



Clinical significance of combined liver function and high-sensitivity C-reactive protein measurement in children with hand-foot-mouth disease

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Genet. Mol. Res. 15 (3): gmr.15038744

Received April 28, 2016

Accepted June 10, 2016

Published September 23, 2016

DOI <http://dx.doi.org/10.4238/gmr.15038744>

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ABSTRACT. Hand-foot-mouth disease (HFMD) is a common pediatric disease responsible for the development of rashes or herpes on the hand, foot, and mouth. Severe complications of HFMD include myocarditis, pulmonary edema, aseptic meningoencephalitis, and even death. Therefore, early diagnosis of HFMD is of particular importance. In this study, we determined the clinical value of the combined detection of liver function and high-sensitivity C-reactive protein (hs-CRP) expression in children with HFMD. Three hundred children with HFMD were recruited to this study between July 2013 and July 2015 and divided into the mild and severe HFMD groups (N = 150 per group). The liver function [aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) levels]

and hs-CRP expression were evaluated using standardized tests, and the clinical value of combined detection of these indices (in parallel and serially) was determined. Patients in the severe HFMD group showed significantly higher levels of ALT, AST, ALP, and hs-CRP compared to those in the mild HFMD group ($P < 0.05$). The hs-CRP and liver function tests had low specificity and sensitivity, respectively. However, parallel combined detection improved the sensitivity and negative predicted value of these indices, whereas serial combined detection improved the specificity and positive predicted value. In conclusion, hs-CRP and liver function play a major role in the diagnosis of HFMD (and identifying its severity), and serial combined detection of these indices enhances the positive predicted value, and could be employed to diagnose severe HFMD at an earlier stage.

Key words: Hand-foot-mouth disease; Liver function; High-sensitivity C-reactive protein; Alanine transaminase; Aspartate aminotransferase; Alkaline phosphatase

INTRODUCTION

Hand-foot-mouth disease (HFMD) is a common pediatric infectious disease that is chiefly transmitted through the respiratory and digestive tracts, and via intimate contact; HFMD chiefly affects children aged below 3 years, or those who are not of school age (McMinn, 2002; He et al., 2013b). Coxsackievirus A16 (CoxA16) and Enterovirus 71 (EV71) are chiefly responsible for HFMD. The virus proliferates in the intestinal tract post-infection; subsequently, it enters the blood stream, and drifts away and colonizes in the hand and foot, inducing local pathological changes such as fever and herpes in the hand, foot, mouth, and hip. While a majority of the infected children show mild symptoms and a relatively good prognosis (Reina et al., 2000; Ho, 2000), the disease develops rapidly in the others, leading to severe complications, such as meningitis, liver function damage, acute flaccid paralysis, and even death (Stalkup and Chilukuri, 2002). HFMD has been classified as a legally reported disease in China. She et al. (2006) reported that a 2008 outbreak of HFMD in a large number of Chinese provinces had a fatality rate of 0.026%. Therefore, future research should focus on the early diagnosis and treatment of HFMD.

C-reactive protein (CRP), an acute-phase protein synthesized in the liver, combines with polysaccharides, lecithin, and nucleic acids from various microorganisms (such as bacteria, fungi, and protozoa) to activate the complement system, in order to induce an inflammatory response to the immunomodulatory and phagocytic invasion of host cells (Hsia et al., 2005; Ma et al., 2013). Low concentrations of CRP can be detected by a highly sensitive method called the high-sensitivity CRP (hs-CRP) test. Jaye and Waites (1997) suggested that viral infections induced transient changes in myocardial enzymes. hs-CRP, a sensitivity index for inflammatory injury, is of great value in diagnosing pediatric infectious diseases. Detection of hs-CRP is more rapid and sensitive compared to that of white blood cells. However, CRP expression is upregulated under severe pathological conditions, such as acute myocardial infarction and traumatic inflammation; therefore, CRP expression is a non-specific diagnostic index (Pan et al., 2012) and must be supplemented by other indices for early diagnosis of pediatric HFMD.

In this study, we compared CRP expression and liver function between patients with mild and severe HFMD; we also evaluated the significance of combined CRP and liver function detection in the early diagnosis and improved prognosis of severe HFMD.

