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ORIGINAL ARTICLE

Investigation of the relationships between knee osteoarthritis and obesity via untargeted metabolomics analysis

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Abstract

Objective Osteoarthritis (OA), the most encountered arthritis form, result from degeneration of articular cartilage. Obesity is accepted as a significant risk factor for knee OA (KOA). In this study, it is aimed to determine the variation of metabolites between control and patients with KOA and observe the effect of obesity on KOA via untargeted metabolomics method.

Methods Serum samples of following groups were collected: patient group including 14 obesity (OKOA) and 14 non-obesity (NOKOA) ($n = 28$) and control group ($n = 15$) from orthopedics and traumatology policlinic. Serum proteins were denatured by acetonitrile and chromatographic separation of metabolites was achieved by LC/Q-TOF/MS/MS method. Data acquisition, classification, and identification were achieved by METLIN database. Cluster analysis was performed with MATLAB2017a-PLS Toolbox 7.2.

Results Obtained results showed that 244 (patient vs control) and 274 (OKOA vs NOKOA) *m/z* ratios were determined in accordance with LC/Q-TOF/MS/MS analysis. Multivariate data analysis was applied 41 and 36 *m/z* signal ($p \leq 0.01$; fold analysis > 1.5) were filtered for patient vs control group and OKOA vs NOKOA, respectively. Twenty-one different metabolites were identified for patient vs control group and 15 metabolites were determined for OKOA vs NOKOA group.

Conclusion Acid concentration and oxidative stress agents were high in inflammation group and their levels were much higher in obesity. It is claimed that obesity cause oxidative stress and acidosis in arthritis patients. Valine was found to be the only BCAA molecule whose concentration has significantly different in KOA patients. The relation between KOA and obesity was firstly investigated with metabolomics method.

Keywords Knee osteoarthritis · LC/Q-TOF/MS/MS · Untargeted metabolomics

Introduction

Osteoarthritis (OA) is a multifactorial, chronic and degenerative disease characterized by degeneration of articular cartilage and disruption of the normal balance between the events of making and destroying the periarticular bone, together with metabolic, genetic and other influencing factors [1]. Knee OA (KOA) is the most common form of OA [2]. KOA, triggered by biochemical and mechanical factors, is a metabolic active and dynamic process in which destruction and repair are observed together. In KOA formation, not only joint cartilage but also processes such as eburn, sclerosis, osteophyte, subchondral cyst and synovial inflammation are seen together in the subchondral bone [3, 4]. The destruction of the balance between matrix construction and degradation leads to the eventual tissue damage in KOA [5–7].

However, KOA 's pathophysiology is still not fully understood [8]. The most important risk factors for developing KOA are age, sex, obesity and inflammation [9]. Obesity, an important risk factor for KOA development and progression, has recently been considered a low-grade inflammatory disease [10]. Abnormal mechanical loading on the joint with obesity may result in changes in cartilage matrix composition and joint degeneration. At the same time, obesity causes deterioration in the joint

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biomechanics [11]. In the recent past, strong epidemiological data showed that people with obesity had about twice the risk of hand OA compared to normal weight people [12]. For this reason, the role of obesity in OA is much more complex than previously thought because it does not fully explain the risk of overload [13]. The presence of a new phenotype associated with obesity-related osteoarthritis and low-grade inflammation has become a focus of interest in recent years in the areas of pathophysiology, diagnosis, prognosis, treatment, and prevention [14].

Metabolomics were described as a comprehensive investigation of small molecules, i.e. metabolites, in cells, biofluids, tissues, food samples, plants and any suitable medium [15, 16] by using different analytical methods (mass spectrometry (MS) coupled with gas chromatography (GC) or liquid chromatography (LC) and nuclear magnetic resonance (NMR)). In osteoarthritis, various metabolites can be identified in plasma or synovial fluid by different methods. But, there were various advantages and disadvantages of determining these biomarkers in synovial fluid or peripheral blood. In most clinical studies, plasma/serum samples were preferred for metabolomics research due to its advantage that are easily obtainable and monitoring overall metabolic behavior [17]. Synovial fluids were specific sample that are only show metabolic behavior of local region that is hard to collect. Furthermore, collecting synovial fluid from healthy volunteers is risky and has ethical drawbacks [18]. Therefore, serum samples are more preferred in most studies [17].

Among these analytical methods, LC/MS methods coupling quadrupole time-of-flight (Q-TOF) permit analysis of more metabolites such as from peptides to hydrophilic organic acids, even to hydrophobic lipids [19]. Accordingly, metabolites and their interaction with this medium were called “metabolome” [20]. The fingerprinting strength of metabolomics makes it a crucial tool for explaining living systems, health and diseases. Metabolomics applications maintain to improve and provide unprecedented opportunity for both scientists and clinicians in different field. This developing technology is currently applied in disease research, gene therapy, pharmaceutical drug discovery, multi-omics studies, food and agriculture. Metabolomics also take an important role in clinical applications in understanding the mechanism of any case, diagnose, and treatment [21].

