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Original Article

Significance of T lymphocyte in the serum of cytomegalovirus-induced hepatitis

Yan-Ping Zhu*, Yue-Qin Han*, Zhen Yu, Yue-Qin Bu, Xin-Xin Chen, Qiao-Zhi Yang

*Department of Pediatrics, Liaocheng People's Hospital, Liaocheng 252000, Shandong, China. *Equal contributors and co-first authors.*

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Abstract: The aims was to investigate the influence of cytomegalovirus infection on the expression of T lymphocyte. 70 children with cytomegalovirus-induced hepatitis were enrolled in this study. According to the different viral loads, children were divided into high viral load group (n=16), medium viral load group (n=31) and low viral load group (n=23), meanwhile, 40 healthy children were as control group enrolled in this study. Furthermore, the expression of T-lymphocyte subsets in peripheral blood were observed and Spearman method was used to analyze the relationship between the expression of T-lymphocyte subsets and cytomegalovirus load. Compared with control group, the total amount of T lymphocytes were fewer and results showed that the numbers of CD4+ T cells and the ratio of CD4 and CD8 reduced. Moreover, in different subgroups, the numbers of T cells were more in medium viral load group than that in high viral load group, and both groups were fewer than low viral load group, which was significant difference ($P<0.05$). Additionally, Spearman analysis showed that the cytomegalovirus load was negative correlation with CD3, CD4 numbers and the ratio of CD4/CD8. Cytomegalovirus infection reduced the numbers of T cells, mainly manifesting the reduction of CD4 cells, which got worse with the progression of disease.

Keywords: T lymphocyte, cytomegalovirus, infection, children with hepatitis, serum

Introduction

Hepatitis is a medical condition defined by the inflammation of the liver and one of the commonest liver diseases, which is induced by virus, bacterial and chemical toxins. Usually, viral hepatitis is the most common cause of hepatitis worldwide [1]. T cells play an important role in controlling the progression of viral hepatitis, including help T cell, cytotoxic T cell and dendritic T cell [2]. Cytomegalovirus belongs to be taherpesvirinea subfamily and cytomegalovirus (CMV) can be transmitted through breast milk [3], and CMV could secrete many kinds of factors to make cells larger after infection, which significantly suppresses immunological function and reduces the ability of destructive immunocytes [4]. CMV usually is not cleared completely and tends to hide in the host [5]. Once infected, CMV could be activated [6]. When patients have normal immune function, patients have no clinical symptoms, but it could be activated when immune function of T cell is impaired [7]. Because children are immature, their liver function and immune function fast reduced when liver is infected by CMV, which causes severe impairment for liver tissue and systemic infection through blood transmission, in addition, CMV is the primary infectious cause of hearing loss [8]. T lymphocyte plays a central role in cell-mediated immunity. when body is infected, T cells present related immune response to maintain the homoeostasis [9]. Some studies indicated that CMV infection is the common factor of hepatitis in patients conducted solid organ transplantation [10, 11] and stem cell transplantation [12], thus T cells is an important immunological index [13]. This study detected the expression of T-lymphocyte subsets in peripheral blood in children with hepatitis induced by cytomegalovirus to explore the relationship between T lymphocyte and CMV.

Methods

Clinical data

70 children with cytomegalovirus-induced hepatitis during June, 2010 to October, 2013 were

Table 1. Comparison of expression of T lymphocyte subsets between two groups (%)

Group	n	CD3	CD4	CD8	CD4/CD8
Hepatitis group	70	59.27±5.34	34.19±3.52	31.06±2.42	1.01±0.22
Control group	40	68.35±6.21	42.21±4.81	28.41±1.94	1.36±0.24
t		5.72	5.61	1.07	5.28
P		<0.05	<0.05	>0.05	<0.05

as hepatitis group in this study, including 41 males and 29 females, aged from 6 to 15 years old (mean age: 12.35±3.57 years old). According to the different viral loads, children were divided into high viral load group (n=16), medium viral load group (n=31) and low viral load group (n=23); meanwhile, 40 healthy children were as control group, including 22 males and 18 females, aged from 5 to 14 years old (mean age: 11.82±3.47 years old). Furthermore, there were no significant differences of sex and age between hepatitis group and control group (P>0.05). This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Liaocheng People's Hospital. Written informed consent was obtained from all participants.