MATERIAL AND METHODS

Clinical data

Three hundred children who were treated for HFMD at the Binzhou People's Hospital between July 2013 and July 2015 were recruited to this study. HFMD was diagnosed according to the diagnostic criteria of the Guidance for Prevention and Control of Hand-Foot-Mouth Disease (2010) formulated by the Ministry of Health. Patients with other diseases that can induce abnormal liver function were excluded. The patients were grouped into the severe and mild HFMD groups (N = 150 per group). The severe HFMD group was composed of 90 male and 60 female patients aged 7 months to 6 years (average age = 2.7 ± 1.3 years), and weighing 4-15 kg (average = 10.5 ± 5.2 kg). The mild HFMD group was composed of 84 male and 66 female patients aged 6 months to 5 years (average age = 2.7 ± 1.3 years), and weighing 4-16 kg (average = 10.6 ± 5.3 kg). We observed no significant differences in the gender ratio, weight, and age between the two groups. Signed informed consent forms were obtained from the parents of all patients prior to the study. The study was also approved by the Medical Ethics Committee of the Binzhou People's Hospital.

Diagnostic criteria

HFMD was diagnosed when the patients tested positive for at least one of the following (He et al., 2013a): specific nuclease of EV71 or CoxA16, or characterization of the isolated enterovirus as the HFMD-causing EV71 or CoxA16. The patients were classified into the ordinary and severe HFMD groups according to disease severity. Ordinary or mild HFMD manifests as acute-onset fever, herpes, papula on the hand, foot, and hip, inflammatory flush around the herpes, liquid-filled blisters, running nose, cough, and lack of appetite. Some cases of mild HFMD only manifest as herpangina or rashes. Severe HFMD is characterized by rapid disease progression, continuous hyperpyrexia, significant increase in the peripheral white blood cell level, hypertension, hyperglycemia, cold sweat, and poor peripheral circulation. Some patients also develop complications associated with the nervous, respiratory, and circulatory systems, such as encephalomyelitis, meningitis, encephalitis, emesis, limb tremor, pulmonary edema, and circulatory disturbance.

Testing for hs-CRP and liver function

Fasting peripheral venous blood (5 mL, early morning) was collected from the recruited patients in tubes loaded with ethylenediaminetetraacetic acid anticoagulant, and transported to the laboratory within 30 min. Serum, isolated by centrifuging the blood samples at 1000 g at 37°C for 3 min, was tested for the relevant indices. All blood samples conformed to the detection criteria. hs-CRP was detected by immune scatter turbidimetry using reagents (Bairui Biotech Co., Ltd., Shanghai, China) according to the manufacturer protocols. The liver function was detected with a fully automated biochemical analyzer (Mindray Co., Ltd.,

Shenzhen, China) using the corresponding reagents. Normal liver function was characterized by aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine transaminase (ALT) levels within the range 0-42 U/L, 26-117 U/L, and 0-41 U/L, respectively. Expression for the positive rate of liver function is as follows.

$$r = d/n \times 100\% \quad (\text{Equation 1})$$

where r refers to the positive rate of liver function, d refers to the number of patients with positive result, and n refers to the number of patients.

Evaluating the value of combined detection

The specificity (Sp), sensitivity (Se), accuracy (Ac), and positive and negative predicted values (+PV and -PV, respectively) of the ALT, AST, ALP, and hs-CRP assays were evaluated. The ALT, AST, ALP, and hs-CRP levels were detected in parallel in accordance with the following parallel detection rule: the combined detection score is considered positive when the score of an (any one) individual assay is positive. The hs-CRP and liver function tests were performed in series, according to the following combined detection rule: the combined detection score is considered positive when the scores of all individual assays are positive.

Data analysis

The ALT, AST, ALP, and hs-CRP levels were compared between patients included in the mild and severe HFMD groups. The positive rates of these indices, as well as the Sp, Se, Ac, +PV, and -PV of ALT, AST, ALP, and hs-CRP detection, were calculated and compared between the two groups. These indices were then analyzed using a combinatorial method, and the Sp, Se, Ac, +PV, and -PV of this method were analyzed.

