Clinical Characteristics and Metabolomics Investigation for Patients with Dengue Virus 2 Infection

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Abstract

OBJECTIVES

Dengue is an endemic viral disease affecting tropical and subtropical regions around the world. The aim of this study was to retrospectively study the clinical characteristics of dengue fever (DF) and evaluate the metabolomic changes.

METHODS

The medical records of 50 patients presented with dengue virus 2 (DENV-2) RNA positive were reviewed. Serum was collected from 20 patients diagnosed with DF and 20 healthy volunteers. The changes in serum metabolites were explored by UPLC-Q-TOF MS spectrometer.

RESULTS

Fever, nausea and vomiting, asthenia, skin hyperemia were the most common clinical symptoms. The results of the laboratory examinations showed that leukopenia (76%, 38/50), thrombocytopenia (92%, 46/50), increase of aspartate aminotransferase (96%, 48/50), increase of free fatty acids (FFA) (68%, 34/50), increase of creatine kinase (CK) (44%, 22/50) were more common. Based on orthogonal projection to latent structures-discriminant analysis (OPLS-DA), 2 up-regulated and 15 down-regulated metabolites were identified, contributing to DF progress to some extent. Among them, LysoPC (18:2(9Z,12Z)) were highly positive correlated with PE (21:0/20:5(5Z,8Z,11Z,14Z,17Z)) and 13'-Hydroxy-alpha-tocopherol. The identified biomarkers were mainly involved in glycerophospholipids metabolism pathway.

CONCLUSIONS

The metabolic abnormalities of glycerophospholipids involved in the occurrence of DF caused by DENV-2.

1. Introduction

Dengue is one of the important mosquito-borne viral diseases, posing a global health threat to public health. The accurate data on prevalence rate is difficult to obtain due to a vast majority of asymptomatic and self-managed patients. It is estimated that there are 390 million dengue virus (DENV) infections (60 million symptomatic infections) per year in 130 countries [1-2]. Most notably, the mortality is increased from 960 to more than 4032 during 2000-2015 [3]. In addition, economic burden of dengue is substantial. A systematic analysis showed the annual global cost of dengue was \$8.9 billion (95% UI, 3.7 billion-19.7 billion) in 2013 [4]. In China, high risk areas of dengue outbreaks were Guangzhou and Yunnan Provinces [5]. However, three outbreaks, especially large epidemic in 2017, were reported in Zhejiang Province with a typical subtropical climate [6].

Dengue virus (DENV) with 4 serotypes (DENV-1, DENV-2, DENV-3, DENV-4) could cause a wide spectrum of disease, including dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [7]. Delayed diagnosis of dengue could increase the risk of severe dengue. Recently, virological tests and serological tests are used for diagnosis of DENV infections in clinic. However, these methods vary in their sensitivity and specificity at different stages [8]. Therefore, it is urgent to develop a rapid and accurate tool for detection of DENV infections in the early stage. The aim of present study was to analyze the clinical characteristics of DF retrospectively and identify metabolites of serum in order to provide rapid diagnosis of DF.

2. Methods

2.1 Medical Records

The medical records of 50 hospitalized patients presented with DENV-2 RNA positive were reviewed during the outbreak attacked Hangzhou, China in 2017. The data of demographics, clinical characteristics, laboratory results, treatment history, hospitalization and clinical outcomes were collected.

2.2 Sample preparation and UPLC-Q-TOF MS spectrometer test

Serum samples were collected from 20 hospitalized DF patients (DF group) and 20 healthy volunteers (HC group) at The First Affiliated Hospital, College of Medicine, Zhejiang University from August to November in 2017. Serum samples were pretreated with triple volumes of acetonitrile and centrifuged at 15,000 rpm for 15 min to extract supernatant. The samples were tested by UPLC-Q-TOF MS spectrometer (Waters, Manchester, UK) using ACQUITY UPLC HSS T3 C18 column (100 mm × 2.1 mm, 1.8 μm) (Waters, Manchester, UK).

2.3 Data processing and multivariate analysis

Nuclear Magnetic Resonance (NMR) data were recorded on AV-400 spectrometer and proton coupling patterns were subjected to multivariate analysis. Metabolites quantification were identified using Chroma TOF (V 4.3x, LECO, United States) for deconvolution and peak matching. Further sorting and annotation were interpreted with Human Metabolome Database (HMDB) (<http://www.hmdb.ca/>) and LIPID MAPS (<http://www.lipidmaps.org/>). Differential metabolites responsible for distinguishing between DF patients and healthy volunteers were analysis by principal component analysis (PCA), partial least squares discrimination analysis (PLS-DA), and orthogonal projection to latent structures-discriminant analysis (OPLS-DA) using metaX (<http://metax.genomics.cn>). R²_Y was used to estimate the goodness of fit, and Q²_Y was used to assess the quality of prediction [9]. In addition, the

value of variable importance in the projection (VIP) greater than 1 and log2FC greater than 2 or lower than 0.5 was considered as significant contribution to classification. Furthermore, the potential biomarkers involved in metabolic pathways was performed based on Metabolomics Pathway Analysis (MetPA) (<https://www.metaboanalyst.ca/>). A p value < 0.05 was considered statistically significant.

