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Metabolomic profiling of active and inactive liver cystic echinococcosis

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ABSTRACT

Cystic Echinococcosis (CE) is one of the life-threatening diseases worldwide. It is a parasitic zoonosis caused by tapeworms of the species *Echinococcus granulosus sensu lato* (s.l). The treatment options of CE vary from simple "watch and wait" approach to invasive treatment, based on the type and especially the nature of the cyst (active/inactive). Serological tests are inadequate to distinguish between active and inactive CE. A diagnostic reference that can determine whether the cyst is active or inactive can easily guide the treatment strategy.

We aimed to test whether gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-quadropole time of flight mass spectrometry (LC-qTOF-MS) based metabolomics can establish a plasma metabolic fingerprint of CE patients and identify a diagnostic reference to discriminate active and inactive CE cysts.

Metabolite concentrations were measured in plasma samples of 36 active CE patients, 17 inactive CE patients and 31 healthy controls. Multivariate statistical analysis on 232 identified metabolites obtained from two analytical platforms was performed by using principle component analysis (PCA) and partial least square-discriminant analysis (PLS-DA) methods. The PLS-DA scores plot of the combined data set demonstrated a good separation between the groups. Compared to the healthy control group, decreased levels of squalene and increased levels of glyceric acid, 3-phosphoglycerate, glutamic acid, palmitoleic acid and oleic acid were determined in the CE patients. However, decreased levels of 3-phosphoglycerate and increased levels of 4-hydroxyphenylacetylglutamine, docosahexanoic acid were determined in active CE patients compared to the inactive CE patients.

Determination of differences in metabolites may provide detailed understandings of potential metabolic process associated with active and inactive CE patients, and altered specific metabolic changes may provide some clues to obtain diagnostic reference for CE. This study has certain limitations: a. various factors affecting results of metabolomic studies such as lifestyle and dietary habits of the patients could not be fully controlled b. other infectious or malignant diseases of the liver should also be included as a positive control to evaluate the specificity of the diagnostic references.

1. Introduction

Cystic Echinococcosis (CE) is one of the life-threatening diseases in the world. It is a parasitic zoonosis caused by tapeworms of the species *Echinococcus granulosus sensu lato*(s.l) (Moro and Schantz, 2009). CE usually affects liver (50–70%) followed by lung (20–30%) and less frequently by spleen, kidneys, bones, central nervous system and other organs(Khanfar, 2004; Moro and Schantz, 2009; Nunnari et al., 2012). This endemic disease is more common in the Mediterranean countries, Asia, Australia, Europe, the Middle East and South America and an

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annual incidence ranged from 1 to 200 per 100,000 population (Nunnari et al., 2012). According to the World Health Organization (WHO), echinococcosis is one of the neglected tropical diseases that should be managed and eliminated by the year 2050 (Giri and Parija, 2012).

After getting infected by with *E. granulosus* s.l., the disease may remain silent for a long time, however complications such as the presence of biliary communication and spontaneous or post-traumatic intraperitoneal rupture may appear in some patients. It was previously shown that the majority of patients are diagnosed with CE by an incidental finding, while being examined for other reasons (Tamarozzi et al., 2018).

According to the WHO classification system, five types of CE (CE1-5) cysts are reported based on ultrasound (US) images: active cysts (CE1 and CE2), transitional cysts (CE3a-CE3b) and inactive cysts (CE4 and CE5). Treatment options for CE include surgery, percutaneous management, "watch and wait" approach, and drug therapy. Appropriate treatment should be chosen after determining the type of cysts by US. While active cysts (CE1, CE2, CE3a and CE3b) should be treated generally by invasive techniques, inactive cysts should be monitored regularly by the US along with "watch-and-wait" approach (Akhan et al., 2020; Akhan et al., 1996; Akhan et al., 2017; Brunetti et al., 2010). The standard approach for clinical diagnosis of liver CE is based on particularly US, which is also the first-choice imaging modality to make a classification. Magnetic resonance imaging (MRI) and computed tomography (CT) scans can be used when required. Although imaging techniques provide sufficient data to diagnose CE in most cases and serological tests are performed, the diagnosis of early CE1 and late CE4-CE5 cyst types are even difficult. Serological tests are used in cases with non-characteristic imaging findings. However, these tests are inadequate to distinguish between active and inactive CE (Tamarozzi et al., 2016). Since a permanent correlation between serological results and cyst type (active or inactive) is not established, clinicians who are unfamiliar with CE may immediately consider positive result as a need for treatment (Piccoli et al., 2014). In addition, it is difficult, even for experienced radiologists, to determine the activity of the cyst, particularly in CE4 cysts. Due to difficulties in determining the type and activity of the cyst, patients with CE may not receive appropriate treatment or may receive unnecessary interventional radiological or surgical treatment. Therefore, a diagnostic reference that can determine whether the cyst is active or inactive can easily guide the treatment strategy, of which can be invasive treatment options or "watch and wait" approach.

