Untargeted Metabolomics Analysis using LC-MS-QTOF for Metabolite Profile Comparison between Patients with Myofascial Pain of Upper Trapezius Muscle versus Controls

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ABSTRACT

Objective: This study aims to identify different biomarkers of Myofascial pain syndrome (MPS) using untargeted metabolomics screening.

Materials and Methods: In a case-control study, serum samples from MPS patients (n = 19) and healthy controls (n = 10) were analyzed using reverse-phase liquid chromatography and mass spectrometry quadrupole time-of-flight (MS-QTOF). The resulted raw data was processed with Progenesis QI data analysis software. The HMBD database was used to identify the metabolites based on their fold change (>1.2), variable importance plot (>1) with $P < 0.05$. MetaboAnalyst 5.0 was used to generate metabolic network analysis for all identified metabolites.

Results: The MPS group reported significantly higher pain on visual analog scale when compared with control while most of the other routine blood chemical profiles were not different. Twenty-seven metabolites were analyzed and identified with untargeted metabolomics analysis which could distinguish MPS patients from healthy controls. Inosine and chenodeoxycholic acid were abundant in the MPS group, whereas the others were low. Metabolites were divided into three categories: lipids, nucleotides, and organic compounds. Possible MPS metabolites included lysoSM (sphingomyelin), lysoPC (lysophosphatidylcholine), lysoPE (lysophosphatidylethanolamine), triglyceride, and inosine.

Conclusion: These metabolite profiles, including glycerophospholipids mechanism and purine metabolism, indicate that the inflammatory process might be related to the mechanisms of MPS. A larger sample size, a different trigger point location, and modifications in therapy afterward should all be further explored.

Keywords: Myofascial pain; trigger point; metabolomics; untargeted metabolomics; mass spectrometry (Siriraj Med J 2022; 74: 792-803)

INTRODUCTION

Myofascial pain syndrome (MPS) is a type of musculoskeletal discomfort that is a widespread health issue and a cause of employee sick leave.[1] The prevalence of MPS is approximately 85% of the population. A clinical characteristic of MPS is the appearance of myofascial trigger points (TrPs). Trigger point is a hyperirritable muscular region accompanied by a sensitively detectable nodule in a taut band. The diagnostic criteria for identifying TrPs

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are a taut band, a hypersensitive area, and referred pain.[2] Trigger point also can cause spontaneous pain, referred pain, fatigue, reduced range of motion, and autonomic dysfunction, such as sweating or piloerection.[3,4] Trigger point can form in a variety of locations throughout the body. The neck and shoulder are the most prevalent sites for TrPs. In a previous study, TrPs of the upper trapezius muscles were observed in more than 80% of chronic nonspecific neck pain patients.[5] Trigger point could be triggered by overuse, muscle trauma, or psychological stress. The most typical causes include poor ergonomics, improper postural alignment, and continuous repetition of daily activities.[6] However, the initiation of TrPs remains unclear.

The mechanism of MPS and the pathogenesis of TrPs are not completely understood. In the TrP region, hypercontracture of the sarcomere was discovered.[6,7] Ultrasound techniques can also be used to determine anatomical and physiological changes relating to TrPs. In the non-TrP region, the tissue echo strength is the same as the surrounding structures or tissues. The TrP region shows solid tissue and a reduction in blood flow. It revealed a localized stiffness region of TrPs.[8] According to the integrated theory, it originated with excessive acetylcholine release from the motor endplate.[9] Increased acetylcholine release results in metabolic stress and an energy crisis. A deficit of energy causes longer sarcomeres to shorten, reducing blood and oxygen supply.[6] In addition, local hypoxia produces physiological abnormalities and cell stress; myokines, inflammatory cytokines, and neurotransmitters, such as bradykinin, substance P, tumor necrosis factor α, interleukin 1β (IL-1β), interleukin 6, serotonin, and norepinephrine, are produced.[10-12] The release of biochemical substances produces pain-activating nociceptors and alters autonomic regulation. This change produces tissue sensitivity and contributes to the persistence of the TrPs and MPS.[6]

Metabolomics is an omics analytical technique that identifies thousands of low-molecular-weight compounds using high-throughput technology.[13] It can be used for detecting metabolites representing human phenotypes in normal and pathological states and also suggesting the pathophysiological processes. It is widely used to identify biomarkers in biological samples that are relevant to disease biomarkers, pharmaceutical development, nutrition, and physiological process. Biomarkers illustrate why disease risk factors and etiologies have changed.[14] The mapping of biomarkers in the metabolic pathway enables diseases to be addressed.[15] Previous studies have found systemic changes in the metabolite profiles of patients suffering from nonspecific neck-shoulder pain and chronic widespread pain. Several metabolite changes suggest the relation with lipid metabolism and energy consumption.[16] Because there is no solid evidence of MPS pathogenesis, untargeted metabolomics research was performed to discover different metabolites of MPS. The objectives of this study are to (1) investigate possible differences in metabolomics profiles between MPS patients and a control group, and (2) analyze the pathways associated with MPS patients. It could be used to explore alterations in a variety of biochemical pathways, as well as to understand further about pathophysiology and treatments.

MATERIALS AND METHODS

Subjects

Twenty-nine subjects aged between 18 and 65 years were recruited at Siriraj Hospital, Bangkok, Thailand (10 control subjects and 19 MPS patients). The MPS group was recruited based on the diagnosis with myofascial pain of the upper trapezius muscle.[4] The followings criteria were considered for the MPS to be eligible for the study:

- Severity of pain greater than 40 on a visual analog scale for at least 3 months
- Presence of at least 1 TrP in the upper trapezius muscle identified by palpation that included palpable taut bands with local hypersensitivity and referral pain

Patients with other musculoskeletal diseases, tumors, psychological problems, metabolic diseases, pregnancy or lactation, previous neck or shoulder pain therapies or medicine, history of alcohol abuse and smoking, and abnormal liver or renal function at the time of screening were all excluded from the study. Healthy control subjects were non-MPS with the same exclusion criteria as those of the MPS group.