Several untargeted metabolomics studies were reported about OA [22–24]. However, this study has become the first untargeted metabolomics research to discuss the relationship between obesity and KOA.

The purpose of this study was to determine the variation of metabolites between control and patients suffered from KOA and then observe the effect of obesity on KOA via untargeted metabolomics method.

Materials and methods

Patients selection and serum isolation

Twenty-eight patients with KOA (14 obesity (OKOA) and 14 non-obesity (NOKOA)) of Erzurum Regional Training and Research Hospital (Erzurum, Turkey) were participated in the study between April 2018 and June 2018. The ethical committee of medicine faculty of the Atatürk University approved the study (Ethics committee approval date: 25.04.2018, decision no. 164). Written informed consent before starting this study was obtained from all participants. The patient group composed between 40 and 60 years and diagnosed with KOA in accordance with the American College of Rheumatology clinical criteria [25]. Patients who had autoimmune diseases, post-infectious or post-traumatic arthropathy active malignancy, systemic inflammatory or infectious diseases, cardiovascular diseases, chronic liver diseases, renal diseases, a history of blood transfusion during the last 3 months, and patients who underwent total knee prosthesis were excluded from the study. According to BMI, patients were divided into two groups as patients with BMI ≥ 30 (OKOA) and as patients with BMI < 30 (NOKOA). The same orthopedic physician examined both groups and blood samples were taken from each patient in morning after fasting for 8 h to avoid metabolite alteration caused by nutrition. Fifteen healthy people (any arthrosis findings were detected in accordance with KL radiological score) in the same age group who applied to the same orthopedics clinic were included in the study as a control group. Routine hemogram and biochemistry parameters (sedimentation rate (ESR), C-reactive protein (CRP), white blood cell (WBC)) were recorded and blood samples were centrifuged at 3000*g* for 15 min and 300 μL of resulting serum samples were aliquoted and stored at − 80 °C until LC/Q-TOF/MS/MS analysis.

Untargeted metabolomics study with LC/Q-TOF/MS/MS

Preparation of serum samples

For untargeted metabolomics studies of all serum samples with LC/Q-TOF/MS/MS, we employed the following protocols: 100 μL of thawed serum samples homogenized on vortex mixer, mixed with 300 μL of acetonitrile and incubated on ice for 15 min to denature the serum proteins. Centrifugation was performed at 16.000*g* for 15 min, 4 °C. The supernatant was carefully separated to Eppendorf vials and dried out at 45 °C in concentrator. Carbamazepine was selected as the internal standard (IS) to improve data quality and checking the autonomous integration success of the software. These dried samples were re-dissolved in 1000-μL mixture of acetonitrile and ultra-purified water (0.1% formic acid)

containing IS (1 μM). Blank sample was prepared with mobile phase mixture containing only IS. In addition to this, quality control (QC) and QC-Support Vector Regression (SVR) samples were prepared according to the above procedure after 5 μL of each samples was collected. Blank sample was injected to observe any interference due to instrument and media. QC and QC-SVR samples were injected on LC/Q-TOF/MS/MS system in order to remove false positives from the system.

Instrumentation and conditions

The LC/Q-TOF/MS/MS system considered of an Agilent 1290 Infinity LC system coupled with Agilent 6530 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) mass spectrometer (Agilent, USA) and Hypersil C18 column (100 mm × 2.1 mm, 1.9 μm) (Agilent, USA). LC mobile phase consisted of 0.1% formic acid ultra-purified and acetonitrile. A mobile phase flow rate of 0.4 mL/min was employed for gradient elution at total 15-min run time. The column temperature was set 55 °C during the analysis. The positive ion scan mode at 3.5-kV capillary voltage was applied during the study. Mass scanning was ranged from 50 to 1000 *m/z*. The ion source was Dual Agilent Jet Stream (AJS) Electron Spray Ionization (ESI). MS absorbance threshold was set at 200. The instrument acquired data by optimized parameters as: drying gas temperature, 350 °C; drying gas flow, 11 L/min; nebulizer, 40 psi; sheath gas temperature, 350 °C; sheath gas flow, 11 L/min. A constant flow of Agilent TOF reference solution through the reference nebulizer allowed the system to continuously correct for any mass drift by using the reference mass ions purine at 121.05087 and HP-921 at 922.00979 *m/z*.

Multivariate data analysis for metabolite profile and statistical analysis

Blank, QC, QC-SVR and all serum samples chromatograms were exported to Agilent Mass Hunter Software version B.02.00 (Agilent Technologies, USA) and then obtained results were transferred into XCMS software programs for peak correction. Features which fulfill the criteria ($p \leq 0.05$ and fold analysis > 1.5 from *m/z* scores) considered to be biomarker metabolites candidate. Identification of the selected important *m/z* scores were evaluated by Human Metabolome Database (HMDB) and METLIN database. Debate in *m/z* score were overcome by MS/MS results of blank, QC and QC-SVR samples. Metabolic pathway analysis was achieved by using Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database and MetaboAnalyst 4.0. In order to carry out cluster analysis, raw data have to be pre-processed. Peak picking and peak list alignment were successively performed by XCMS online software. Obtained pre-processed data were exported to MATLAB 2017a software for Orthogonal Partial Least Square Discriminant Analysis (OPLS-DA) analysis. Three latent variables were selected to explain the whole model.