In the last decades, untargeted metabolomics analyses have been widely used for many purposes, such as to diagnose diseases, to assess the treatment effectiveness or to determine the effect of gut flora (Eylem et al., 2020b; Gonulalan et al., 2019; Kart et al., 2020; Nemutlu et al., 2019a; Zeki et al., 2020). The purpose of untargeted metabolomic studies is to measure the widest range of metabolites in a sample without a *priori* knowledge and to find new biomarkers for identification of phenotypes (Johnson et al., 2016).

The aim of this study was to evaluate the metabolomic profiles of CE patients having active and inactive cysts by two orthogonal analytical platforms (GC-MS and LC-qTOF-MS) in order to 1) determine distinct metabolic patterns to differentiate CE patients from healthy individuals and establish a plasma metabolic fingerprint of CE patients; 2) identify a diagnostic reference to discriminate active and inactive CE cysts.

2. Materials and Methods

2.1. Ethics statement

Ethical approval for the study was granted by the Institutional Ethics Committee (GO 17/711). Informed consent from all patients was obtained.

2.2. Recruitment of participants

The recruited CE patients were diagnosed at the Hacettepe University Hospitals, Non-Vascular Interventional Radiology Unit. The diagnosis of CE was based on integrated use of imaging examination and serologic tests. All CE patients were categorized according to the WHO-IWGE classification. Details of the classification and US image of one of the patients for each cyst type are presented in the Table 1. Fifty-three patients (n=36 with active cyst and n=17 with inactive cyst; mean age: 38.1 years, 21 male and 32 female) diagnosed with liver CE and have not treated previously were included in the study. None of the patients had undergone interventional procedure prior to sample collection. Patients with other metabolic, infectious, autoimmune and malignant diseases were excluded in order to prevent potential overlapping metabolic effects of other diseases. Patients' details and clinical characteristics are summarized in Supplementary Table 1.

Control subjects (n=31; mean age: 37.48 years, 15 male and 16 female) matched with the patients in terms of sex and age and who have no other underlying diseases (such as metabolic, infectious, autoimmune and malignant diseases) were recruited during routine consultation in the same unit.

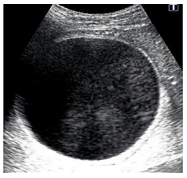
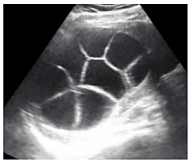
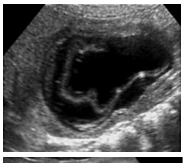
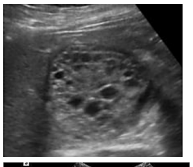
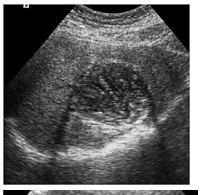
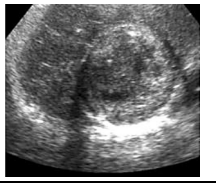
2.3. Metabolomics analysis

The blood samples of CE patients and control subjects were centrifuged at 3000 rpm at 4°C for 15 min and the obtained plasma was transferred into tubes. Quality control (QC) samples were prepared by pooling 0.1 mL of every individual study sample. Two orthogonal analytical platforms described earlier in our studies were used for metabolic analysis (GC-MS and LC-qTOF-MS) (Chukhrienko et al., 1975; Cutler, 1991). Briefly, 0.1 mL plasma and QC samples were extracted using 0.9 mL methanol:water mixture (8:1, v/v) and two separate 0.4 mL aliquots were speed vacuum dried for GC-MS and LC-qTOF-MS analyses.

GC–MS based metabolomics studies were analyzed as described previously (Eylem et al., 2020a; Nemutlu et al., 2019b; Nemutlu et al., 2015; Xia et al., 2012). Briefly, the residues were methoxyaminated and derivatized with MSTFA (N-Methyl-N-(trimethylsilyl) trifluoroacetamide with 1% TMCS (trimethylchlorosilane). After the derivatization, the samples were transferred into GC–MS vials and analyzed using GC–MS (Shimadzu GCMS-QP2010 Ultra) with a DB-5MS stationary phase column (30 m + 10 m DuraGuard × 0.25 mm i.d. and 0.25-μm film thickness). LC-qTOF-MS-based metabolomics studies were analyzed as described previously (Nemutlu et al., 2019b; Nemutlu et al., 2015). Briefly, the residues were reconstituted using acetonitrile:water mixture (1:1, v/v). The metabolites were separated in the C18 column (150 × 4.6 mm, 3 μm) and analyzed in the LC-qTOF-MS system (Agilent 6530). The mobile phase included solvent A (water- 0.1% formic acid) and solvent B (acetonitrile- 0.1% formic acid) with gradient elution. The flow rate was adjusted to 0.3 mL/min. The injection volume was 10 μL. Positive and negative ionization modes were applied in the ESI source with a 4000 V of capillary voltage and 300°C of capillary temperature. The auto MS-MS data of metabolites at low (10 eV), medium (20 eV) and high voltage (40 eV) were recorded between 100 and 1700 m/z above the 2000-count threshold. In the batch design, a QC sample was analyzed in GC-MS and LC-qTOF-MS after every eighth and tenth sample respectively. All samples were injected in a random order in the analytical sequence.

