



HOSTED BY



ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: <http://ees.elsevier.com/apjtm>Original research <http://dx.doi.org/10.1016/j.apjtm.2016.05.009>

Ultrasonic diagnosis of patients with clonorchiasis and preliminary study of pathogenic mechanism

Mei Fan^{1*}, Lin Lu², Chun Su³, Mei Xue⁴, Ji-Mei Dou³, Pei Li⁵, Han-Qi Feng⁶, Yan-Bing Fan⁷¹Department of Ultrasound, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China²Department of Radiology, The Third Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China³The Fifth Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China⁴Henan Experimental Kindergarden, Zhengzhou 450052, China⁵Department of Pathophysiology, Zhengzhou University, Zhengzhou 450052, China⁶The Third Affiliated Hospital of Zhengzhou University, Zhengzhou 450000, China⁷Anyang Hospital of Traditional Chinese Medicine, Anyang, China

ARTICLE INFO

Article history:

Received 15 Apr 2016

Received in revised form 16 May

2016

Accepted 23 May 2016

Available online 30 May 2016

Keywords:

Clonorchiasis

Ultrasonic diagnosis

Inflammatory reaction

Cell apoptosis

ABSTRACT

Objective: To discuss the liver function damage mechanism of patients with clonorchiasis by analyzing the ultrasound characteristics, liver function, change of the serum inflammatory factors and cell apoptosis factors.

Methods: Color Doppler ultrasound technique was adopted to detect the portal vein and blood flow change of patients with clonorchiasis; ELISA was used to determine the level of different serum inflammatory factors. The levels of serum total bilirubin, serum albumin and glutamic-pyruvic transaminase were detected by automatic biochemical analyzer. Western blot was used to determine the expression of proteins relevant to apoptosis.

Results: Compared with the health control group, the trunk diameter of portal vein and the thickness of spleen, as well as the hepatic artery pulsation index of clonorchiasis patients increased obviously, the mean blood flow velocity of portal vein ($P < 0.05$ or $P < 0.01$) decreased. The content of total bilirubin and transaminase in plasma increased significantly, but albumin decreased ($P < 0.05$). Levels of TNF- α , IL-6 and IFN- γ increased remarkably, and the level of every factor was significantly different among patients with Child-Pugh I, Child-Pugh II and Child-Pugh III classification of liver function ($P < 0.05$ or $P < 0.01$). With the exacerbation of liver dysfunction, levels of TNF- α , IL-6 and IFN- γ gradually increased ($P < 0.05$). Compared with the healthy control group, the expression quantity of apoptosis protein Fas, FasL, Bax and Caspase-3 increased significantly ($P < 0.05$ or $P < 0.01$), but Bcl-2 decreased ($P < 0.05$).

Conclusions: Changes of ultrasonic characteristics and liver dysfunction, caused by liver fluke infection, may be related to that both inflammatory response and apoptosis response have participated in the pathogenic process and liver damage course of clonorchiasis.

1. Introduction

With the improvement of living standards and diversification of diet, the incidence of the clonorchiasis is increasing year by

year. Clonorchiasis is a kind of food-borne parasitic disease which is caused by the parasitism of *Clonorchis sinensis* on the human intra-hepatic bile duct. And clonorchiasis was ever ranked as the first kind of risk factors by the World Health Organization [1]. At present, common examinations for clonorchiasis diagnosis include hematology, immunology, parasitism, ultrasound and CT examination [2]. Among which, the ultrasound diagnosis has the advantages of noninvasive, shortcut and low cost. Color Doppler ultrasound used for diagnosing clonorchiasis is based on the ultrasonogram of patients' pathological change, which has provided the basis for the early diagnosis of clonorchiasis.

*Corresponding author: Mei Fan, Department of Ultrasound, The First Affiliated Hospital of Zhengzhou University, No.1 East Jianshe Road, Erqi District, Zhengzhou 450052, China.

Tel: +86 13673665086

E-mail: fanmei772@163.com

Peer review under responsibility of Hainan Medical College.

Foundation Project: It was supported by Trans-Century Training Programme Foundation for the Talents by the Ministry of Education (NO. NCET-11-0949).

In the parasitic infection, the role of cytokines has attracted more and more attention. Adult liver fluke damages the liver tissue and leads to the change of the patient's cellular immunity and humoral immunity function [3]. It has reported that the clonorchiasis patient may produce specific immune response [4] which is often accompanied by complications like jaundice, cholangitis, cholelithiasis, liver cirrhosis, biliary duct cancer, liver cell cancer and other liver and gall diseases [5–8]. This seriously threatens the life of patients. The cytokines TNF- α , IL-6 and IFN- γ are important mediums of immunoreaction in various infectious diseases. Their functions in the pathogenesis and pathological changes of clonorchiasis, and the relations with liver function injury remain unclear. This study will preliminarily explore their clinical significance in the clonorchiasis. In addition, when the liver cells suffer damage, the cytokines FasL/Fas, TNF- α , TGF- β 1 and INF- γ are activated to induce apoptosis by combining with the receptor from the target cells [9]. Studies have suggested that the pathogenesis of many kinds of liver damage, including hepatic failure, viral hepatitis, cirrhosis, autoimmune liver disease and liver tumors are closely related to the apoptosis [10]. To study the liver cell apoptosis of clonorchiasis patients is of important significance on the analysis of the liver damage mechanism and the treatment of related diseases.