Inclusion and exclusion criteria

Inclusion criteria [14]: 1) following the rules for prevention schemes of viral hepatitis revised by Chinese medical association branch of infectious diseases and parasitic diseases in September, 2009; 2) without severe diseases; 3) without history of liver diseases, immune systemic dysfunction; 4) with obvious clinical symptoms of viral hepatitis; 5) patients and their families signing up informed consent and approved by ethics committee.

Exclusion criteria: 1) children with hepatitis did not induced by CMV; 2) history of using immunological medicines within 1 year; 3) children confronted with liver function impairment caused by alcohol or drugs; 4) without antivirus therapy within half of a year; 5) refusing this study or dropping out during the study.

Blood collection

According the previous report [15], all patients should keep empty stomach in the morning to perform blood collection. 20 ml blood was collected and stored in the freezer (Haier refrigerator BCD-215KS, Qingdao, China) for one day and used at the next day. Serum specimens were isolated by auto-centrifuge instrument (5424R, Eppendorf, Juelich, Germen) and each blood specimen was divided into two groups (group A and group B). Specimen from group A was used to detect the T lymphocyte subsets and specimen from group B was used to measure the CMV load. All experiments were completed within 2 h.

Measurement of T lymphocyte subsets

According the previous report [16], all specimens from group A were conducted real-time PCR assay through fluorescent quantitative PCR instrument (Perkin Elmer Company, USA) and labeled by fluorescent antibodies of T lymphocyte subsets and then added anti-coagulate reagents into the specimens and mixed for 15 min, followed by hemolysis via COULTER Q-PREP auto-hemolysis instrument (Beckman coulter Company, USA), and then T lymphocyte subsets were measured through Epics XL flow cytometry (FACSAriaTM II BD, New Jersey, US) and calculated the percentage of CD3, CD4 and CD8 among 1000 T lymphocytes. All procedures were followed by instructions.

CMV load testing [17]

All specimens from group B were divided into group C and group D, and group C was treated with K3EDTA and group D was not any treatment. CMV was labeled by PCR reagents (Shenzhen PIJI Bio-tech, Ltd) and then DNA of CMV in the plasma was amplified through Light Cycler amplification instrument (DYCZ-20E, Nanjing, China) and measured CMV load. All procedures were followed by instructions.

Grading the CMV load

Low viral load: CMV DNA load was less than 1×10^4 copies/ml; medium viral load: CMV DNA load was from 1×10^4 to 5×10^4 copies/ml; high viral load: CMV DNA load was more than 5×10^4 copies/ml.

Table 2. Comparison of expression of T lymphocyte subsets among three groups (%)

Group	n	CD3	CD4	CD8	CD4/CD8
High load group	16	44.27±4.26	29.73±2.84	32.29±2.20	0.93±0.23
Medium load group	31	58.36±5.12*	33.52±3.07*	30.91±2.31[a]	1.01±0.16*
Low load group	23	64.54±5.62*,#	38.06±3.35*,#	29.81±1.14[a,b]	1.21±0.25*,#
F		6.837	6.128	1.262	5.631
P		<0.05	<0.05	>0.05	<0.05

Vs. high load group, *P<0.05; vs. medium load group, #P<0.05; vs. high load group, [a]P>0.05; vs. medium load group, [b]P>0.05.