Once the analysis was completed, complex chromatograms were deconvoluted and aligned using MS-DIAL software with following parameters: The MS1 and MS2 tolerance values were set 0.01 and 0.05 Da, respectively. Minimum peak height was 1000 amplitudes and MS/MS abundance cut-off values are set at 10 amplitudes to reduce MS noise. The data matrices were transferred to an Excel work file and normalized to total peak area in order to eliminate day to day variations from multiple analytical batches. Any metabolite traits having more than 50%

Table 1
WHO-IWGE Classification of CE cysts.

WHO-IWGE Classification	Gharbi Classification	Ultrasonographic Features	Stage	US Apppereance
CE1	Type I	Simple round or oval unilocular cyst with anechoic content and a visible double cystic wall	Active	
CE2	Type III	Completely filled with daughter vesicles, appears as "septa"; not true septa but the cyst walls of the daughter vesicles adjacent to one another. ("rosette-like" "honeycomb" sign)	Active	
CE3a	Type II	The endocyst detached from the cyst outer wall pericyst ("water-lily" sign).	Transitional	
CE3b	Type III	Predominantly solid lesion with daughter vesicles	Transitional	
CE4	Type IV	Coarse variable (hyper, hypo) echogenic echotexture without daughter vesicles. The detached endocyst as a hypoechoic folded structure embedded in a hyperechoic matrix ("ball of wool" sign)	Inactive	
CE5	Type V	Partially (with an egg-shell calcified wall) or completely calcified with shadowing.	Inactive	

of the values missing in the QC samples or >20% RSD of QC samples in LC-qTOF-MS and >30% RSD of QC samples in GC-MS data were excluded from the data matrix. Missing values in the data table were filled with the half value of the smallest concentration in the metabolite group. Metabolite identification for GC-MS was done using commercially available retention index library (Fiehn Retention Index Library) with 70% of higher identification cutoff score. Metabolite identification for LC-qTOF-MS was done using MS/MS data for accurate identification of the metabolites using MS-FINDER (±0.01 Da for MS1 and ±0.05 Da MS2 tolerance) and metabolites with a score of greater than 6 were accepted as being accurately identified. The metabolites with $p < 0.05$ in t-test results and AUC> 0.8 in ROC analysis were considered for discussion in the study.

2.4. Statistical data analysis

The data matrices were merged and transferred to the SIMCA-P+ (v13.0, Umetrics, Umea, Sweden) for multivariate analysis for PCA and PLS-DA. The variable importance in projection (VIP) values estimated to distinguish the most important metabolites for the stratifications of the groups and regression coefficients were exploited to illustrate effects of metabolites on the group. The Student's unpaired t-test was used to statistically compare changes in mean expression per metabolite between the groups. A receiver operating characteristic (ROC) curve analysis with support vector machines (SVM) were used for identifying potential diagnostic references and evaluating their performance.

3. Results

In total 232 metabolites were identified with the GC-MS-based (144 metabolites) and LC-qTOF-MS based analysis (99 metabolites; 10 of them are overlapping with metabolites detected with GC-MS-based analysis) in plasma samples. The list of metabolites; the mean and standard errors of these metabolites in the groups, together with their p values, are presented in Supplementary Table 2. Moreover, the individual results for each metabolite are given in Supplementary Table 3.

In order to investigate the differences in metabolomic profiles between groups, multivariate statistical analysis of the combined GC-MS and LC-qTOF-MS dataset was performed by using PCA (unsupervised) and PLS-DA (supervised) methods. These methods were used to reduce variations and to visualize the complex metabolomics datasets. R^2 and Q^2 were calculated as R^2= 0. 83, Q^2= 0.61 and R^2= 0.84, Q^2= 0.63 for all patients and the patient group excluding those with multiple cysts, respectively. Since it was determined that the differences between the groups were not affected by the exclusion of the patients with multiple cysts, all patients' data were included in the analysis.

PCA scatter plot was used for an initial visual representation of the full dataset and has demonstrated the differences between the metabolomic profiles of the active, inactive CE patients and the healthy control group (Fig. 1A). Furthermore, PLS-DA analysis were performed in order to investigate differences between these 3 groups (Fig. 1B). Score plots, VIP graphs and the regression coefficient plots of PLS-DA analysis are presented in Fig. 2A, 2B and 2C for healthy control versus all CE patients, active versus inactive CE patients and healthy control versus active CE patients, respectively.

