increase in LCFA and VLCFA in the active pattern of SLE patients in general, although it was not different statistically ($p>0.05$). The total fatty acid fraction was 38% higher in an active condition, compared to inactive.

The prominent increases of fatty acid fractions were alpha-linolenic acid (inactive vs active: 23.25±17.97 vs 48.25±38.58 μmol/l), oleic acid (1300±190.4 vs 1774±866.3 μmol/l) and myristic acid (31.25±12.76 vs 59.25±40.4 μmol/l). Conversely, omega 6 and Omega 3 and AA/EPA index decreased in the active state (21.75±6.70 vs 13.5±3.70 and 3.25±2.22 vs 4.25±1.26, respectively).

DISCUSSION

SLE is an autoimmune disease that more often affects women of productive age [2]; this was in line with our results. We also showed renal abnormalities found in most of the patients. Renal abnormality is often found in SLE patients [7, 10]; this was also evident in the previous study, wherein they determined that 41.9% of the total 813 SLE patients suffered renal abnormalities [3].

In this current study, there was an increasing trend of LCFA and VLCFA in the active pattern of SLE patients in general. A higher level of total fatty acid was also found in this study. FFA was released from adipose tissue as a result of lipolysis; an increase of FFA can also be found in fasted [16, 17], in obesity that is the result of an increase of adipose tissue [18] or triggered by inflammation [19]. The concentration of FFA is higher in SLE patients compared to healthy subjects; this elevation is correlated with metabolic syndrome and insulin resistance, but not inflammation response [20]. Our study showed a further increase in total fatty acid, suggesting inflammation in the active condition triggers lipolysis. This condition can further increase the risk of metabolic syndrome and cardiovascular disease.

The prominent increases in fatty acid fractions were alpha-linolenic acid, oleic acid and myristic acid. Alpha-linolenic acid is a member of the omega-3 polyunsaturated fatty acid family and functions as an anti-inflammatory agent, neuroprotectant and antidepressant [21]. Oleic acid is a monounsaturated omega-9 fatty acid that can modulate inflammation responses [22]. The increase of both alpha-linolenic acid and oleic acid might be a response to inflammation in active conditions. Myristic acid is a saturated LCFA with a 14-carbon backbone, which has a role as a lipid anchor in bio-membranes [23]. Increasing myristic acid was positively correlated to higher cholesterol and triglyceride level in humans and increases the risk of coronary atherosclerosis [24]. A study by Posadas-Romero *et al.* showed high levels of saturated fatty acids in SLE patients, which is related to inflammatory and autoimmunity processes in the SLE disease [9]. We found a prominent increase in myristic acid in active condition of SLE patients; this condition could contribute to the risk of coronary atherosclerosis in these patients.

CONCLUSION

The serum of LCFA and VLCFA in SLE patients are tending to increase in flare condition.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

All authors have none to declare.

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