For statistical comparison of demographic and laboratory parameters from patient and control groups, independent-samples Student's *t* test was used and associations between categorical variables were evaluated using the χ^2 or Fisher's exact test. All analyses were performed using SPSS 24 software for statistical analysis. Results were presented as mean ± standard deviations and $p \leq 0.05$ was considered as statistically significant.

Results

Participants

The comparison of demographic characteristics (gender, age, and BMI) and laboratory parameters (platelet, neutrophil, lymphocyte and monocyte counts, ESR, CRP, and WBC values) of patient and control group are shown in Table 1. No statistically significant difference was evaluated in demographic characteristics ($p > 0.05$) and only lymphocyte count was statistically found to be different in accordance with the results of laboratory parameters between both groups ($p < 0.05$).

Identification of metabolites

Two parameters were fulfilled to identify metabolites which were retention time (RT) and mass to charge ratio (*m/z*). In order to evaluate the RT and *m/z* ratio, all analysis data were collected and exported to Agilent Mass Hunter Software version B.02.00 to determine peak information then processed results were transferred into XCMS software programs for peak correction and peak list alignment. In this study, two different comparison analysis (patients-control and OKOA-NOKOA) was carried out by TOF detector. According to the obtained results, 244 (patient vs control) and 274 (OKOA vs NOKOA) *m/z* ratio were determined. Following criteria, $p \leq 0.01$ and fold analysis > 1.5 from *m/z* scores were taken into account to filter the multivariate data. It is observed that 41 and 36 *m/z* signal passed these criteria for patient vs control group and OKOA vs NOKOA, respectively. These features were compared with the reference database of METLIN. Unfortunately, *m/z* scores of several features overlapped by more than one metabolites. This problem was overcome by checking MS/MS results of blank, QC and QC-SVR samples. False peaks were also removed by QC and QC-SVR samples. Twenty-one different metabolites were identified for patient vs control group (Table 2) while 15 metabolites were determined to be statistically significant for OKOA vs NOKOA group

Table 1 Comparison of demographic characteristics and laboratory parameters patients and control groups

	Patient group ($n = 28$)	Control group ($n = 15$)	p[a]
Age (years)	60.89 ± 9162	59.13 ± 4.83	0.493
Gender (female %)	67.85	66.6	0.937
BMI	32.19 ± 8.33	28.58 ± 4.38	0.143
Platelet count (K/μL)	288.52 ± 70.36	252.93 ± 40.69	0.079
Neutrophil count (K/μL)	4.99 ± 1.80	4.71 ± 1.42	0.608
Lymphocyte count (K/μL)	2.10 ± 0.58	2.48 ± 0.32	0.026*
Monocyte count (K/μL)	0.509 ± 0.17	0.502 ± 0.12	0.882
CRP	0.54 ± 0.67	0.43 ± 0.19	0.522
ESR	12.92 ± 9.48	8.20 ± 4.12	0.074
WBC	7.91 ± 2.51	7.82 ± 1.96	0.904

The findings are given as mean ± standard deviation

[a] Independent-samples Student's t test was used. *$p < 0.05$ was accepted as significant

CRP, C-reactive protein; *ESR*, erythrocyte sedimentation rate; *WBC*, white blood cell

(Table 3). Intercepted metabolites that were found in both obese patients with OA vs non-obese with OA and OA vs control were given in Table 4. By this way, our findings become more clear. We have underlined especially the alteration in acidic metabolites much more better via Table 4. Several pathways could be affected due to these metabolites. In order to discuss effects of metabolites on metabolic pathways, KEGG and MetaboAnalyst 4.0 software were utilized. According to the database, 32 different pathways for patient vs control group and 16 different pathways for OKOA vs. NOKOA group could be affected from the variations in metabolites. Glycerolipid, nitrogen, glycerophospholipid, glycine, serine and threonine metabolism, tryptophan metabolism, and fatty acid biosynthesis were found as the mostly affected pathways for both comparison analysis. Overview of pathway impacts was monitored in Figs. 1 and 2.