The PLS-DA scores plot of the combined data set demonstrated good separation between healthy control and CE patient groups with R^2= 0. 83 and Q^2= 0.61 (Fig. 2A). The reliability of PLS-DA analysis was tested using variance testing of cross-validated predictive residuals (CV-ANOVA) analysis and p value of the method (2.1×10^{-15}) proved that the predictive power was high. Metabolites that are the most useful in discriminating all CE patients from healthy controls were displayed in the VIP plot (Fig. 2A). Six metabolites were detected and identified as promising diagnostic references for discriminating healthy controls and all CE patients. These metabolites included squalene (AUC=0.869, p=3×10^{-6}), 3-phosphoglycerate (AUC=0.848, p=2×10^{-4}), glutamic acid (AUC=0.841, p=1×10^{-6}), glyceric acid (AUC=0.805, p=2×10^{-4}), palmitoleic acid (AUC=0.804, p=7×10^{-5}) and oleic acid (AUC= 0.780, p=3×10^{-4}). Compared to the healthy control group, decreased levels of squalene and increased levels of glyceric acid, 3-phosphoglycerate, glutamic acid, palmitoleic acid and oleic acid were determined in the CE patients.

According to the misclassification analysis, PLS-DA method has a very high predictive power which was over 96% and only two CE patients were misclassified (Table 2). These misclassified cysts were found to be inactive; all active cysts were successfully discriminated by this method. Fischer's probability of the misclassification of the PLS-DA method was 5.6×10^{-21}.

The comparison of the metabolomic profiles of active and inactive CE patients are presented in the Fig. 2B. The R^2 and Q^2 of the model were found to be 0.78 and 0. 28, respectively. The p value of the CV-ANOVA analysis was 0.003. The VIP plot is presented in the Fig. 2B. By comparing the metabolomic profiles of the active and inactive CE patients, three metabolites were detected and identified as being potential diagnostic reference. These metabolites are (in order of importance) 4-hydroxyphenylacetylglutamine (AUC=0.899, p=0.009), 3-phosphoglycerate (AUC=0.822, p=0.001) and docosahexanoic acid (AUC=0.753, p=0.032). Compared to the inactive CE patients, decreased levels of 3-phosphoglycerate and increased levels of 4-hydroxyphenylacetylglutamine, docosahexanoic acid were determined in active CE patients.

Misclassification analysis of the PLS-DA model with 2.7×10^{-11} Fischer's probability is presented in the Table 2. In this analysis, one of the CE4 cysts was misclassified as an active cyst additionally one of the active cysts (CE 3a) was misclassified as inactive.

The metabolomic profiles of the active CE patients and the healthy control groups are presented in the Fig. 2C. The R^2 and Q^2 of the model were found to be 0.88 and 0. 67, respectively. The p value of the CV-ANOVA analysis was 4.90×10^{-14}. In order to discriminate active CE patients and healthy controls, 8 metabolites were detected and identified as being potential diagnostic reference (Fig. 2C). The metabolites in order of importance include squalene (AUC=0.902, p=4×10^{-6}), 4-hydroxyphenylacetylglutamine (AUC=0.886, p=6×10^{-4}), glutamic acid (AUC=0.838, p=6×10^{-6}), oleic acid (AUC=0.830, p=1×10^{-4}), palmitoleic acid (AUC=0.818, p=1×10^{-4}), docosahexanoic acid (AUC=0.797, P=0.010), 3-phosphoglycerate (AUC=0.795, P=0.002)

