

## **Relationship between murine cytomegalovirus infection and late-onset vitamin K deficiency intracranial haemorrhage.**

Lin Jin<sup>1#</sup>, Qiang Liu<sup>2#</sup>, Lechi Zhang<sup>3\*</sup>

<sup>1</sup>Department of Neurosurgery, The First Affiliated Hospital of Xi'an Medical University, Xi'an, Shaanxi, PR China

<sup>2</sup>Department of Neurosurgery, Affiliated Hospital of Yan'an University, Yan'an Shaanxi, PR China

<sup>3</sup>Department of Rehabilitation, Suzhou Municipal Hospital, Suzhou, Jiangsu, PR China

#These authors contributed equally

### **Abstract**

**Objectives:** To discuss relationship between murine cytomegalovirus infection and late-onset vitamin K deficiency intracranial hemorrhage.

**Methods:** Twenty healthy mice were randomly divided into control group (n=10) and cytomegalovirus infection model group (n=10). The changes of biochemical indexes such as PT, APTT, FIB and serum TBil, DBil, GGT and TBA were measured and compared at 2 months and 4 months of age.

**Results:** At the age of 2 months and 4 months, the levels of PT, APTT, TBil, DBil, TBA, GGT and AST in the cytomegalovirus infection group were significantly higher than those in the control group (P<0.05). There was no significant difference in the levels of FIB and ALT between the two groups (P>0.05).

**Conclusion:** Cytomegalovirus infection can lead to abnormal blood coagulation and serum biochemical indicators in mice, induced vitamin K deficiency in the occurrence of intracranial hemorrhage.

**Keywords:** Cytomegalovirus infection, Intracranial haemorrhage, Hemagglutination, Biochemical markers.

*Accepted on December 30, 2017*

### **Introduction**

Cytomegalovirus infection is an infectious disease induced by human cytomegalovirus (CMV) [1]. Related information shows [2,3], most of CMV infection occurs in the stage of the infants and young children. In clinical practice, CMV infection induced spontaneous intracranial hemorrhage in recent years has been increasing, causing the attention of clinicians. At present, there are few researches on the relationship between cytomegalovirus infection and late vitamin K deficiency intracranial hemorrhage. The aim of this study was to establish a model of cytomegalovirus infection in mice. To study the relationship between cytomegalovirus infection and late-onset vitamin K deficiency intracranial hemorrhage according to animal experiments; observe the changes of hemagglutination and serum biochemical indexes in cytomegalovirus, which are to provide a better understanding of the pathogenesis of spontaneous intracranial hemorrhage in infants with CMV infection and to provide a valuable reference for the treatment of children with such diseases.

### **Materials and Methods**

#### ***Experimental animals***

Twenty healthy mice were randomly selected. They were male clean animals with the body weight of  $8.5 \pm 2.5$  g, provided by our laboratory animal research center. Twenty mice were randomly divided into control group (n=10) and cytomegalovirus infection model group (n=10). Cytomegalovirus infection model of the construction method: injecting human cytoplasmic virus containing human cytoplasm. One month later, urinary test cytomegalovirus-DNA was positive infection, and head craniotomy confirmed the presence of late vitamin K deficiency intracranial hemorrhage. All the mice were fed with normal feed, and the tap water was free to drink. After 2 months and 4 months, researchers weighed mice respectively, did pentobarbital anesthesia, drawn inguinal blood and collected blood samples. Serum was separated and kept in refrigerator with -20°C.

#### ***Observation index***

At the age of 2 months and 4 months, the mice's plasma PT, APTT, FIB and serum TBil, DBil, GGT, TBA and other biochemical indicators were measured by using

hemagglutination analyzer (Shenzhen Shengxin Kang Technology Co., Ltd.) and automatic biochemical analyzer (Jinan Geli Te Technology Co., Ltd.).

### Statistical analysis

With SPSS 19.0 software, we compared the difference between two groups by using t test.  $P < 0.05$  suggests that the difference is statistically significant.