Table 2 List of the significantly different metabolites identified in patient vs control group

Metabolites	Fold	p value	Regulation (up/down)	m/z med	rtmed	Pathways
PA(18:2(9Z,12Z))	1.6	0.02927	Up	674.5122	14.00	Ether lipid metabolism
PA(16:0/16:0)	1.6	0.02927	Up	674.5122	14.00	Glycerophospholipid metabolism
Phosphatidylethanolamine	1.5	0.02927	Up	674.5122	14.00	Glycerophospholipid metabolism
Propane-1,3-diol	1.6	0.03552	Up	224.1318	4.39	Glycerolipid metabolism
Sulfate	1.5	0.03552	Up	224.1318	4.39	Cysteine and methionine metabolism
Benzoic acid	1.5	0.00015	Up	344.1389	5.84	Phenylalanine metabolism
Phosphoric acid	1.6	0.01181	Up	299.9636	4.44	Oxidative phosphorylation
Butyric acid	1.6	0.03704	Up	165.0575	5.62	Butanoate metabolism
Acetic acid	1.6	0.03704	Up	165.0575	5.62	Glycolysis or gluconeogenesis
L-Valine	1.7	0.01002	Up	566.3295	13.73	Aminoacyl-tRNA biosynthesis
L-Alanine	1.6	0.01686	Up	137.0482	6.65	Alanine, aspartate and glutamate metabolism
Stearic acid	1.5	0.01136	Up	388.2599	10.9	Fatty acid biosynthesis
Benzeneethanamine	1.5	0.00241	Up	294.1595	7.47	Phenylalanine metabolism
Carbamic acid	1.5	0.00792	Up	344.1393	5.51	Nitrogen metabolism
Hydroxylamine	2.5	0.02207	Up	125.9882	3.13	Nitrogen metabolism
Indoleacetic acid	1.5	0.02793	Up	294.1596	8.27	Tryptophan metabolism
Urea	1.6	0.02910	Up	262.1328	4.64	Arginine and proline metabolism
Glycine	1.7	0.0317	Down	261.1353	10.24	Glycine, serine and threonine metabolism
Glycerol	1.5	0.0352	Up	341.3047	13.22	Fatty acid metabolism
Oleic acid	1.5	0.0065	Up	523.3648	14.44	Fatty acid metabolism
Lyso PC (18:2(9Z,12Z))	1.5	0.0379	Up	503.4200	13.58	Glycerophospholipid metabolism

Table 3 List of the significantly different metabolites identified OKOA vs NOKOA

Metabolites	Fold	P value	Regulation (up/down)	*m/z* med	rtmed	Pathways
Phosphatidylethanolamine	1.8	0.01950	Up	674.5127	13.99	Glycerophospholipid metabolism
PA(16:0/16:0)	1.5	0.01014	Up	820.6067	14.29	Glycerophospholipid metabolism
Lyso PC (18:2(9Z,12Z))	1.7	0.00984	Up	520.3419	13.58	Glycerophospholipid metabolism
Stearic acid	1.5	0.02163	Up	388.2599	10.90	Fatty acid biosynthesis
Oleic Acid	1.7	0.03148	Up	523.3642	14.27	Fatty acid biosynthesis
Propane-1,3-diol	1.9	0.02212	Up	224.1318	4.39	Glycerolipid metabolism
Carbamic acid	1.5	0.02976	Up	344.1393	5.53	Nitrogen metabolism
Hydroxylamine	1.5	0.11486	Up	125.9880	14.79	Nitrogen metabolism
L-Valine	1.7	0.027073	Up	566.3294	13.73	Aminoacyl-tRNA biosynthesis
Leucine	1.7	0.052215	Up	261.1353	10.23	Aminoacyl-tRNA biosynthesis
Isoleucine	1.6	0.07417	Up	643.4653	13.9	Aminoacyl-tRNA biosynthesis
L-Ornithine	1.5	0.04159	Down	353.1506	12.63	Glutathione metabolism
D-Alanine	1.7	0.04159	Up	353.1506	12.63	Vitamin B6 metabolism
Butyric acid	1.9	0.00102	Up	182.0842	5.6	Butanoate metabolism
Urea	1.5	0.04544	Up	273.1717	11.81	Arginine and proline metabolism

Multivariate data analysis

Untargeted metabolomics studies aimed to plot a whole map to observe the variation of small compounds in any biological medium or other analytical situation without observational biases. Nevertheless, there is huge amount of data to encounter in a challenge as forming biologically relevant conclusions from any dataset and complex algorithms is needed to organize the whole information [26]. In order to maintain data reorganization in a confident way, several multivariate data analysis algorithms were presented in literature. One of the most used algorithm was OPLS-DA where class memberships are coded in *Y* as a dummy variable and analytical responds were embedded into *X* block. These two blocks were formed the data matrix and categorization were carried out.