3. Results

3.1 Clinical characteristics of DF

The clinical characteristics of the enrolled 50 patients showed in Table 1. There were 21 males and 29 females. The average admission days was 4.9 (3.9-7) days. Fever, nausea and vomiting, asthenia, skin hyperemia were the most common clinical symptoms. Regarding the comorbidities, hypertension (26%, 13/50), fatty liver (14%, 7/50), diabetes mellitus (10%, 5/50) were the most prevalent comorbidities. The results of the laboratory examinations showed that leukopenia (76%, 38/50), thrombocytopenia (92%, 46/50), increase of transaminase (alanine aminotransferase 29/50, aspartate aminotransferase 48/50, γ -glutamyl transpeptidase 23/50), increase of free fatty acids (FFA) (68%, 34/50), increase of creatine kinase (CK) (44%, 22/50) were more common. The management for most symptomatic DF patients were supportive treatments including.

3.2 Quality Control (QC) analysis and metabolites annotation

There was no statistical difference in age and gender between DF group and HC group. The QC samples were used to evaluate the repeatability and stability of UPLC-Q-TOF MS spectrometer. The Pearson correlation coefficient was calculated based on the peak area. The results of QC analysis indicated the experiment conditions exhibited high reliability (Supplementary Figure 1).

A total of 20 classes of metabolites were annotated and 631 types were lipids and lipid-like molecules (Figure 1a). According to the LIPID MAPS, flavonoids, glycerophosphoinositols and glycerophosphocholines were three most metabolites (Figure 1b).

3.3 Multivariate analysis

PCA was initially applied to evaluate the distribution pattern among all samples. As shown in Figure 2a, DF group and HC group showed good aggregation and classification. PLS-DA was further carried out to investigate the discrimination between the two groups (Figure 2b). The model parameters for the classification were $R^2Y = 0.87$, $Q^2Y = 0.79$ by 7-fold cross-validation. There was no obvious overfitting in model validation by randomly shuffled 200 times of the class membership (Figure 2c). The well fit PLS-DA models showed a significant separation in the scores plot between DF patients and HC, indicating the endogenous metabolites were changed as a result of DENV infection. Based on VIP and log2FC, a total of 619 metabolites significantly up-regulated and 64 metabolites down-regulated (Figure 2d).

3.4 Identification of potential biomarkers

Based on OPLS-DA, 17 metabolites in the upper-right quadrant of S-plot were tentatively identified, with 2 up-regulated and 15 down-regulated (Figure 2e) (Table 2). The relationship of 17 potential metabolites was evaluated by correlation analysis. Among them, LysoPC (18:2(9Z,12Z)) were highly positive correlated with PE (21:0/20:5(5Z,8Z,11Z,14Z,17Z)) and 13'-Hydroxy- α -tocopherol. Further metabolic pathways were analyzed by MetPA. The results from pathway analysis were presented in Figure 2e. Glycerophospholipid metabolism pathway was the main pathway of metabolic disorder in DF.

4. Discussion

The global burden of dengue has increased 400% during 13 years, presenting a great challenge to prevention and control [10]. The current diagnostic procedures for DENV infections had different their own limitations [8]. The results of our retrospective study indicated that the clinical symptoms were mild-to-moderate for most hospitalized patients during the outbreak in Hangzhou. Some degree of leucopenia and thrombocytopenia, increase of transaminase, FFA and CK were common during the febrile phase. In addition, the changes in serum metabolites were investigated by UPLC-Q-TOF MS spectrometer. There were 17 potential biomarkers were identified, mainly involved in glycerophospholipid metabolism pathway.

In the present study, all enrolled patients were infected by DENV-2. Previous study also demonstrated DENV-2 was the dominant serotype in Zhejiang Province, however, other three serotypes were identified during the same period [11]. Most people infected by DENV remain asymptomatic or self-limited [10]. We found fever, nausea and vomiting, asthenia, skin hyperemia were the most common clinical symptoms in hospitalized patients. These symptoms were relieved after supportive treatments. However, critical phase of dengue could be developed from febrile, especially for infants, old age, pregnant women with hypertension or vascular disorders.[10]. Although there were 26% patients with hypertension in our study, none of hospitalized patients progressed to severe dengue.