**Table 1.** Comparison of results of hemagglutination indexes between two groups.

Group	Number	PT (s)		APTT (s)		FIB (g/L)		
		2 months	4 months	2 months	4 months	2 months	4 months	
Control group	10	10.4 ± 1.7	10.5 ± 1.5	31.8 ± 4.7	31.9 ± 4.9	394.2 ± 75.9	392.6 ± 73.7	
Cytomegalovirus group	10	21.2 ± 4.5	30.6 ± 5.3	52.5 ± 6.3	63.7 ± 7.5	387.6 ± 74.3	381.1 ± 71.5	
T		7.10	7.45	6.93	7.00	0.12	0.11	
P		0.023	0.019	0.027	0.021	0.375	0.366	
				4 months	47.1 ± 75.9	63.4 ± 14.3	8.92	0.013

### Comparison of serum biochemical indexes between two groups

At the age of 2 months and 4 months, the levels of TBil, DBil, TBA, GGT and AST in the cytomegalovirus infection group were significantly higher than those in the control group ( $P < 0.05$ ). There was no significant difference in the level of ALT between two groups ( $P > 0.05$ ) (Table 2).

**Table 2.** Comparison of serum biochemical indexes between the two groups.

Group	Control Group	Cytomegalovirus group	t	P	
Number	10	10			
Tbil ( $\mu\text{mol/L}$ )	2 months	4.7 ± 2.05	52.4 ± 13.5	7.09	0.039
	4 months	4.7 ± 2.05	59.2 ± 14.7	8.85	0.02
Dbil ( $\mu\text{mol/L}$ )	2 months	2.2 ± 0.8	27.5 ± 9.8	6.17	0.048
	4 months	2.2 ± 0.8	29.1 ± 7.6	6.84	0.04
GGT (IU/L)	2 months	18.7 ± 6.7	51.6 ± 21.1	7.23	0.035
	4 months	18.7 ± 6.7	57.5 ± 19.7	8	0.022
TBA (IU/L)	2 months	7.2 ± 3.4	41.6 ± 6.7	9.04	0.01
	4 months	7.2 ± 3.4	48.3 ± 9.6	9.66	0.007
ALT (IU/L)	2 months	24.7 ± 8.4	25.1 ± 7.6	0.15	0.362
	4 months	24.7 ± 8.4	25.57 ± 8.2	0.16	0.355
AST (IU/L)	2 months	47.1 ± 75.9	58.7 ± 12.5	7.4	0.031

## Results

### Comparison of results of hemagglutination indexes between two groups

At 2 months and 4 months, the PT and APTT of the cytomegalovirus infection group were significantly higher than those of the control group ( $P < 0.05$ ). There was no significant difference in the levels of FIB between two groups ( $P > 0.05$ ) (Table 1).

## Discussion

Clinically, infants and young children are prone to late-onset vitamin K deficiency, and lack of vitamin K-dependent factors, which will induce bleeding [4]. Studies show that [5,6] vitamin K belongs to a derivative containing naphthoene gene. The coagulation factor II and VII and other factors in the body must experience carboxylation process. Only after this process can its own glutamic acid residues be carboxylated into  $\gamma$ -carboxy glutamic acid, and then combined with more  $\text{Ca}^{2+}$ , provided with Coagulation biological activity. It is worth mentioning that the carboxylation process often requires the participation of carboxylase in vitamin K, otherwise it cannot occur carboxylation [7]. If it is lack of vitamin K, a variety of coagulation factor in the body is only a non-bioactive function of the protein which will not participate in the body coagulation process. Therefore, it will cause bleeding [8]. In clinical practice, we found that the situation of late vitamin K deficiency of intracranial hemorrhage is serious and rapid with high mortality. What's more, most of survivors will also be in central nervous system sequelae. Clinical prognosis is relatively poor, seriously affecting the quality of life of patients.

In recent years, CMV infection can't be ignored because of the increasing cases. Relevant data show that [9], pregnant women on CMV infection antibody positive rate are as high as 95%. CMV is generally transmitted to infants through the placenta, birth canal, breast milk and saliva and other ways. At present, on the cytomegalovirus infection and late vitamin K deficiency of intracranial hemorrhage *in vitro* animal experiments have not been reported. In this study, mice were injected with cytomegalovirus containing human blood to carry out the experimental study. The model established has late vitamin K

deficiency of intracranial hemorrhage symptoms, which confirmed that CMV infection and late vitamin K deficiency of intracranial hemorrhage is closely related. One of the possible reasons is when the body absorbs vitamin K, bile must be involved actively, and the occurrence of CMV infection will make the body cholestatic, excretion obstruction, thereby affecting the body's absorption of vitamin K [10]. Another potential reason is that the liver is the main synthesis sites of II, VII and other coagulation factors, and the occurrence of CMV infection will cause damage to the liver, making abnormal liver function, thereby affecting a variety of coagulation factors in the liver synthesis [11,12]. Moreover, related data [13-15] show that CMV can induce vascular endothelial cell inflammatory changes, improve vascular permeability, which increases intracranial hemorrhage risk.