The study was approved by the Siriraj Institutional Review Board at Faculty of Medicine Siriraj Hospital, Mahidol University (Si259/2017).

Sample collection

Fasting blood samples were collected between 7 and 9 am to reduce variation in sample collection. Basal clinical laboratory profiles were assessed by central laboratory of the Siriraj Hospital. For metabolomics analysis, the blood was obtained in BD Vacutainer serum blood collection tubes (Becton Dickinson, Franklin Lakes, New Jersey) to initiate clotting. After centrifugation for 10 minutes at 4,500g at 4°C, the supernatant was collected, aliquoted, and kept at −80°C until analysis. The sample collection was performed in accordance with good clinical practice, which included ethical and safety considerations.

Sample preparation for metobolomics analysis

The MPS and healthy control samples were analyzed in random order. The protocol was modified from a previous study for metabolic profiling of serum using LCMS.[17] Serum samples were retrieved from the –80°C freezer and allowed to thaw on ice. After that, 50 µL of 1 ng/µL mixed internal standard solution (l-phenylalanine [13CC8H11N1O2], caffeine [13CC7H10N4O2], cholic acid [D4C24H36O5]) was added to a 200 µL serum sample. Serum samples were extracted by adding 600 µL of methanol. The samples were continuously mixed at 4°C for 15 minutes at 2000 revolutions per minute using a Multitube vortexer (BenchMixer; Benchmark Scientific Inc, Sayreville, New Jersey) and then frozen for 1 hour at –80°C. The samples were then centrifuged for 15 minutes at 4°C at 15,800g. Then, the supernatant was collected and dried in a speed vac (CentriVap Mobile System Console, Labconco, USA) for 300 mins at 30°C. After completely dry, samples were store at -80°C for analysis.

Quality control samples were utilized in the study to examine technical stability and repeatability. For quality control, 29 serum samples were pooled and extracted using the same methods as the individual samples.

Liquid chromatography and mass spectrometry quadrupole time-of-flight analysis

On the day of analysis, dried samples were reconstituted with 100 µL of water and shaken at 2000 revolutions per minute for 10 minutes at 4°C. The samples were centrifuged for 10 minutes at 4°C at 15,800g. The supernatant was transferred to a total recovery vial (Waters Corp, Milford, Massachusetts). Extracted serum samples were maintained the temperature at 4°C during analysis.

Extracted serum samples were analyzed using ultraperformance liquid chromatography system (Waters Corp, Wilmslow, United Kingdom) coupled with Synapt G2-Si QTOF mass spectrometer (Quadrupole Time-of-Flight; Waters Corp). The ultraperformance liquid chromatography system was performed using an Acquity HSS T3 column with a 100-mm length 2.1-mm internal diameter and 1.8 microns in particle size. The protocol for LCMS-QTOF analysis was modified from a previous study.[17] The temperatures in the column and sample manager were 40°C and 4°C, respectively. The gradient elution was mobile phases A1 and B1 at a flow rate of 0.4 mL/min. In the mobile phase, solvents A and B were 0.1% formic acid in water (vol/vol) and 0.1% formic acid in MeOH (vol/vol), respectively. The elution gradient was as follows: initial conditions at 100% A1; from 1 to 16 minutes, increased to 100% B1; from 16 to 20 minutes, maintained at 100% B1; from 20 to 22 minutes, increased to 100% A1; and from 22 to 24 minutes, maintained at 100% A1.

For mass spectrometer, the instrument was used in resolution mode with the MSe data acquisition function with electrospray ionization in positive and negative ion modes. The parent ion's trap collision energy was 6 electron volts (eV), whereas the fragmentation's ramp trap collision energy was 15 to 40 eV. The capillary voltage was set to 3 kV, whereas the sampling cone voltage was set to 40 V. The desolvation gas temperature was set at 500°C, with the source temperature set at 150°C. The desolvation gas flow rate was set at 1000 L/h, whereas the cone gas flow rate was set at 50 L/h.

Biomarker identification and pathway analysis

Progenesis QI data analysis software (Nonlinear Dynamics, Newcastle, United Kingdom) was used to import and process the raw data. A search of the Human Metabolomics Database, HMDB 5.0 (https://hmdb.ca) was used to identify compounds. MetaboAnalyst 5.0 (www.metaboanalyst.ca) was used to perform metabolic network analysis on all chemical identifications.

Progenesis QI data analysis software (Nonlinear Dynamics, Newcastle, UK) was used to import and process the raw data. All of the samples were aligned to ensure that retention duration, mass measurements, isotopic composition, and fragmentation were all correct. The experiment comprised a between-subject design with an unpaired T-test for the different groups (CON versus MPS). The following criteria were used to select compounds: p-value < 0.05, fold change between two groups >1.2. The significant metabolites were selected.

Multivariate Analysis (MVA) and Univariate Analysis were used to examine all chemical values in normalized compound abundances (UVA). The scale was Pareto for principal component analysis (PCA) and orthogonal partial least-square discriminant analysis (OPLS-DA). S-plots were used to filter metabolites biomarkers when p (1) and p (corr) (1) from the OPLS-DA model were > ±0.04. The variable importance plot (VIP) > 1 was chosen. After matched the significant metabolites with selection biomarkers from S-plot. The metabolites biomarkers were searched from the online Human Metabolomics Database (HMDB 5.0). The precursor tolerance and fragmentation tolerance were both set to 20 ppm, with a retention time of less than 0.1 minute. When the isotope similarity was greater than 70%, the identification was confirmed.

MetaboAnalyst 5.0 (www.metaboanalyst.ca) was used to perform metabolic network analysis on all chemical identifications. Significant pathways had a p-value less than 0.05 and impact more than 0.05.