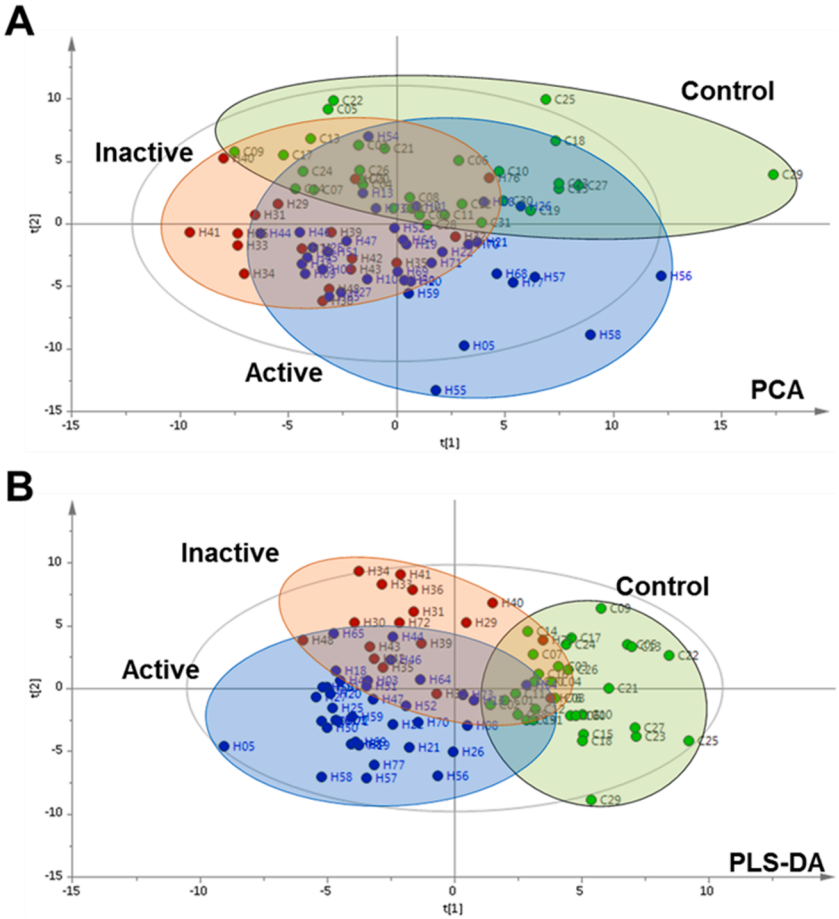


Fig. 1. PCA (A) and PLS-DA (B) score plots of metabolic profiles of control, active and inactive cyst groups.

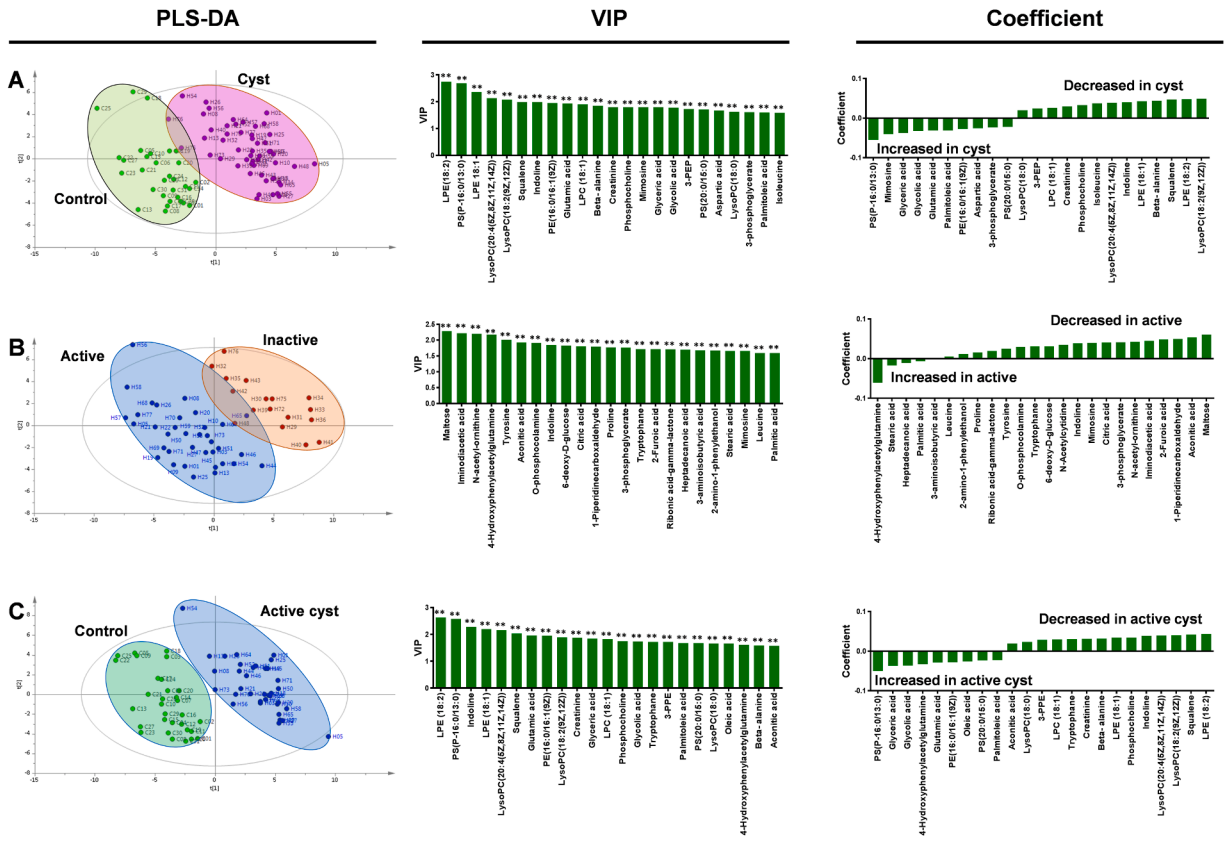


Fig. 2. Comparison of individualized metabolomic profiles of (A) all CE patients and control group, (B) active CE patients and inactive CE patients, (C) active CE patients and control group. Left panels: PLS-DA score plots showing metabolomic profiles. Middle panels: Plots of variable importance in the projection (VIP) showing the most significant metabolites in discriminating between metabolomic profiles. Right panels: The regression coefficient plot of PLS-DA analysis indicating metabolite levels in groups.

Table 2
Misclassification analysis of the PLS-DA models.