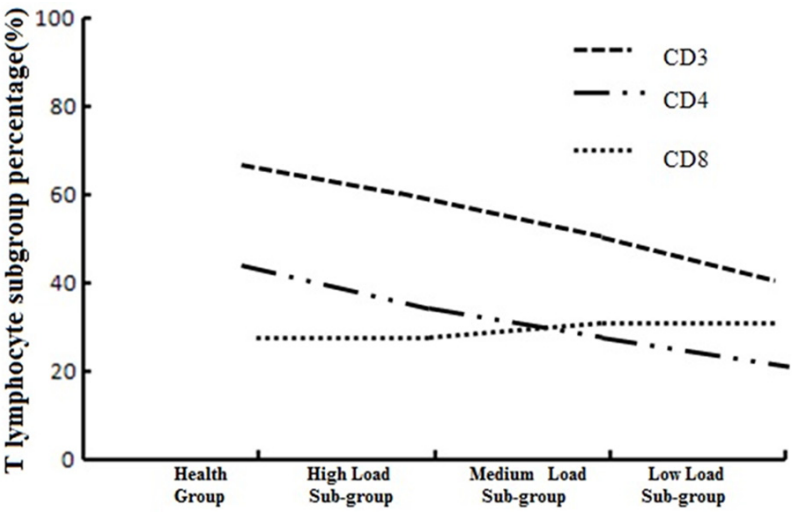

Figure 1. Spearman's rank correlation analysis between CMV load and T lymphocyte subsets percentage.

Statistical analysis

Statistical analysis was performed using the SPSS15.0 software (IBM, Armonk, NY, USA). Measured data was presented as means ± SD. Student t test was used to compare data between two groups, and Spearman' rank correlation assay was used to evaluate the relationship between CMV load and T lymphocyte subsets levels in peripheral blood. *P*<0.05 denoted a significant statistical difference.

Results

Expression of T lymphocyte subsets between hepatitis and control group

CD3 and CD4 levels and the ratio of CD4 and CD8 were obviously lower in hepatitis group than those in control group, which was a statistically significant difference (*P*<0.05), but there was no significant difference of CD8 level between two groups (*P*>0.01, **Table 1**).

Expression of T lymphocyte subsets among high viral load, medium viral load and low viral load group

CD3 and CD4 levels and the ratio of CD4 and CD8 were obviously lower in high CMV load group than those in medium CMV load group. Meanwhile, CD3 and CD4 levels and the ratio of CD4 and CD8 were obviously lower in medium CMV load group than those in low CMV load group, which was a statistically significant difference (*P*<0.05), and there was no significant difference of CD8 level among three groups (*P*>0.05, **Table 2**).

Correlation analysis between CMV load of children with hepatitis and the T lymphocyte subsets percentage

Spearman analysis showed that CMV load had negative correlation with percentage of CD3 and CD4 and the ratio of CD4 and CD8 (r1=-0.563; *P*=0.003; r2=-0.502; *P*=0.006; r4=-0.751; *P*<0.001) and CMV load had no correlation with CD8 level (r3=0.073; *P*=0.472) (**Figures 1**, **2**).

Discussion

Human cytomegalovirus is the common virus to cause viral hepatitis and could invade urinary system, central system and liver system to lead to diseases and inhibit the immune function of the human, moreover, it bring more complications after infection by CMV, even death [18].

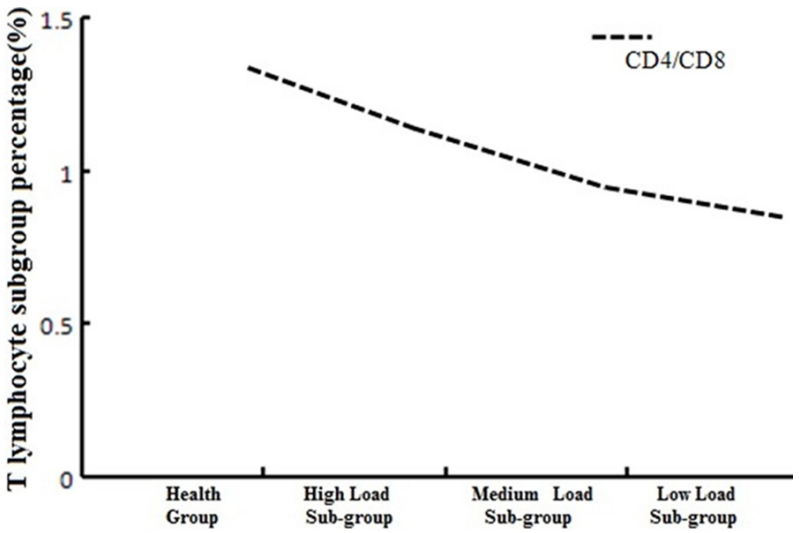

Figure 2. Spearman's rank correlation analysis between CMV load and the ratio of CD4 and CD8.