Statistical analysis

The metabolite profile was assessed using Multivariate Analysis and Univariate Analysis, as previously described. All demographic and other parameters were evaluated using SPSS software (PASW version 18; IBM Corp, Chicago, Illinois). To test the normalization of data distribution, the Shapiro-Wilk test was performed. An unpaired t test or Chi-square was used to compute the differences between groups. $P < 0.05$ was considered statistically significant.

RESULTS

Subject demographics and clinical profiles

The demographic parameters and blood chemical profiles of the subjects under study are shown in Table 1. Subject characteristics differed between MPS and healthy controls in age and gender, but not in body mass index. Myofascial pain syndrome and healthy controls had significantly different pain intensity and pain duration. Most of the blood profiles were not different between groups. Creatinine in the MPS group was significantly lower than that of the healthy control group ($P = 0.015$). Multiple linear regression was used to adjust for age and gender, and the results revealed that there is no association between biomarkers and these variables ($P = 0.573$ for age, $P = 0.393$ for sex).

TABLE 1. Demographic and Clinical Characteristics of the Study Population.

Characteristics	Healthy controls (CON)	Myofascial pain syndrome (MPS)	*P*-value
Number of subjects	10	19	-
Male/female (%)	30/70	0/100	0.033#
Age (years)	26.90 (3.45)	33.89 (8.80)	0.005*
Body mass index (kg/m^2)	22.04 (2.14)	21.46 (3.22)	0.612
Pain parameter			
Visual analog score	0	49.55 (11.40)	<0.001*
Pain duration (months)	0	5.53 (2.51)	<0.001*
Blood chemical profiles			
Aspartate aminotransferase (U/L)	17.20 (3.97)	18.77 (8.04)	0.567
Alanine aminotransferase (U/L)	16.96 (11.52)	17.61 (12.10)	0.890
Alkaline phosphatase (U/L)	58.22 (9.54)	58.13 (18.33)	0.988
Albumin (g/dL)	4.64 (0.17)	4.58 (0.21)	0.458
Blood urea nitrogen (mg/dL)	10.50 (2.16)	9.60 (1.83)	0.244
Creatinine (mg/dL)	0.81 (0.14)	0.69 (0.10)	0.015*
Glucose (mg/dL)	89.82 (5.65)	88.41 (6.28)	0.558
Triglyceride (mg/dL)	64.41 (19.91)	80.52 (50.80)	0.347
High-density lipoprotein-cholesterol (mg/dL)	64.55 (16.82)	66.01 (15.44)	0.816
Cholesterol (mg/dL)	175.91 (19.93)	175.68 (25.55)	0.981
Creatine kinase (U/L)	104.20 (45.50)	81.79 (43.40)	0.204
Mb isoenzyme of creatine kinase (ng/mL)	1.10 (0.49)	1.10 (0.41)	0.985
Lactate (mmol/L)	1.16 (0.76)	1.06 (0.59)	0.689

Data are presented with mean (SD) unless otherwise noted.
*Significant $P < 0.05$ with unpaired *t* test.
#Significant $P < 0.05$ with χ^2.

Metabolomics differences between MPS and healthy control subjects

A data matrix of 19,643 peak compounds was obtained after alignment and filtering processes. A clustering of samples was observed using MVA. PCA was employed to summarize data and present an overview of each sample, each plot on the PCA represents a sample. The OPLS-DA approach was utilized to compare two groups, In the OPLS-DA model, the x-axis represented a score scatter plot. The horizontal direction of the provided score scatter plot represents group variation. The principal component analysis score plot was unable to differentiate between MPS and healthy control groups (Figs 1A, B). The metabolomics data were analyzed using supervised MVA and visualized with OPLS-DA. The OPLS-DA revealed that MPS and healthy controls could be differentiated (Figs 1C, D). S-plots were generated from the OPLS-DA model to visualize the distance between differential metabolites (Figs 1E, F).

A total of 40 metabolites were investigated as possible biomarkers. Twenty-seven metabolites were analyzed and identified using HMDB to differentiate individuals with MPS from healthy controls (Table 2). In the MPS group, inosine and chenodeoxycholic acid were highly abundant, whereas the others were lower.

The hierarchical clustering and heat maps of the 27 metabolites were different in their peak intensity between healthy controls and MPS (Fig 2). The heat map displays the relative abundance of each identified compound in each sample. The association between samples represented by the color and horizontal dendrogram. It splits samples into two primary groups, with the left showing high intensity of metabolites or mean increase metabolites that were largely associated to the CON group, and the