In order to carry out a successive cluster analysis, raw data have to be filtered via discarding interferences, false peak and low-intensity signals. Data processing (filtering peak picking and peak list alignment) was successively applied by XCMS online software [27]. Obtained pre-processed results were exported to MATLAB 2017a software for OPLS-DA model. In both model, orthogonal signal correction (OSC) was preferred for pre-processing of *X* block. *Y* block of the matrix was formed as dummy variables of 1 and 0. Cross validation was applied to assess predictivity of model. Venetian blinds with 10 splits and 1 samples per split were preferred for cross validation. Score plot of latent variable exhibit two clusters in each case. Root mean square error of calibration (RMSEC) was found to be 0.16 while regression coefficient of calibration was 0.87 for patient vs control. In OPLS-DA analysis of

Table 4 Intercepted metabolites between patient vs control group and OKOA vs NOKOA

Compound name	HC vs OA	OKOA vs NOKOA	*m/z*	Retention time	Pathways
Phosphatidylethanolamine	Up	Up	674.5127	13.99	Glycerophospholipid metabolism
PA(16:0/16:0)	Up	Up	820.6067	14.29	Glycerophospholipid metabolism
Lyso PC (18:2(9Z,12Z))	Up	Up	520.3419	13.58	Glycerophospholipid metabolism
Stearic acid	Up	Up	388.2599	10.90	Fatty acid biosynthesis
Oleic acid	Up	Up	523.3642	14.27	Fatty acid biosynthesis
Propane-1,3-diol	Up	Up	224.1318	4.39	Glycerolipid metabolism
Carbamic acid	Up	Up	344.1393	5.53	Nitrogen metabolism
Hydroxylamine	Up	Up	125.9880	14.79	Nitrogen metabolism
L-Valine	Up	Up	566.3294	13.73	Aminoacyl-tRNA biosynthesis
Leucine	Up	Up	261.1353	10.23	Aminoacyl-tRNA biosynthesis
Butyric acid	Up	Up	182.0842	5.6	Butanoate metabolism
Urea	Up	Up	273.1717	11.81	Arginine and proline metabolism

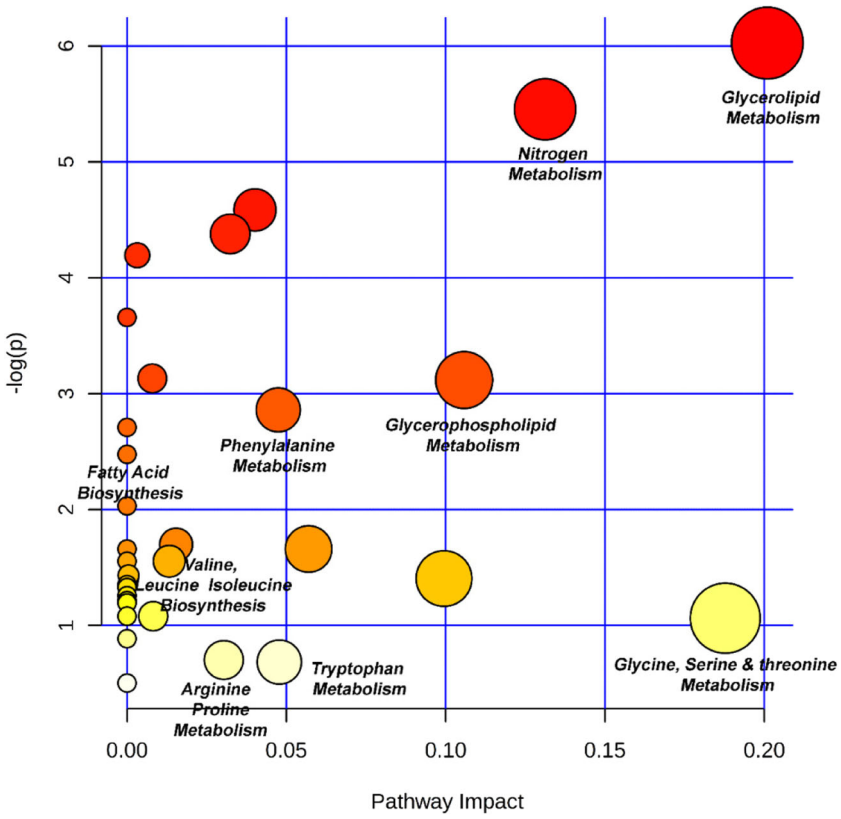

Fig. 1 Pathway analysis generated by MetaboAnalyst online: the major pathways varied in patient vs control group

OKOA vs NOKOA, three latent variables were selected to explain the model. RMSEC and R^2 values were calculated to be 0.013 and 0.99, respectively. These results showed that goodness of fit values were satisfactory for each group.