Meanwhile, human cytomegalovirus belongs to a high specific virus and only infects human and threats people's health [19].

T lymphocyte originates from hematopoietic stem cell in the bone marrow and differentiates into different immune T cells and is involved in normal immune response under the stimulation of thymin [20]. The several subsets of T cells each have a distinct function, including T helper cells, cytotoxic T cells, memory T cells, regulatory T cells, natural killer T cells [21]. T cell subsets are effective differentiated through detecting cellular superficial markers, which helps doctors to know immune function of patients so as to make effective measures [22].

This study detected the expression of T-lymphocyte subsets of peripheral blood in children with hepatitis induced by cytomegalovirus and the results showed that the CD3 and CD4 levels and the ratio of CD4 and CD8 were lower in children with hepatitis than those in healthy children, but the CD8 level was the same. Furthermore, the CD3 and CD4 levels and the ratio of CD4 and CD8 in peripheral blood were obvious lower in patients with high CMV load group than those in patients with medium CMV load group. Moreover, the CD3 and CD4 levels and the ratio of CD4 and CD8 in peripheral blood were obvious lower in patients with medium CMV load group than those in patients with low CMV load group, but there was no significant difference of CD8 among three groups. Spearman analysis indicated that CMV load had a negative correlation to CD3 percentage and CD4 percentage and the ratio of CD4 and CD8, but no correlation to CD8 percentage. CD3, CD4 and CD8 subsets represent helper T cell, effective T cell and cytotoxic T cell respectively, and the ratio of CD4 and CD8 could reflect the immune status of effective response cells [23]. After infection by CMV, the toxic substances inhibited the proliferation of regulatory T cell and reduced the regulatory effect of regulatory T cell on immune cells, and indirectly inhibited the active factor that could activate the T cell subsets, such as CD3 and CD4, which further make the disorder of T cell differentiation so as to reduce the T cell subsets levels and help effect of body's immune function, moreover, destructive immune cells had not enough ability to kill CMV [24]. CD8, cytotoxic T cell, can inhibit the infectious cells, and when infected by CMV, infectious cells numbers increased and T cell CD8 also increased. However, CMV inhibited the regulatory T cell to cause immune dysfunction so as to decline the CD8 activity mediated by regulatory T cell. Thus, the T cell CD8 level increased a bit in children with hepatitis. In addition, CMV had strong capacity of proliferation during infection period and could secrete a great number of toxic substances, low CD3, CD4 levels and ratio of CD4 and CD8 in peripheral blood severely declined the immunological function, which increased the risk of complications [25]. Therefore, measurement of CD3, CD4 levels and ratio of CD4 and CD8 had important significance on the diagnosis of children with hepatitis induced by CMV. Moreover, it was helpful to understand the condition and immunological function, most of important, doctors could plan individual therapeutic measurements.

One of our study limitation was the sample size was not sufficient, and the confounders may

also should be reconsidered carefully, which need further study to achieve more objective and reasonable conclusion.

In conclusion, hepatitis induced by CMV could inhibit the T cell levels, such as CD3, CD4 and the ratio of CD4 and CD8 in peripheral blood and decline the immune function. Expressions of related T cell subsets are the important indicators to evaluate the CMV infection and help doctors determine the therapeutic regimens, thus, it was worthy for being popularized further in clinical practice.

Disclosure of conflict of interest

None.

Address correspondence to: Yan-Ping Zhu, Department of Pediatrics, Liaocheng People's Hospital, No. 67 Dongcangxi Road, Liaocheng 252000, Shandong, China. Tel: +86 0635 8272667; E-mail: yanpingzhucn@126.com

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