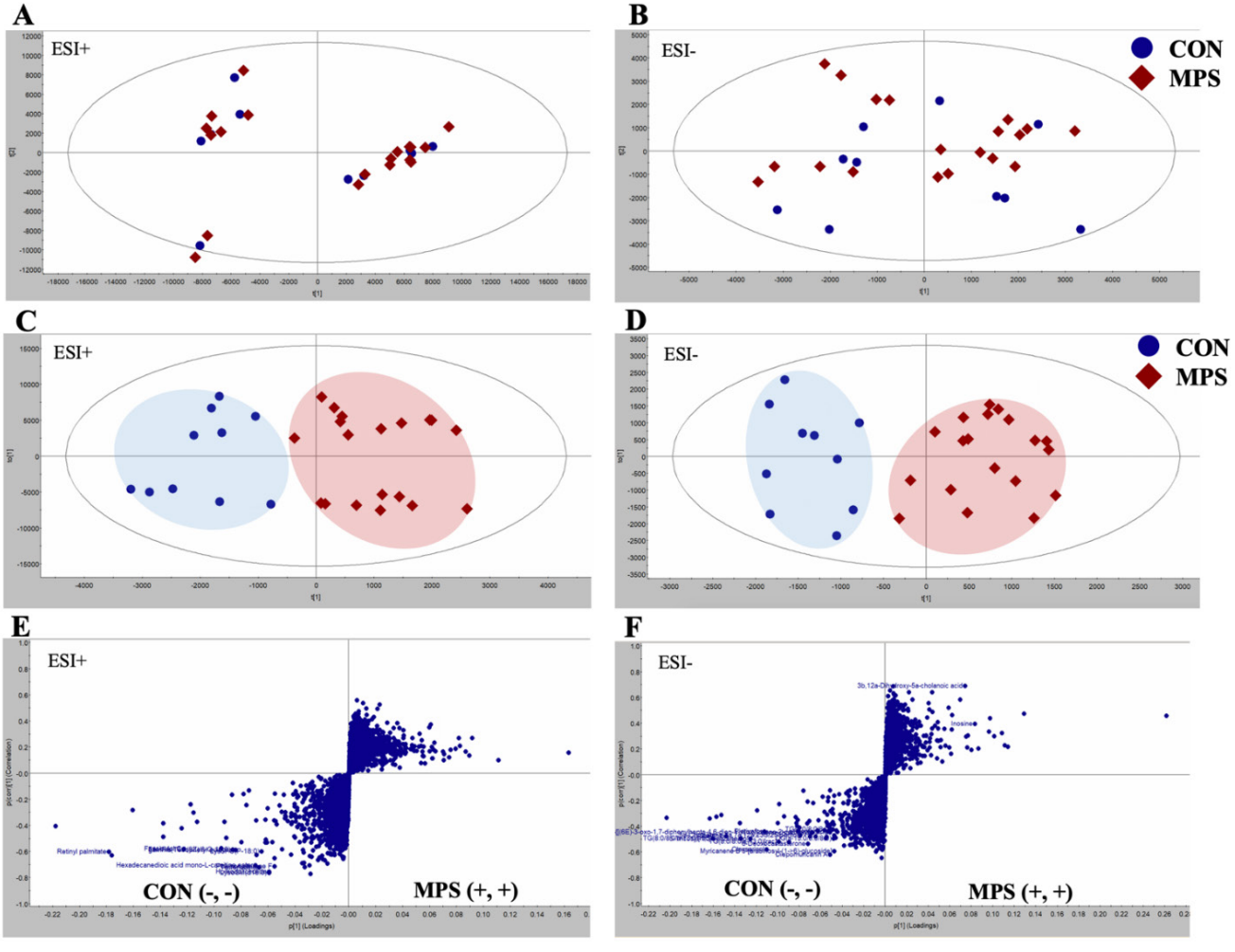

Fig 1. Multivariate analysis for the myofascial pain syndrome (MPS) patient group differentiates from the healthy control group based on all detected metabolites. A and B, Principal component analysis score plot of (A) positive and (B) negative electrospray ionization (ESI) modes. C and D, Orthogonal partial least-squares discriminant analysis score plot of (C) positive and (D) negative ESI modes. Healthy control group (CON, blue dots). Myofascial pain syndrome group (red diamonds). E and F, S-plot of (E) positive and (F) negative ESI modes. In positive and negative ESI, the dependent variables (*Y*) were $R^2 = 0.849$, $Q^2 = -0.058$ and $R^2 = 0.796$, $Q^2 = 0.381$, respectively. The model had a moderate prediction accuracy, and the negative value of Q^2 prevented overfitting. It was also stable and reliable.

TABLE 2. Untargeted liquid chromatography and mass spectrometry indicating significant differences in metabolites between MPS patients and healthy controls.

Metabolite identification	m/z	*P*-value	Fold change	Trend in MPS	Metabolite class
Inosine	267.0719	0.039	1.73	Up	Purine nucleosides
Chenodeoxycholic acid	391.2828	0.007	2.05	Up	Steroids and steroid derivatives
Sunitinib	397.2034	0.017	1.63	Down	Organoheterocyclic compounds
Clausarinol	413.1984	0.003	1.67	Down	Coumarins and derivatives
Momordol	421.3355	0.039	1.3	Down	Fatty Acyls
gamma-Tocopheryl quinone	433.3663	0.029	1.31	Down	Prenol lipids
3,4,5-trihydroxy-6-{[(6E)-3-oxo-1,7-diphenylhepta-4,6-dien-1-yl]oxy} oxane-2-carboxylic acid	435.1444	0.036	1.34	Down	Phenylpropanoids and polyketides
DG(8:0/15:0/0:0)	441.3553	0.042	1.47	Down	Glycerolipids
Trihydroxycoprostanoic acid	447.344	0.046	1.2	Down	Steroids and steroid derivatives
6-Deoxocastasterone	449.3617	0.022	1.37	Down	Steroids and steroid derivatives
5b-Cholestane-3a,7a,12a,23S,25-pentol	451.3413	0.036	1.33	Down	Steroids and steroid derivatives
Polyporusterone F	463.3357	0.024	1.28	Down	Steroids and steroid derivatives
5-Tricosyl-1,3-benzenediol	467.3707	0.014	1.29	Down	Phenols
TG(8:0/8:0/8:0)	469.352	0.039	1.2	Down	Glycerolipids
Cholestane-3,7,12,24,25-pentol	475.3364	0.018	1.31	Down	Steroids and steroid derivatives
LysoSM(d18:0)	489.3488	0.016	1.37	Down	Sphingolipids
LysoSM(d18:0)	489.3518	0.017	1.3	Down	Sphingolipids
Fasciculol C	491.3678	0.025	1.26	Down	Prenol lipids
LysoPE(0:0/20:4(5Z,8Z,11Z,14Z))	500.2784	0.041	1.22	Down	Glycerophospholipids
LysoPC(P-18:0)	508.3731	0.037	1.28	Down	Glycerophospholipids
TG(8:0/8:0/13:0)	521.4131	0.01	1.34	Down	Glycerolipids
Plerixafor	537.4156	0.024	1.39	Down	Benzene and substituted derivatives
TG(13:0/8:0/8:0)	539.4305	0.022	1.43	Down	Glycerolipids
FAHFA(16:1(9Z)/8-O-18:0)	559.466	0.043	1.43	Down	Fatty Acyls
TG(8:0/8:0/a-13:0)[rac]	563.4279	0.034	1.4	Down	Glycerolipids
Diepomuricanin A	581.4449	0.002	1.25	Down	Fatty Acyls
Myricanene B 5-[arabinosyl-(1->6) -glucoside]	615.2468	0.002	1.39	Down	Phenylpropanoids and polyketides