Statistical analysis

The obtained data were analyzed using the SPSS software platform (v.20.0; IBM, Armonk, NY, USA). The measured data are reported as means \pm standard deviations (SD), and analyzed by the t -test. The enumerated data were analyzed by the chi-square test. Differences with P values < 0.05 were considered statistically significant.

RESULTS

Liver function and hs-CRP levels in the HFMD groups

Patients in the severe HFMD group showed significantly higher levels of ALT, AST, ALP, and hs-CRP compared to those in the mild HFMD group ($P < 0.05$; Table 1).

Detection of positive rate of liver function and hs-CRP

Patients in the mild HFMD groups showed a significantly higher positive rate of liver function and hs-CRP compared to the patients in the severe HFMD group ($P < 0.05$; Table 2).

Table 1. Comparison of the liver function and high-sensitivity C-reactive protein (hs-CRP) test results between the mild and severe hand-foot-mouth disease (HFMD) groups [means \pm standard deviation (SD)].

Group	AST (U/L)	ALT (U/L)	ALP (U/L)	hs-CRP (mg/L)
Severe HFMD group (N = 150)	56.21 \pm 3.66	60.07 \pm 25.38	58.19 \pm 14.24	14.21 \pm 2.76
Mild HFMD group (N = 150)	25.03 \pm 8.03	21.02 \pm 13.53	27.76 \pm 2.66	5.93 \pm 2.71
t-value	12.28	10.31	9.85	7.24
P value	<0.05	<0.05	<0.05	<0.05

AST, aspartate aminotransferase; ALT, alanine transaminase; ALP, alkaline phosphatase.

Table 2. Comparison of positive rate of liver function and high-sensitivity C-reactive protein (hs-CRP) expression between the severe and mild hand-foot-mouth disease (HFMD) groups N (%).

Group	AST (U/L)	ALT (U/L)	ALP (U/L)	hs-CRP (mg/L)
Severe HFMD group (N = 150)	98 (65.3)	133 (88.7)	138 (92.0)	144 (96.0)
Mild HFMD group (N = 150)	6 (4.0)	4 (2.7)	44 (29.3)	56 (37.3)
χ^2	110.26	60.90	63.36	59.19
P value	<0.05	<0.05	<0.05	<0.05

AST, aspartate aminotransferase; ALT, alanine transaminase; ALP, alkaline phosphatase.

Evaluation of value of liver function and hs-CRP detection

An evaluation of the results of liver function and hs-CRP testing suggested that the hs-CRP and liver function tests had low specificity and sensitivity, respectively. Parallel combined detection improved the sensitivity and the negative predicted value, whereas serial combined detection improved the specificity and positive predicted value of the liver function and hs-CRP tests (Tables 3-5).

Table 3. Evaluation of detected value of liver function and high-sensitivity C-reactive protein (hs-CRP) (%).

Index	Sp	Se	Ac	+PV	-PV
AST	95.8	65.4	80.0	94.4	71.8
ALT	97.2	88.5	92.7	97.2	88.6
ALP	70.8	92.3	82.0	77.4	89.5
hs-CRP	62.5	96.3	80.0	73.4	93.6

AST, aspartate aminotransferase; ALT, alanine transaminase; ALP, alkaline phosphatase; Sp, specificity; Se, sensitivity; Ac, accuracy; +PV, positive predicted value; -PV, negative predicted value.

Table 4. Evaluation of liver function and high-sensitivity C-reactive protein (hs-CRP) values (%) detected by parallel combined detection.

Index	Sp	Se	Ac	+PV	-PV
ALT combined with AST	100	61.5	85.3	100	76.6
ALT combined with ALP	100	85.9	92.7	100	86.7
ALT combined with hs-CRP	97.2	88.5	92.7	97.2	88.6
Combination of four indices	100	61.5	80.0	100	70.6

AST, aspartate aminotransferase; ALT, alanine transaminase; ALP, alkaline phosphatase; Sp, specificity; Se, sensitivity; Ac, accuracy; +PV, positive predicted value; -PV, negative predicted value.

Table 5. Evaluation of liver function and high-sensitivity C-reactive protein (hs-CRP) values (%) detected by serial combined detection.