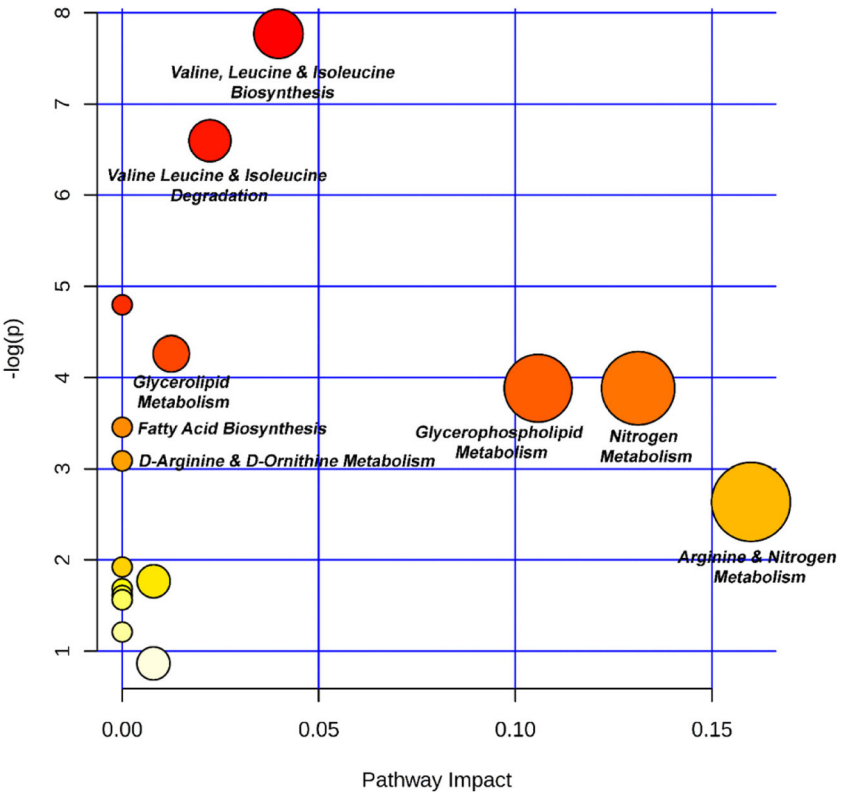

Fig. 2 Pathway analysis generated by MetaboAnalyst online: the major pathways varied in OKOA vs NOKOA

Patient vs control and OKOA vs NOKOA exhibit two clusters and their differences were monitored in score Fig. 3a, b, respectively. LV1 vs LV2 score plot were monitored for OKOA vs NOKOA in Fig. 3a and leverage score plots for patient vs control group were exhibited in Fig. 3b.

Discussion

OA defined as the heterogeneous group of disorders that have unsimilar etiologies whereas quite similar clinical and biological results. Articular cartilage, subchondral bone, synovium, capsules and ligaments could be degenerated due to such disorders [1]. Age and obesity are accepted as significant risk factors [9]. Obesity is thought to be one of the major cause of KOA, because mechanical overload on weight-bearing joints stimulate chondrocytes and leads to cartilage degeneration [28]. In addition to this, variation in fatty acid metabolism (increasing the release of fatty acid) could cause several diseases like diabetes mellitus, gallbladder disease, OA, heart disease and some forms of cancer [29]. As a result of imbalance in matrix formation-degradation, tissue degeneration was occurred in KOA. Catabolic process, as decrease in matrix synthesis, increases in proteinase activity with chondrocyte apoptosis. In addition, morphological, biochemical and molecular variations; cytokines secreted from synovium, chondrocyte and inflammation mediators lead to augment in cartilage degradation [7]. However, physiopathology of KOA