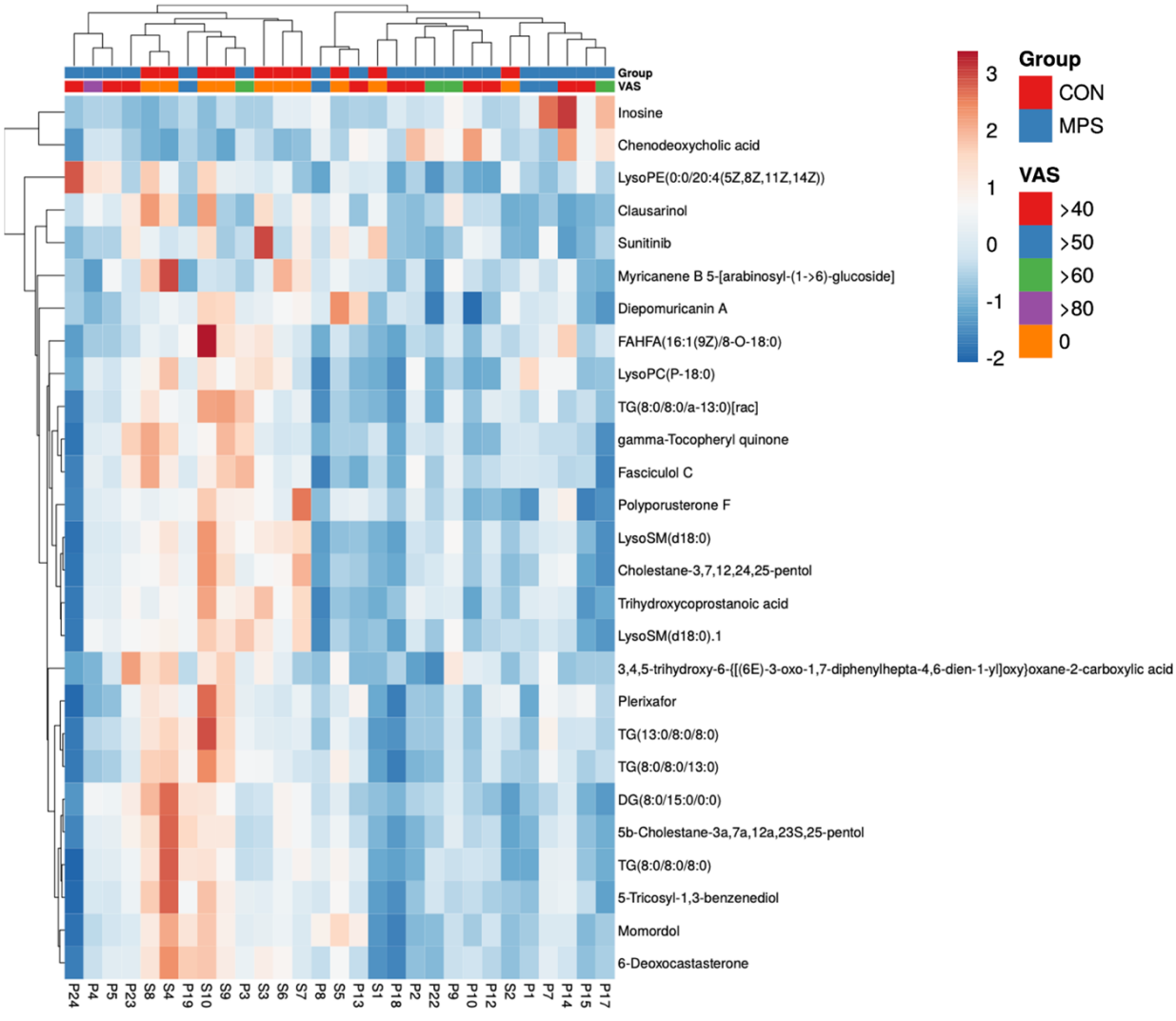

Fig 2. Heat maps of the significantly different metabolites between the healthy control (CON) (n = 10) and myofascial pain syndrome (n = 19) groups. *X* axis represents individual samples. *Y* axis represents 27 metabolites. Red represents high normalized intensity, and blue represents low normalized intensity of the metabolite. Color bars on *X* axis show visual analog scale (VAS) score.

right showing low intensity or decrease metabolites that were mostly related to the MPS group. The color bars representing each subject's VAS score were mapped to the X-axis. A horizontal dendrogram and a VAS scale were used to investigate the relationship between two groups. The VAS scale had a range of >40 to >80. In the MPS group, pain severity may be related to increased metabolite intensity. Vertical dendrograms indicated a relationship between compounds that our results could not be observed.

There were significant differences in the amount of possible biomarker metabolites that were linked to the MPS mechanism when comparing the MPS group with the control group as shown in Fig 3. Using MetaboAnalyst 5.0, the metabolites revealed MPS-related pathways, including glycerophospholipids mechanism, primary bile acid biosynthesis, and purine metabolism (Fig 4).