Index	Sp	Se	Ac	+PV	-PV
ALT combined with AST	95.8	92.3	94.0	96.0	92.0
ALT combined with ALP	70.8	97.4	84.7	78.4	96.2
ALT combined hs-CRP	62.5	100	82.0	74.4	100
Combination of four indices	43.1	43.1	80.0	65.0	100

AST, aspartate aminotransferase; ALT, alanine transaminase; ALP, alkaline phosphatase; Sp, specificity; Se, sensitivity; Ac, accuracy; +PV, positive predicted value; -PV, negative predicted value.

DISCUSSION

HFMD usually manifests in a mild manner in children, in the form of a fever, rash, or herpes on the hand, foot, and mouth (Guimbao et al., 2010). However, some patients also develop encephalitis, acute flaccid paralysis, encephaledema, and myocarditis induced by impairment of central nervous system and respiratory system. Mild cases that rapidly develop these conditions have a short therapeutic time window and high fatality rate (Wang et al., 2007). The enteroviruses EV71, CoxA, and Echo, that are characterized by strong infectivity, a high probability of unapparent infection, a complex (and rapid) transmission approach, and high risk of outbreak, are known to cause HFMD (Zhang et al., 2016). Early diagnosis of severe cases and timely intervention could lower the disability and fatality rates in, and improve the prognosis of, patients with HFMD. Therefore, a highly efficient, accurate diagnostic method must be developed to accurately diagnose severe cases at an early stage.

The severity of HFMD is closely associated with the activation of an inflammatory cytokine cascade. CRP is a typical inflammatory marker secreted by an acute phase protein that is closely correlated with the oxidative stress response (Wang et al., 2007). The half-life period of CRP is 5 to 7 h; moreover, it is free from the influence of whole blood, anti-inflammatory drugs, and hormones, and is known to change synchronously with the inflammation. For example, the serum hs-CRP level increases rapidly during the early stages of infection, the increase being positively correlated to the degree of infection (Shekhar et al., 2005). Chen et al. (2007) reported that hs-CRP played a major role in determining the severity of HFMD based on the observation that the level of hs-CRP was much higher in the severe cases than that in the mild cases (which in turn was higher than that in normal children). In this study, patients with severe HFMD showed higher hs-CRP levels compared to those with mild HFMD, which was consistent with the results of the previous study. This indicated that hs-CRP has a considerable diagnostic value in determining the severity of HFMD in children. However, the specificity of the hs-CRP test was quite low.

Recent studies (Huang et al., 2006; Zhao et al., 2011) have reported that liver function abnormality is a major complication of HFMD; in fact, studies have indicated that the liver cells of children with acute-phase HFMD are more likely to be injured, which might be correlated with the proliferative capacity and toxicity of viruses that invade the liver cells during the early stages of the disease. In this study, the AST, ALT, and ALP levels were significantly higher in severe HFMD cases compared to the mild cases. Therefore, mild HFMD was characterized by an absence of liver function impairment, whereas severe cases were likely to develop liver function injury. Currently, it is universally recognized that impaired liver presents an abnormal hepatic enzyme spectrum. ALT, AST, and ALP are important indices that reflect impairments in the liver function; therefore, an increase in the hepatic enzyme spectrum is usually a predictor of HFMD aggravation (Hsueh et al., 2000; Wang et al., 2003). Patients with severe HFMD showed significantly higher levels of ALT, AST, and ALP compared to those with mild HFMD. However, this analysis was not very sensitive. Serial combined detection of hs-CRP and liver function indices significantly improved the specificity and positive predicted value, whereas parallel combined detection improved the sensitivity and negative predicted value of the tests. Therefore, combined detection of hs-CRP and liver function has high diagnostic applicability.

In conclusion, hs-CRP and liver function could be used to characterize the severity of HFMD. Combined detection of these indices can improve the positive predicted value of these tests, thereby facilitating early diagnosis and characterization of HFMD.

This study was conducted in samples obtained from HFMD patients only; that is, a control group, comprising healthy children, was not included. This necessitates further research with a larger sample size and a control group comprising healthy children.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

The authors sincerely thank the service from Binzhou People's Hospital.

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