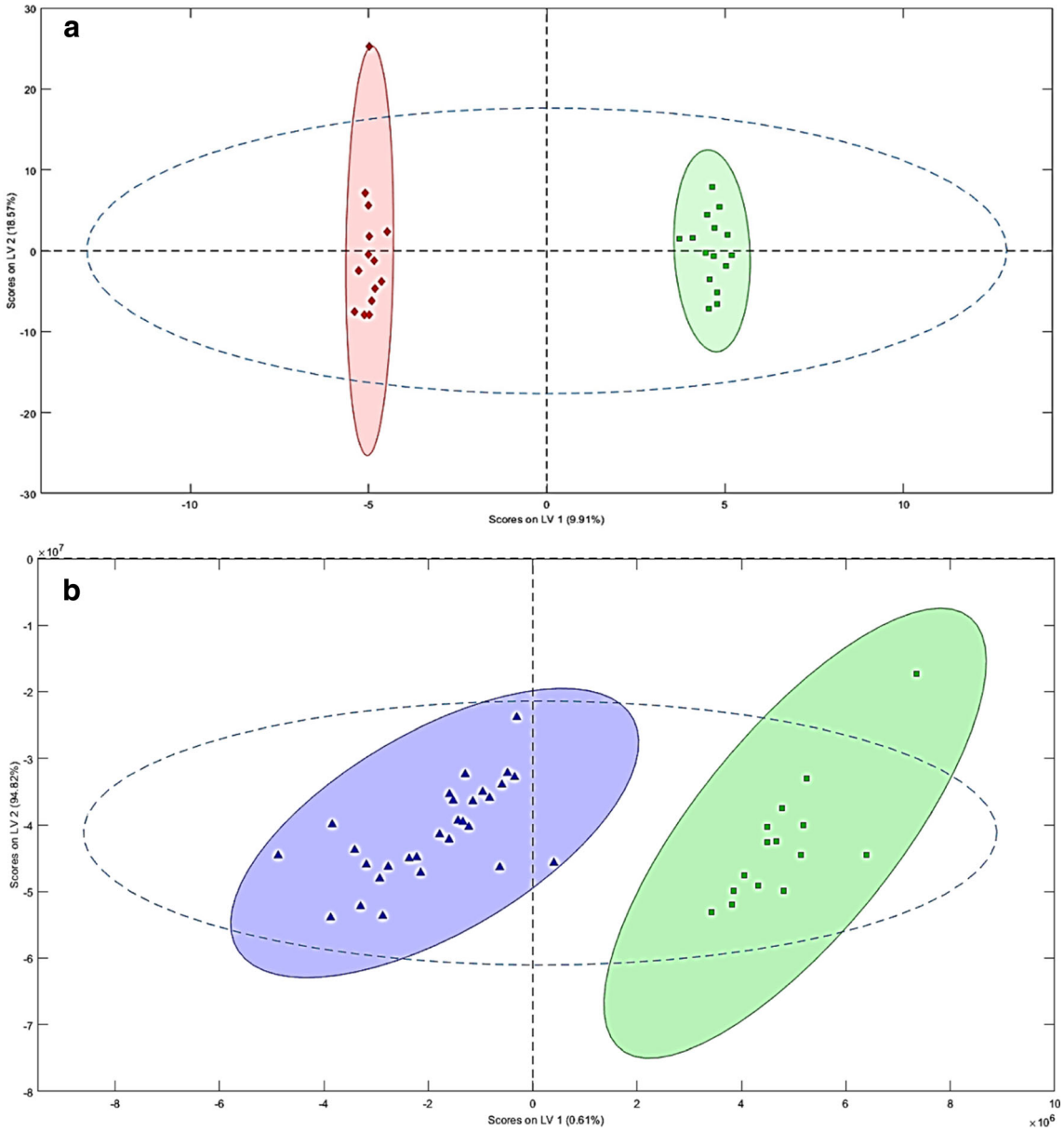

Fig. 3 OPLS score plot for **a** OKOA (red) vs NOKOA (green). **b** Patient (blue) vs control (green)

could not be clearly demonstrated yet [8]. Due to the increase in incident of obesity and elderly population, KOA is getting bigger problem and early diagnosis is much more important. Laboratory diagnosis does not used due to lack of sensitivity and specificity [30]. In early diagnosis, pre-radiographic level has to be determined. Due to that reason, specific biomarkers were required to detect KOA in blood. Thus, analysis of alternative biomarkers was quite important that monitor prognosis and disease.

In this study, it is aimed to understand the relationship between KOA and obesity in molecular level. In order to perform this analysis, various different small molecules, called as metabolites, have to be evaluated. Therefore, mass spectroscopy (MS) methods were used. MS is mostly deal with mass-to-charge ratio (*m/z*) and abundance of any detected ion. MS accepted as one of the most powerful and widespread probes in identification of biological compounds. Both gas and liquid chromatography techniques were developed for MS analysis [31]. In this study, LC/Q-TOF/MS/MS instrumentation was preferred to collect as much data as possible for identification of endogenous metabolites [19].

In human serum, there are at least 4000 different metabolites [32]. Simultaneous evaluation of these data including identification, pathway analysis and enrichment analysis could be achieved by the aid of multivariate data analysis models and this whole process mostly known as metabolomics.

It is found that 41 and 36 *m/z* signal were statistically significant for patient vs control and OKOA vs NOKOA, respectively. These candidates were compared with the METLIN database and 21 different metabolites were identified for patient vs control (Table 2) and 15 metabolites were determined to be statistically significant for OKOA vs NOKOA (Table 3). Intercepted metabolites that were found in both OKOA vs NOKOA and OA vs control were given in Table 4. By this way, our findings become more intelligible. The major recognized metabolic pathways that were taken into account are related to fatty acid, tryptophan, nitrogen, glycerophospholipids, glycerolipid metabolism, and acidotic-hypoxic situation related to the inflammation on knee parts of the body both comparison analysis.

It is reported that some glycerophospholipids were higher in KOA and can be used as biomarker [33]. These molecules take into place in glycerophospholipid metabolism and could be responsible for such increase. It is claimed that alterations in glycerolipid concentration could lead to KOA. Phospholipids and enzymes contribute to lipid metabolism and they play an important role in regulating neutrophil activation. Furthermore, lysophosphatidylcholine may bring about inflammation. In this study, similarly, phosphatidylethanolamine, lyso PC (18:2 (9Z,12Z)) and PA(16:0/16:0) levels were high in patient with respect to the control. Also, concentration of these molecules was higher in OKOA regarding to NOKOA. Moreover, propane-1,3-diol level was significantly increased in KOA that play an important role in glycerolipid metabolism [34].