DISCUSSION

Mechanism of MPS is not well understood. This study used the untargeted metabolomics analysis with liquid chromatography and mass spectrometry quadrupole time-of-flight to identify possible biomarkers of MPS of the upper trapezius muscle in a case-control study. Nineteen subjects with chronic MPS of upper trapezius muscle for at least 3 months were recruited. All subjects have an average pain intensity of 50 on the visual analog scale, whereas there was no pain in the control groups. All MPS subjects were female of the young-adult group (average age, 33 years), which was the common age group for MPS.[18] The control groups were mostly female (70%) with lower average age than the MPS group. The baseline clinical laboratory profiles were within reference range and mostly similar between the two groups except the creatinine, which was lower in the MPS group.

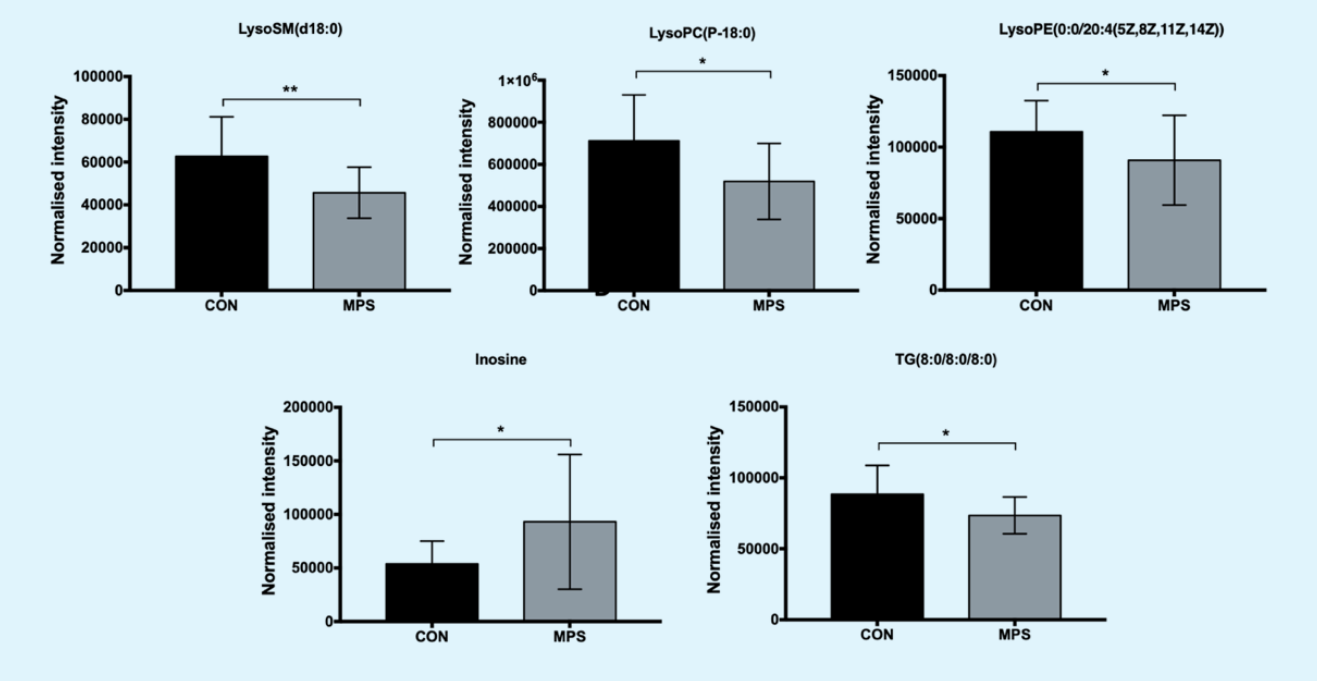

Fig 3. Significant differences in the amount of possible biomarker metabolites in the myofascial pain syndrome group (MPS) compared with the control group (CON) (*$P < 0.05$ and **$P < 0.01$).

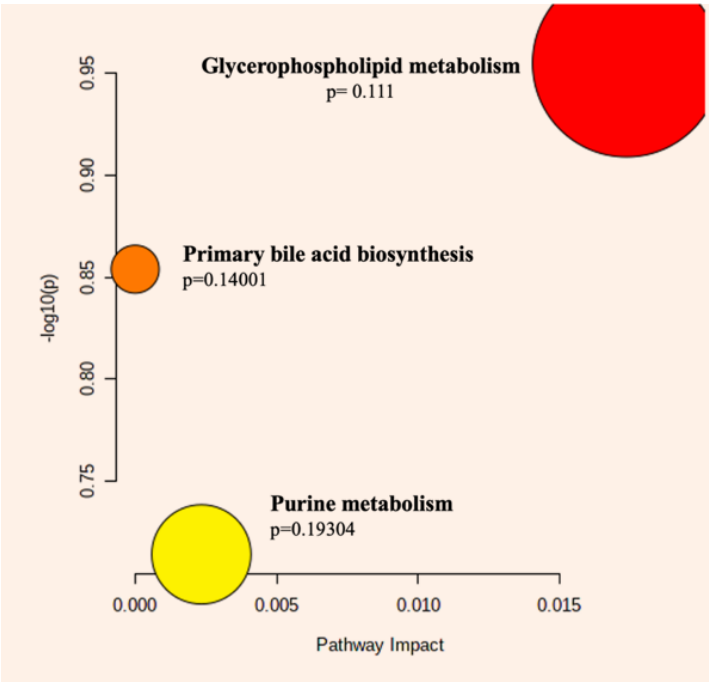

Fig 4. Pathways involving 27 metabolites that distinguish MPS patients from healthy controls were identified. Metabo Analyst 5.0 was used to create the plot. The *x* axis shows impact of the discovered metabolites on the indicated pathway. The *y* axis shows relatively abundant discovered metabolites are in the designated pathway. The significance of pathway enrichment is shown by the color of the circles. The size of the circle shows the pathway's impact.