Significantly lower white blood cell and platelet were observed in present and previous studies [12]. In addition, laboratory investigations showed transaminase increased, especially for aspartate aminotransferase, indicating the combination of liver and musculoskeletal involvement [13].

Previous metabolomic studies detected serum metabolome and lipidome changes in patients infected with DENV [14]. The changes in host lipid homeostasis could not only satisfy the replication of offspring virus, but also support the energy of lipolysis supply [15-16]. In present study, 2 up-regulated and 15 down-regulated serum metabolites were contributed to DF. The down-regulated serum metabolites were phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin (SM), lysophospholipids (LPL), which might be attributable to the alternation of cell membrane composition caused by DENV, consistent with previous results in human serum [14]. In addition, glycerophospholipid metabolism pathway was the main pathway of metabolic disorder in DF. Furthermore, the potential glycerophospholipids metabolites have been identified as useful biomarkers for detection of DHF from DF and HC [17]. The glycerophospholipids

were linked to thrombocytopenia, vascular permeability and viral spherule membrane formation during DENV-2 infection [18]. In addition, the decrease of glutathione and total antioxidant capacity in patients with dengue fever leads to mild disorder of antioxidant system [19]. 13'-Hydroxy-alpha-tocopherol regarding as an antioxidant was reported to be beneficial to thrombocytopenia due to the increase of platelets [20]. The reason for this phenomenon is associated with the antioxidant effect of vitamin E on the inflammatory response of viral infection. The increase of FFA and the abnormality of glycerin phospholipid in clinic further confirmed the important relationship between lipid metabolism and virus reproduction after dengue virus infection.

Although the differences of metabolites were identified for DF from HC, there were several limitations in our study. Firstly, all patients were infected by DENV-2 due to the outbreaks in 2017. Second, the clinic data were collected and analyzed retrospectively. Third, the sample size was small and no patients progressed to severe dengue. Therefore, the results of this study need higher quality studies for confirmation in the future.

5. Conclusions

In conclusion, the major clinical symptoms of DF were fever, nausea and vomiting, asthenia, skin hyperemia, leukopenia, thrombocytopenia, and increase of transaminase. In addition, DENV-2 induced alterations in human glycerophospholipids, especially for the decrease of PC, PE, SM, LPL. These results should be supported in further animal infection models and even prospective randomized controlled clinical trials.

Declarations

Ethical Approval. This study was approved by the recommendations of the Ethics Committee of The First Affiliated Hospital, College of Medicine, Zhejiang University (No. 2020622). To protect personal privacy, identifying information of each patient in the electronic database was encrypted.

Consent for publication. All authors have seen and approved the content and fulfil the journal's requirements for authorship.

Availability of data and materials. All data in the manuscript.

Competing Interests. None

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Author Contributions. WY and YQQ developed the concept and designed the experiments. JPT and ZW collected clinical data from the patient records. JPT, JJZ and LZ did UPLC-Q-TOF MS spectrometer test. WY and ZW analyzed the metabonomic data. JPT and WY wrote the paper. All authors discussed the results and implications and commented on the manuscript at all stages.

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Tables

Table 1
Clinical characteristics of DF

General Information		Laboratory examinations	
Age (Year)	53.9 ± 16.3	WBC (3.5–9.5)*10E9/L	2.7 ± 1.5
Gender (Males/Females)	21/29	L (20–50) %	31.2 ± 14.7
BMI (kg/m ²)	22.6 ± 3.0	M (3–10) %	10.5 ± 4.3
Temperature (°C)	39.1 ± 0.8	N (40–75) %	57.0 ± 15.3
Admission days	4.9 (3.9-7)	Hb (115–150) g/L	138.1 ± 17.0
Clinical symptoms		PLT (125–350)*10E9/L	65.9 ± 40.8
Rash	36% (18/50)	CRP (0–10) mg/L	8.7 ± 14.2
Petechia	38% (19/50)	Alb (40–55) g/l	36.2 ± 2.8
Nausea and vomiting	68% (34/50)	ALT (7–40) U/L	64.4 ± 53.0
Abdominal pain and diarrhea	64% (32/50)	AST (13–35) U/L	114.1 ± 83.7
Asthenia	98% (49/50)	GGT (7–45) U/L	91.2 ± 127.8
Myalgia	60% (30/50)	Tbil (3.4–24) µmol/L	10.9 ± 5.3
Headache	46% (23/50)	Cr (35–123) µmol/L	69.7 ± 12.4
Skin hyperemia	50% (25/50)	BuN (3.1–8.8) mmol/L	3.8 ± 1.5
Lymphadenectasis	8% (4/50)	FFA (129–769) µmol/L	923.6 ± 353.0
Cough and sputum production	36% (18/50)	LDH (120–250) U/L	358.9 ± 112.1
Dyspnea	10% (5/50)	CK (40–200) U/L	298.1 ± 337.4
Haematemesis	4% (2/50)	TC (3.11–5.96) mmol/L	3.3 ± 0.8
Hemorrhinia and gingival bleeding	6% (3/50)	TG (0.34–1.7) mmol/L	1.6 ± 0.6
Comorbidities		PT (9.7–13.5) s	11.1 ± 1.1
Hypertension	26% (13/50)	Fi (2–4) g/L	2.1 ± 0.4
Diabetes mellitus	10% (5/50)	PCT (0-0.25) ng/ml	0.2 ± 0.2
Fatty liver	14% (7/50)	Treatment	
Hypothyroidism	4% (2/50)	Platelet transfusion	18% (9/50)
Coronary heart disease	2% (1/50)	Hemagglutinase	32% (16/50)
Cancer	4% (2/50)	Human granulocyte-stimulating Factor	38% (19/50)
Ankylosing spondylitis	2% (1/50)	Recombinant human interleukin-11	30% (15/50)
Bronchiectasis	2% (1/50)	Tranexamic acid	18% (9/50)
Chronic hepatitis B	2% (1/50)	Fibrinogen	8% (4/50)