	Number	Correct	Control	CE Patient
Control	31	100%	31	0
CE patients	53	96.23%	2	51
	Number	**Correct**	**Active CE**	**Inactive CE**
Active CE	36	97.14%	35	1
Inactive CE	17	94.12%	1	16
	Number	**Correct**	**Control**	**Active CE**
Control	31	100%	31	0
Active CE	36	100%	0	36

and glyceric acid (AUC=0.789, $p=4 \times 10^{-4}$). Compared to the healthy control group, decreased levels of squalene and increased levels of 4-hydroxyphenylacetylglutamine, docosahexanoic acid, glutamic acid, oleic acid and 3-phosphoglycerate were determined in CE patients with active cysts. Misclassification analysis of the PLS-DA model with 8.4×10^{-20} Fischer's probability is presented in the Table 2.

The statistical goodness and robustness of the models were evaluated using R^2 (the fraction of variance explained by a component) and Q^2 (the fraction of the total variation predicted by a component) values, respectively. These values for models were > 0.6 except for the active CE cyst versus inactive CE cyst model. These high values indicated the validity of the methods and showed that the models were stable and might be used for the predictions. Moreover, the lower p values (<0.001) in the PLS-DA methods supported that the separations between groups were significant.

The sensitivity and specificity of the panel of the potential diagnostic reference were verified by a receiver operating characteristic (ROC) curve analysis. In the ROC curve analysis, SVM models that were created using different number of metabolites (5, 10, 15, 25, 50 and 100) were examined. Higher predictive accuracies of the models with 5 metabolites were 92.8% for all CE patients from control, 92.6% for active CE patients from control and 69.8% for active CE patients from inactive CE patients (Fig. 3). Selected metabolites were found to discriminate all groups from each other successfully and the panel of the selected metabolites showed high sensitivity, specificity and accuracy in diagnosis of CE.

4. Discussion

In this study, we tested whether GC-MS and LC-qTOF-MS-based metabolomics can establish a plasma metabolic fingerprint of CE patients and identify a diagnostic reference to discriminate active and inactive CE cysts. PCA model demonstrated a clear metabolomic profile shifting between 3 groups (active CE patients, inactive CE patients and healthy control).

Zheng et al. have previously reported that *de novo* cholesterol synthesis is not observed in *E. granulosus* due to lack of several enzymes that have role in squalene synthesis pathway (such as squalene synthase, squalene monooxygenase). They concluded that *E. granulosus* utilizes the host's cholesterol ester pool (Zheng et al., 2013). Consistent with previous findings that *E. granulosus* uses the host's squalene pool, a decrease in squalene level was found in all CE patients compared to healthy controls.

Glutamic acid, 3-phosphoglyceric acid and glyceric acid are all involved in glycine and serine metabolism. These compounds are required for proliferation of the immune cells (Ueland et al., 2017). On contrary to the most of the amino acids *de novo* glutamic acid synthesis was reported to take place in *E. granulosus* (Zheng et al., 2013). Furthermore, a study that considers *in vitro* metabolomic footprint of the