Disease status is pathogenic and manifests characteristics that can be identified through biomarkers. As a result, metabolomics is a clinical research tool that may detect components in health or disease in order to understand the mechanism. It can map the metabolites associated with pathologies along the metabolic pathway. In this study, the possible metabolites involved mainly in glycerophospholipid metabolism and purine metabolism differed between healthy subjects and MPS patients. The metabolism of glycerophospholipids is linked to the structure of the cell membrane.[19,20] Metabolites linked to glycerophospholipid metabolism in MPS include lysoSM (sphingomyelin), lysoPC (lysophosphatidylcholine), lysoPE (lysophosphatidylethanolamine), and triglyceride (TG). Purine metabolism has an impact on all living organisms because it produces components for DNA and RNA.[21,22] Metabolite linked to purine metabolism in MPS is inosine.

The study discovered a decrease in lysoSM in MPS patients. LysoSM is involved in signal transduction of the axon nerve. It is a type of sphingolipid that helps to regulate immune cell function during inflammation.[23] Sphingomyelin is regulated by a protein called sphingomyelinase, which breaks down sphingomyelin into ceramide. Ceramide and sphingomyelinase play a role in the enhancement of proinflammatory cytokines, which cause inflammation.[24] Sphingolipids involved in sphingomyelin-ceramide metabolism were shown to be higher in chronic neuropathic pain patients.[25] Ceramide levels and sphingomyelin degradation both increased. The level of sphingomyelin may be affected by the upstream pathway. Total ceramides were also found to be lower in individuals with episodic migraine compared with controls, whereas sphingomyelin species were higher.[26] In patients with nociceptive and neuropathic pain, sphingomyelin and lipid metabolites were altered.[27,28] The decrease in lysoSM could be due to the conversion of sphingomyelin to ceramides, including this study. As a result, a rise in ceramide causes an increase in inflammation. It can lead to pain-related peripheral and central sensitization in MPS patients.

In MPS patients, we discovered a reduction in lysoPC and lysoPE. LysoPC is a lipid signaling molecule that interacts with lysophospholipid receptors.[29] LysoPC and lysoPE are involved in the inflammatory response.[30] A previous study found that, after medication therapy, lysoPC levels increased in arthritis knee patients. An elevation in LysoPC can exacerbate the inflammatory response.[31] LysoPC levels were shown to be higher in fibromyalgia patients in a previous metabolomic investigation. It was hypothesized that it possessed proinflammatory compounds and produced reactive oxygen species, which were linked to fibromyalgia pathogenesis.[32] LysoPC could be used as a fibromyalgia biomarker. It works by causing the release of proinflammatory cytokines including tumor necrosis factor α and IL-1b through the platelet-activating factor receptor. In fibromyalgia, LysoPC may play a role in allodynia and atypical pain.[33] The decreases in lysoPC and lysoPE were similar to those seen in migraine patients in a previous study. In migraine patients, lysoPC and lysoPE concentrations were lower. The decrease in lysoPC and lysoPE was thought to be linked to a reactive oxidant species imbalance. In migraine, oxidants can activate phospholipase A_2, which can affect the production of lysoPC and lysoPE. They have been suggested as migraine biomarkers.[34] Furthermore, the decrease in lysoPC could be due to the conversion of lysophosphatidic acid via autotaxin. Lysophosphatidic acid can cause descending pain inhibition.[35] The drop in lysoPE could be due to a drop in phosphatidylethanolamines, which would result in a drop in endocannabinoids. As a result, pain inhibition is reduced.[36] As a conclusion, the pathophysiology of pain in MPS patients may be explained by a decrease in lysoPC and lysoPE.

In MPS patients, we discovered a reduction in TG. Triglyceride is a kind of triacylglycerol that has a role in the inflammatory process.[37] Inflammatory macrophage function is regulated by TG synthesis, which results in the release of proinflammatory mediators such as IL-1, IL-6, and prostaglandin E_2. The suppression of the TG metabolite is a marker of anti-inflammation and a therapy target.[38] Triglyceride levels were shown to be higher in patients with peripheral and central sensitization in a previous study.[39] Phosphatidylcholine levels can impact TG storage.[40] Because lysoPC levels were found to be lower in MPS patients, lipid products and TG levels may also be lower. As a result, the level of lipid metabolites may be linked to MPS, and therefore more investigation is necessary.

In MPS patients, we discovered an increase in inosine. Inosine is a component of the purinergic system. Adenosine is transformed into inosine. Adenosine is phosphorylated again to form adenosine monophosphate. Adenosine monophosphate is transformed to adenosine triphosphate, which is the primary source of energy in cells.[41] Neuroprotective, cardioprotective, and cytoprotective properties of adenosine have been reported.[42] In tissue damage and muscle injury, adenosine has been shown to suppress the release of proinflammatory cytokines. Antinociception is a property of adenosine. It affected pain behavior and pain modulation by adenosine receptors, as shown in a previous study.[43] This study found a significant elevation in inosine, which was consistent with the previous report in fibromyalgia patients.[44] The rise in inosine is due to adenosine conversion. Increased inflammatory mediators and pain sensitivity are caused by a decrease in adenosine.[41,45] In chronic myofascial pain, an increase in inosine could indicate a decrease in cell proliferation. It induces proinflammatory cytokines to infiltrate the tissue. Furthermore, inhibiting adenosine deaminase may help to balance the adenosine-to-inosine ratio, which can serve to reduce pain.[41] However, this study found a wide range of SD that inosine might be characterized as a possible metabolite biomarker, and future targeted analysis should be investigated.