Table 2
17 metabolites that show differences between DF and HC

Compound	RT (min)	VIP	p	Adducts	Description	m/z	Formula	Fragment Score
17.20_782.5694n	7.86	22.23	*	M + H, M + Na	LysoPC(18:2(9Z,12Z))	520.3417	C26H50NO7P	75.8
16.25_782.5721m/z	16.25	19.06	*	M + H	PC(18:0/18:4(6Z,9Z,12Z,15Z))	782.5721	C44H80NO8P	6.15
17.20_807.5815n	17.20	18.89	*	M + H, M + Na	PE(21:0/20:5(5Z,8Z,11Z,14Z,17Z))	808.5888	C46H82NO8P	17.4
15.96_779.5492n	15.96	11.83	*	M + H, M + Na	PC(20:5(5Z,8Z,11Z,14Z,17Z)/16:0)	780.5565	C44H78NO8P	64.5
20.19_785.5958n	20.19	10.36	*	M + H, M + K, M + H- H2O	PC(18:1(9Z)/18:1(9Z))	786.6031	C44H84NO8P	42.4
20.19_771.6136n	20.19	9.59	*	M + Na, M + K, M + H	PC(18:0/P-18:1(11Z))	810.6036	C44H86NO7P	5.04
17.76_783.5809n	17.76	8.80	0.001	M + H- H2O, M + H	PC(18:1(11Z)/18:2(9Z,12Z))	784.5863	C44H82NO8P	1.44
16.31_756.5570m/z	16.31	7.51	*	M + H	PC(16:0/18:3(6Z,9Z,12Z))	756.557	C42H78NO8P	0.412
15.95_756.5567m/z	15.95	7.11	*	M + H	PC(14:0/20:3(5Z,8Z,11Z))	756.5567	C42H78NO8P	5.61
17.66_847.5400n	17.66	6.75	0.001	M + H, M + NH4	PS(19:1(9Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	865.5344	C47H78NO10P	0.244
7.85_543.3333n	7.85	6.39	*	M + H, M + Na	LysoPC(20:4(8Z,11Z,14Z,17Z))	544.3406	C28H50NO7P	41.6
15.81_729.5335n	15.81	4.87	0.001	M + H, M + Na	PC(14:1(9Z)/18:1(11Z))	752.5357	C40H76NO8P	95.8
18.01_446.3751n	18.01	4.18	*	M + H- H2O, M + H	13'-Hydroxy-alpha-tocopherol	429.3718	C29H50O3	70.9
16.76_744.5570m/z	16.76	3.42	*	M + K	SM(d18:0/16:0)	744.557	C39H82N2O6P+	11.3
9.88_531.2744m/z	9.88	1.71	0.018	M + H	PG(20:5(5Z,8Z,11Z,14Z,17Z)/0:0)	531.2744	C26H43O9P	0
7.01_444.2715n	7.01	1.31	0.001	M + H, M + Na, M + NH4, M + H-H2O	1(3)-glyceryl-6-keto-PGF1alpha	445.2787	C23H40O8	20.6
8.65_730.4595n	8.65	1.28	0.004	M + NH4, M + Na, M + H-H2O	PA(17:2(9Z,12Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	748.4933	C42H67O8P	0

RT, retention time; VIP, value of variable importance in the projection; * p<0.001.

Figures

Figure 1

Metabolites annotation. (a) HMDB annotation; (b) LIPID MAPS annotation.