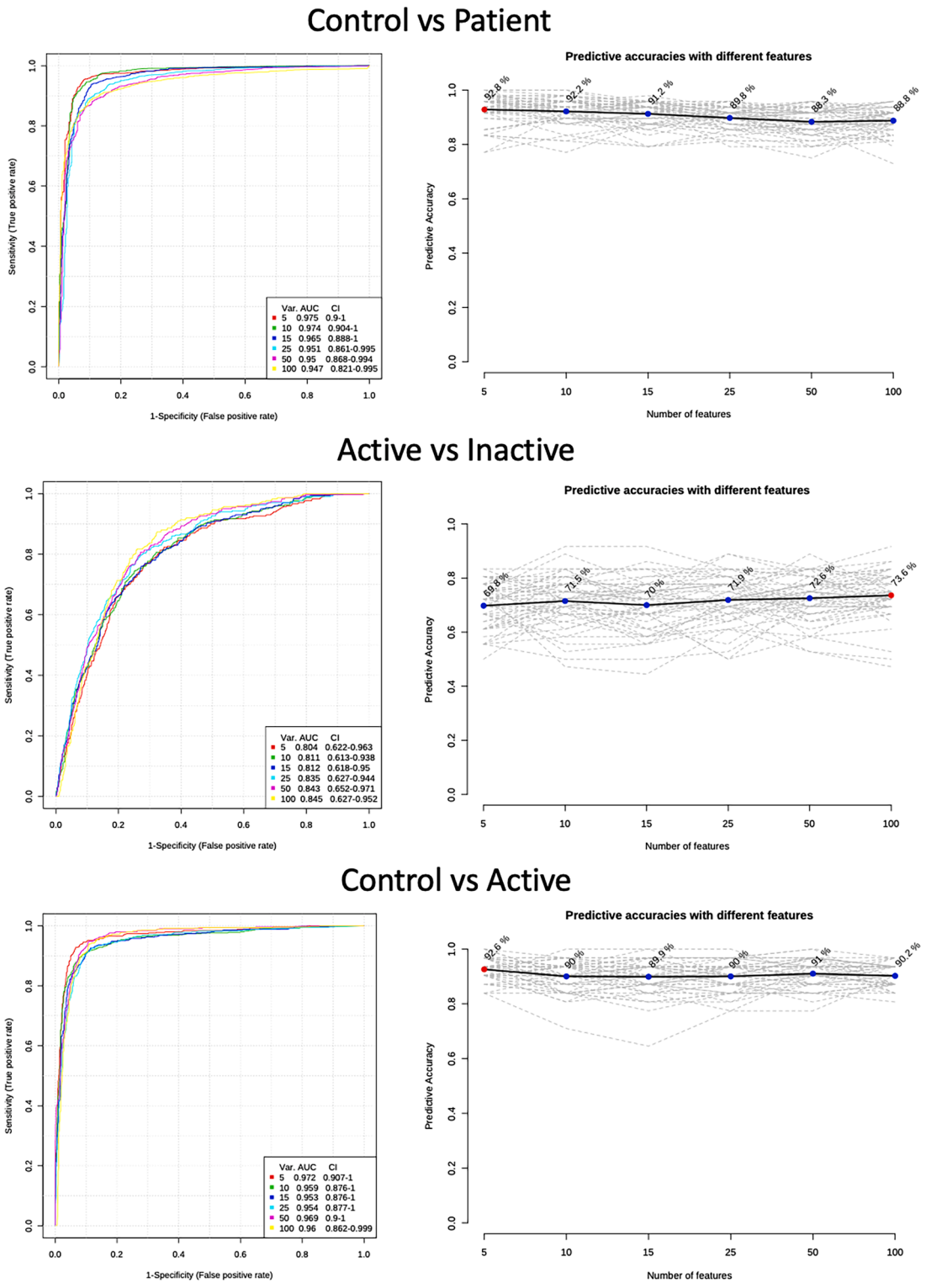


Fig. 3. Plot of ROC curves for all or a single biomarker model based on its average performance across all MCCV runs and plot of the predictive accuracy of biomarker models with an increasing number of features. (A)Comparison of control and CE patients metabolomic profiles. (B) Comparison of active and inactive CE patients metabolomic profiles. (C) Comparison of control and active CE patients metabolomic profiles.

E. multilocularis metacestode which is the cause of alveolar echinococcosis (AE) in humans, has been reported that glutamic acid can be synthesized and released into the medium by *E. multilocularis*. In parallel, an increase in glutamic acid level in CE patients can be attributed to glutamic acid synthesized by *E. granulosus* and released into the environment (Ritler et al., 2019).

Although the level of 3-phosphoglycerate was found to be increased in all CE patients (both active and inactive CE patients) compared to the healthy controls, this increment was found to be higher in inactive CE patients. It has been reported that pentose phosphate pathway occurs in *E. granulosus* scolices ((Agosin and Aravena, 1960), and is inhibited by 3-phosphoglyceric acid. Results of other study aimed proteomic

characterization of larval and adult developmental stages in *E. granulosus* reveals that phosphoglycerate kinase, which is a glycolytic pathway enzyme and responsible from reversible conversion of 1, 3-bisphosphoglycerate to 3-phosphoglyceric acid, is one of the abundant proteins in adult stage of *E. granulosus* (Cui et al., 2013). The increase in 3-phosphoglyceric acid levels can either be due to synthesis and release of this amino acid by *E. granulosus* or reaction of the host to the presence of *E. granulosus*.

Results in this study showed that glyceric acid level is elevated in CE patients. Glyceric acid has a role in three different metabolic pathways; i. amino acid (glycine, serine and threonine) metabolism ii. glycerolipid metabolism and iii. glyoxylate and dicarboxylate metabolism, therefore is required for protein and biological membrane synthesis. As a result, the increment in glyceric acid level may be the consequences of overuse by parasites that triggers the host's glyceric acid production.

The levels of two fatty acids, oleic acid and palmitoleic acid, were found to be increased in CE patients (Field et al., 2002). These increases were thought to be related to immune system activation observed in CE patients. Exosome-like vesicles containing proteins, lipids, RNAs and produced by *E. granulosus* are known to mediate the immune modulation in the host. These vesicles can modify host's responses in order to maintain parasite survival, proliferation and dissemination (Nicolao et al., 2019). It has been reported that *E. granulosus* requires lipids that are synthesized by the host for biological membrane synthesis, biosynthetic processes and cellular signaling which are essential for maintenance and growth of cysts. Furthermore, exosome-like vesicles can transfer molecules between phylogenetically diverse species and the content of the vesicle can be used by parasites to alter the properties of the host environment (Siles-Lucas et al., 2017; Simbari et al., 2016). Thus, metabolic pathways related to lipid synthesis was found to be altered in CE patients, possibly in response to exosome-like vesicles produced by *E. granulosus* (Nicolao et al., 2019; Siles-Lucas et al., 2017; Simbari et al., 2016).