Lipids and purine metabolites were determined to contribute to MPS and could be used as biomarkers and diagnostic tools in this research. Myofascial pain syndrome is a type of localized pain disorder, but this research revealed metabolic biomarkers linked to the

inflammatory process and pain mechanism. This could imply that MPS causes inflammation not only in the muscle but also in the system as a whole. It has the potential to be used as a target therapy. To confirm the identification of metabolites with fragmentation and standard, all possible biomarkers should be investigated for targeted analysis in future research. The average pain score for MPS patients is half of the maximal pain score. One of the biomarkers that could be connected to pain sensitivity is inosine levels. However, we were unable to detect a correlation between metabolite levels and pain severity in our research.

This study used a reproducible workflow for untargeted LCMS analysis in serum samples. It covered polar and non-polar compounds observed from internal standard spikes. The linearity was observed at 3 different concentrations of pooled QCs. The robustness of 60 pooled QC samples was compared. Non-polar compounds were similar to previous studies of fibromyalgia patients[33,45] for the biomarkers that we can detect the difference between CON and MPS. However, we were unable to identify any amino acids that could be linked to glutamate receptor[46,47], such as tryptophan[48], arginine[49], L-leucine[50], and carbohydrate[16], as previously found in chronic musculoskeletal pain studies. Furthermore, the method of amino acid investigation and identification should be further investigated. Additionally, future studies in a broader demographic population could confirm whether amino acid compounds are MPS biomarkers.

In this investigation, there were no significant differences in the laboratory testing of blood chemical profiles between those with no MPS and MPS patients. Metabolomics research was more sensitive in identifying disease biomarkers. Although the relationship between metabolites and MPS pathophysiology could not be demonstrated directly in this study, the findings could imply the involvement of a systemic pain and inflammatory mechanism. Future study should investigate on targeted metabolomics analysis of compounds related to MPS pathogenesis. Our investigation was limited by a small sample size due to the study strict control of all factors that potentially interact with metabolites. Another limitation was that the study only included female MPS of varying ages with CON. The impact of sex and age on the metabolites should be investigated. As a consequence, a larger sample size should be explored to see the pattern of MPS metabolite alterations.

CONCLUSION

This untargeted metabolomics profiling study revealed serum metabolites implicated in lipids and purine metabolites in MPS. LysoSM, lysoPC, lysoPE, TG, and inosine were all possible MPS metabolites. Mechanism of MPS is not well understood. However, the

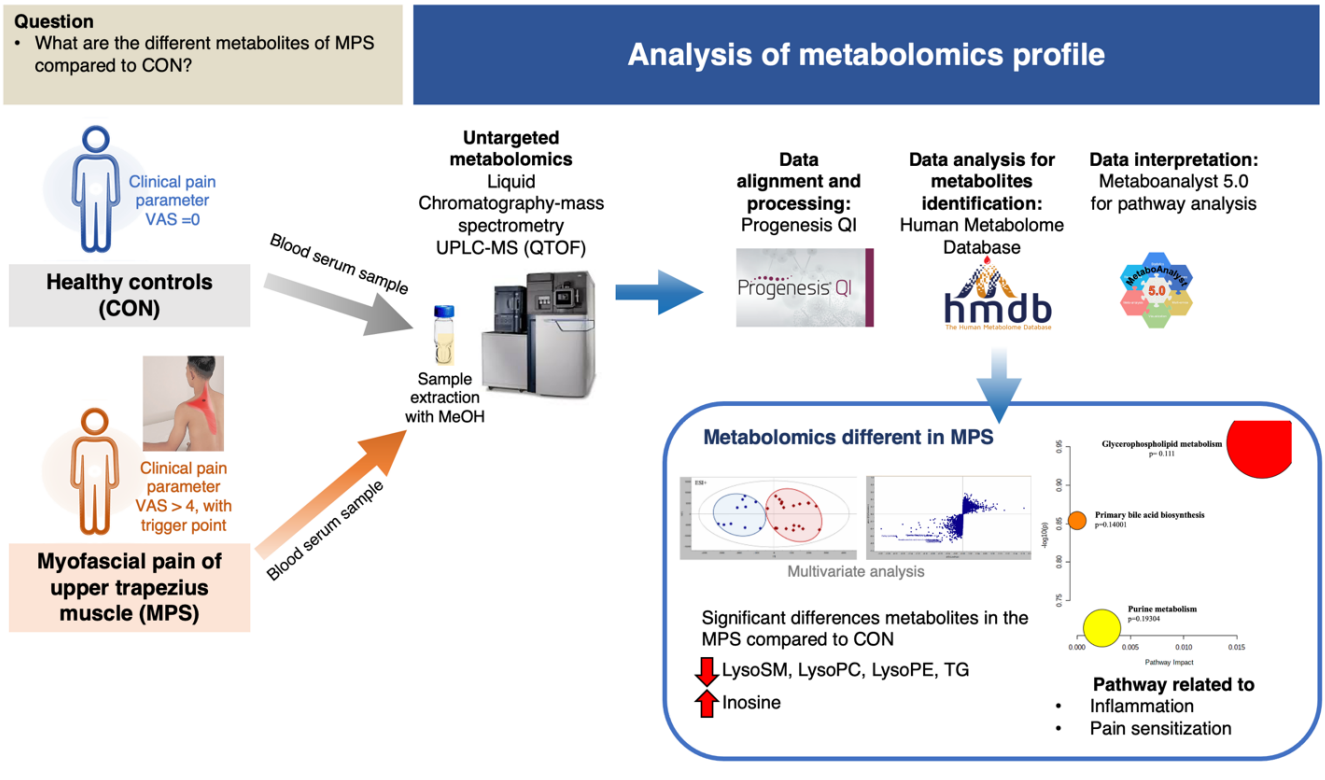

Fig 5. Summary of the study

key biomarkers are linked to the inflammatory process and the mechanism of pain. The findings of this exploratory study reveal that changes in metabolites in the MPS of the upper trapezius muscle differ from those in the control group. A larger sample size, a different TrP site, and changes in subsequent therapy should all be investigated further.

Conflicts of interest: The authors declare no conflict of interest.

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