The presence of excessive fat may cause arthritic variation in both synovial membrane and articular cartilage. In addition to this, increase in fatty acid concentration could be also hazardous for metabolism of prostaglandins synthesis via chondrocytes, reducing collagen type II synthesis and reduce catabolic factors in cartilage degradation. It is found that insulin resistance resulted in adipose tissue cause variated ability of insulin signaling cascade to store triglycerides. This will induce lipolysis and uncontrolled release of free fatty acids (FFA) and glycerol [35, 36]. Similarly, our results exhibit that elevated levels of glycerol and FFA as stearic and oleic acid in the serum of KOA patients. Glycine, proline, and lysine play crucial role in type II collagen synthesis, so higher concentration of these amino acids aid to regenerate the articular cartilage matrix. Besides, this collagen was accepted as the most important essential amino acid among others. Deficiency could cause severe consequences in collagen synthesis [37]. In this study, it is also found that glycine level was lower in OA. It could be suggested to add to the diet of these patients.

The tryptophan metabolism was one of the most studied pathway in rheumatoid arthritis (RA) and OA and give statistically significant difference against control group [38, 39]. In this study, it is reported that indoleacetic acid, which is a breakdown product of tryptophan is much higher in OA patients that point out the tryptophan catabolism.

Another finding from the metabolomics study is that carbamic acid and hydroxylamine levels were much higher in OA patients. These two molecules take part in nitrogen metabolism and highness in their concentration could be caused by inflammation-based reactive nitrogen species [40]. This may cause an increase in oxidative stress. In addition to these, benzoic acid and L-alanine were also high in OA patients. L-Alanine has been already reported in present studies [22, 41]. These molecules could be related to energy consumption demand of inflammation region. OA and other chronical inflammations were mostly associated with acidosis [42]. More hypoxic and acidotic situation, that is an expected case in OA due to inflammation, could make an increase on some acidic metabolites as butyric acid, stearic acid, acetic acid, phosphoric acid and benzoic acid [43]. To compare OKOA and NOKOA patients, it has been observed that carbamic acid and hydroxylamine is higher in OKOA patients. It seems that oxidative stress is quite higher due to obesity. In addition to this, acidosis characteristics get stronger in obesity patients suffered from KOA. As a result of this, butyric acid, alanine, stearic acid and oleic acid levels found an increased level in OKOA patients.

Leucine, isoleucine and valine were known as branched-chain amino acids (BCAA) and have aliphatic and non-linear side chains. Nearly, one third of skeletal muscle consists of these BCAA molecules and they have significant role in

protein synthesis. These small molecules were mostly associated with obesity [44] and they could be used as biomarker in insulin resistance [45]. It is also reported that concentration of these metabolites were getting higher with increasing age (15). In addition to these, higher BCAA concentrations bring about an increase in production of cytokines that may cause and increase in the rate of joint collagen degradation. Due to that reason, OA strongly related with the concentration of cytokines. According to proposed work, OKOA leucine, isoleucine, and valine concentrations were found to be higher than NOKOA. We claimed that these molecules were also associated with KOA because obesity is accepted as an important risk factor for KOA. In addition, valine concentration was found to be higher in patient group regarding to control group. Valine was found to be the only BCAA molecule has a significant difference in its concentration. Because of that reason, this could be a biomarker for KOA diagnosis.

Current studies reveal that metabolomics alteration was investigated in both blood samples and synovial fluids. Rockel et al. reveal that PC-lysoPC-LPA, BCAA-mTOR, and arginine-NO/L-ornithine metabolisms were important in OA in accordance with current studies [46]. In our study, we have also found that acidosis and metabolites related to oxidative stress were influenced by obesity in OA patients. In another study, Anderson et al. reveal that aminoacyl biosynthesis, nitrogen metabolism, taurine, alanine metabolism, valine leucine, and isoleucine degradation metabolisms were also affected by OA which confirms our findings [47].

It is obvious that this study contain some restrictions. Metabolite concentration may be affected by diet, drug consumption, smoking, and presence of other chronic diseases. Secondly, sample size could be wider regarding to highly prevalent disease. Lastly, metabolomics studies, due to its nature, are semi-quantitative methods so exact concentration of these compounds could not be obtained by this technique.

Conclusion

KOA is a common health problem that badly affects health quality. Severe cases may limit all movement of joint. Due to that reason, understanding its mechanism and side effects could be illuminated. In this study, KOA was studied in a comprehensive way via metabolomics approach. In addition to this, the relation between KOA and obesity was firstly investigated with metabolomics method. Valine was found to be the only BCAA molecule that has a significant difference in its concentration in KOA patients. And also, acidotic effect of inflammation was known by other studies and this study was also confirmed this situation. However, it is claimed in this study that obesity-induced KOA shows higher oxidative stress and acidic media. It is observed that especially reactive nitrogen species-based oxidative stress and acidosis problems are getting higher in OKOA with regard to NOKOA.

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Compliance with ethical standards

Disclosure None.

Ethics approval This study was conducted with the approval of the Ataturk University Ethics Committee.

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