2. Material and methods

2.1. General materials

Sixty-eight clonorchiasis patients treated in the First Affiliated Hospital of Zhengzhou University from March 2013 to February 2015 were collected, including 47 males and 21 females, age among 19–69 years old, with average age as (45.7 ± 3.1) years old. Among which, 41 cases were confirmed as positive by liver fluke enzyme mark, 20 cases were confirmed by checking eggs in stool, 7 cases were confirmed by checking eggs in duodenal fluid using gastroscope. The healthy control group had 25 cases, including 15 males and 10 females, aged 21 to 70, with average age as (56.5 ± 1.8) . All the subjects were ruled out other parasitic infections and anaphylactic disease, as well as intemperance, autoimmunity disease, viral hepatitis. And they volunteered to participate and signed the informed consent form. In additional, five human liver tissue species from clonorchiasis patients and five healthy liver tissue species conserved in the hospital were taken respectively to do control experiment.

2.2. Ultrasonic diagnosis

Patients were maintained at supine position. The patients' liver parenchyma, biliary system and the thickness of the spleen were observed by the Color Doppler Ultrasonic Diagnosis Apparatus (Phillips Envisor, 3.5 MHz transducer frequency). Then the portal trunk inner diameter, frequency spectrum and hepatic artery pulsation indexes were measured.

2.3. Measurement of the liver function

The serum total bilirubin (TBIL), serum albumin (ALB) and glutamic-pyruvic transaminase (ALT) content of the subjects were detected by the fully automatic biochemical analyzer. After

obtaining the data, the patients were classified according to the Child-Pugh classification standard [11]. Class I: TBIL <34 $\mu\text{mol/L}$, ALB >35 g/L, ALT <40 IU/L; Class II: TBIL $(34\text{--}51)$ $\mu\text{mol/L}$, ALT $(40\text{--}55)$ IU/L; Class III: TBIL >51 $\mu\text{mol/L}$, ALB <28 g/L, ALT >55 IU/L.

2.4. Determination of serum inflammatory factors level

All the subjects were collected 6 mL elbow venous blood in the morning with an empty belly. Blood samples were sitting for 4 h without anticoagulation. Then, they were centrifuged for 15 min at 2000 rpm to separate the serum and saved under -20 °C for standby application. Serum TNF- α , IL-6 and IFN- γ levels were detected by double antibody sandwich ABC-ELISA method. Human TNF- α , IL-6 and IFN- γ kits were bought from Beijing Jingmei Biological Engineering Company. The measuring instrument was ELx800 fully automatic microplate reader (BioTek, USA). The detailed detection procedures were operated according to the instruction of the kits.

2.5. Apoptosis-related proteins expression in the liver tissue

The liver tissue samples saved in liquid nitrogen were taken out. Fifty mg liver tissue was fetched and grinded. Five hundred μL tissue lysis buffer was added. The sample was ice-bathed for 1 h at 4 °C, and then centrifuged for 30 min at 13000 rpm. The supernate was collected and determined its total protein content with Bradford method. The target protein was separated from the extracted total protein by SDS-PAGE and then was transferred onto the PVDF membrane. It was sealed with TBST liquid containing 50 g/L skim milk under room temperature. Rabbit anti-human polyclonal antibody Fas (1: 200)/FasL (1: 200)/Bcl-2 (1: 500)/Bax (1: 200)/Caspase-3 (1: 100)/ β -actin (1: 500) was added and incubated at 4 °C. After rinsing, the goat anti-rabbit IgG-HRP antibody was added and hybridly incubated, then and rinsed. All the antibodies were bought from Wuhan Boster Biological Engineering Company. Western blot bands were scanned and the gray level was analyzed by Quantity One software. The ratio of the target protein gray level to that of β -actin was regarded as the relative expression level.

3. Results

3.1. Ultrasonic diagnostic features of clonorchiasis patients

According to the analysis of the ultrasonoscopy, it was found that in the clonorchiasis patients, the local vessel expansion appeared in intrahepatic bile duct whose wall became thickened. The echo increased and the liver parenchyma changed. From the results of detecting related indexes by ultrasound, the internal diameter of portal vein, thickness of spleen and hepatic artery pulsation index in clonorchiasis patients had increased obviously compared with the normal group, but mean blood flow velocity of portal vein decreased, and the differences were of statistical significance ($P < 0.05$ or $P < 0.01$).

Comparison of the ultrasonic testing indicators was as follows. In Normal group ($n = 25$) and Clonorchiasis group ($n = 68$): Internal diameter of the portal vein was (8.5 ± 1.9) mm and (14.7 ± 2.8) , respectively; Thickness of spleen was

Download English Version:

<https://daneshyari.com/en/article/3455192>

Download Persian Version:

<https://daneshyari.com/article/3455192>

[Daneshyari.com](https://daneshyari.com)