Compared to the inactive CE patients, 4-hydroxy phenylacetylglutamine and docosahexanoic acid levels were found to be increased in active CE patients. Phenylacetylglutamine is formed by the conjugation of phenylacetate and glutamine and occurs naturally in human urine. However, the role of 4-hydroxy phenylacetylglutamine in the metabolism has not been clearly defined yet. Docosahexanoic acid is a phospholipid derived polyunsaturated fatty acid which can serve as precursor for bioactive oxidation products in mammalian hosts. It has been known that pathogen derived oxidized fatty acids interact with the immune system of the host. Thus, oxidized fatty acids are ideal candidates for host–pathogen interaction (Pohl and Kock, 2014).

There are metabolomic studies in the literature addressing other infectious diseases with parasites. In one of the LC-MS based studies Lakshmanan et al. reported that metabolites of the α-linolenic acid pathway (traumatin, traumatic acid, jasmonate, OPC6-CoA and 9-oxononanoic acid), were found to be elevated in *Plasmodium falciparum* infected patients' plasma and erythrocyte samples (Lakshmanan et al., 2012).

In another GC-MS based study, the increased levels of fatty acids and lipid-related compounds (glycerol, palmitoleic acid, hexadecanoic acid, linoleic acid, oleic acid, stearic acid and 3-hydroxybutyric acid) in severe malaria patients were reported and this elevation was thought to be induced by the parasite to meet its demand or to be related with catabolic host response to the infection (Surowiec et al., 2015). In metabolomic study focused on hepatic alveolar echinococcosis which is caused by the *E. multilocularis,* 21 distinct metabolic differences were identified between hepatic alveolar echinococcosis patients and healthy individuals. They found significant increase in the concentrations of phenylalanine, glutamate, tyrosine, formate, lactate and significant decrease in the concentrations of valine, leucine, isoleucine, lysine, serine, glutamine, betaine, creatine and 1-methylhistidine. In order of importance the top 10 metabolites that can discriminate patients and healthy individuals were found to be glutamate, valine, leucine, phenylalanine, creatine, isoleucine, lysine, formate, betaine and serine (Lin et al., 2019). The authors have concluded that these metabolites are associated with perturbations in amino acid metabolism, energy metabolism, glyoxylate and dicarboxylate metabolism. In a review published in 2019, the authors have reviewed the serum and urine metabolite biomarkers for the diagnosis of hepatocellular carcinoma, hepatitis B and C (Satriano et al., 2019). Among the metabolites that were found to be differentiated between CE patients and healthy subjects in our study, only the glutamic acid was listed as a marker to discriminate hepatocellular carcinoma associated with hepatitis B and C patients in the aforementioned review. An increased level of glutamic acid was reported in hepatitis B associated hepatocellular carcinoma compared to hepatitis C associated hepatocellular carcinoma. Given the previously published metabolomic studies on parasitic or malignant diseases of the liver, although metabolites other than glutamic acid are not common with CE, further studies are still needed to analyze all malignancies simultaneously and to be conducted on more patients for the definitive distinction between CE and control group. Determination of metabolites that differs in active and inactive CE patients (3-phosphoglycerate, 4-hydroxyphenylacetylglutamine, docosahexanoic acid) may provide more detailed understandings of the potential metabolic process associated with active and inactive CE patients. Furthermore, altered specific metabolic changes may provide some clues to obtain diagnostic references for CE.

There are some limitations of this study. First, the patients in this study were limited to CE without positive controls such as other parasitic diseases. Thus, further research including other parasitic diseases or other organ cysts like lungs, kidneys etc. is needed to determine the specificity of the findings. Second, the metabolite levels in the living organism are known to be affected by multiple conditions such as age, gender, life style, disease, gut microbiota, morbidities and hormonal status. We have not fully controlled especially the life style and the dietary habits of the outpatients. To validate these diagnostic references to be used in clinical practice and to determine their feasibility for the diagnosis of CE, metabolomic studies including a larger number of patients with CE and other liver related diseases should be performed.

Author statement

Turkmen T. Ciftci: He is the principal investigator of the project. He, as an experienced interventional radiologist, was the primary consultant for classification of cysts. He was also responsible from necessary literature review for the study.

Samiye Yabanoglu-Ciftci: She was responsible from reporting and interpreting the metabolomic data.

Emre Unal: He took the responsibility in patient follow-up and collection of blood samples.

Devrim Akinci: He provided important contributions to collection of blood samples and reviewed the article scientifically before submission.

Ipek Baysal: She was responsible from reporting and interpreting the data and preparation of first draft of the manuscript.

Gokhan Yuce: He took the responsibility in patient follow-up and collection of blood samples.

Ahmet Bulent Dogrul: He took the responsibility in patient follow-up and collection of blood samples.

Serra Orsten: She was responsible from the correlation of results of serological tests with the clinical data and keeping the demographic data of the patients.

Okan Akhan: He made important conceptual critical corrections for preparation of study design and the content.

Emirhan Nemutlu: He took the responsibility in execution of the experiments, data management and statistical analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2021.105985.

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