In addition, through the cytomegalovirus infection model group of blood coagulation, researchers test biochemical indicators. The results show that, at the age of 2 months and 4 months, PT, APTT, TBil, DBil, TBA, GGT and AST were significantly higher in the model group than in the control group ( $P < 0.05$ ). There was no significant difference in FIB and ALT levels between two groups ( $P > 0.05$ ). It further proved that cytomegalovirus infection can lead to mouse blood coagulation and serum biochemical abnormalities, vitamin K deficiency in the occurrence of intracranial hemorrhage; It also shows that maybe FIB, ALT two indicators have no significant correlation with cytomegalovirus infection and late vitamin K deficiency of intracranial hemorrhage disease.

## References

1. Antona D, Lepoutre A, Fonteneau L. Seroprevalence of cytomegalovirus infection in France in 2010. *Epidemiol Infect* 2017; 145: 1471-1478.
2. Lili G, Hongmei X. Human cytomegalovirus infection epidemiological research progress. *Shandong Med* 2017; 31: 1131-1133.
3. Tanimura K, Tairaku S, Ebina Y. Prediction of congenital cytomegalovirus infection in high-risk pregnant women. *Clin Infect Dis* 2017; 64: 159-165.
4. Davis NL, King CC, Kourtis AP. Cytomegalovirus infection in pregnancy. *Birth Defects Res* 2017; 109: 336-346.
5. Jia L, Liuan Q, Shaozhi X. Vitamin K metabolism related gene CYP4F2 involved in coagulation factor FIX activation study. *Chinese J Multiple Organ Dis* 2017; 16: 208-211.
6. Park BK, Choonara IA, Haynes BP. Abnormal vitamin K metabolism in the presence of normal clotting factor activity in factory workers exposed to 4-hydroxycoumarins. *Br J Clin Pharmacol* 2012; 21: 289-293.
7. Liu J, Wang Q, Gao F. Maternal antenatal administration of vitamin K1 results in increasing the activities of vitamin K-dependent coagulation factors in umbilical blood and in decreasing the incidence rate of periventricular-intraventricular hemorrhage in premature infants. *J Perinatal Med* 2006; 34: 173-176.
8. Ito K, Goto K, Sugiura T. Polymorphisms of the factor VII gene associated with the low activities of vitamin K-dependent coagulation factors in one-month-old infants. *Tohoku J Exp Med* 2007; 211: 1-8.
9. Lijuan C, Xiaodong Z, Yunjing C. Analysis of brainstem auditory evoked potentials in patients with congenital cytomegalovirus infection. *Practical Med J* 2012; 29: 999-1000.
10. Qiang H, Yan Y, Jing M. Combination of traditional Chinese and western medicine treatment of cytomegalovirus infection in infants with cholestatic liver disease. *Beijing Med* 2015; 37: 596-597.
11. Lautenschlager I. CMV infection, diagnosis and antiviral strategies after liver transplantation. *Transplant Int* 2009; 22: 1031-1040.
12. Popovic B, Golemac M, Podlech J. IL-33/ST2 pathway drives regulatory T cell dependent suppression of liver damage upon cytomegalovirus infection. *Plos Pathogens* 2017; 13: e1006345.
13. Read RW, Zhang JA, Ishimoto SI. Evaluation of the role of human retinal vascular endothelial cells in the pathogenesis of CMV retinitis. *Ocular Immunol Inflamm* 1999; 7: 139.
14. Yuan C, Duoduo L, Jilin C. Clinical expression of human vascular endothelial cells associated with renin gene expression in human cytomegalovirus clinical isolates. *Chinese J Mol Cardiol* 2009; 9: 193-198.
15. Lei Y, Zheng Z, Jieju D. Experimental study of cytomegalovirus induced apoptosis of human vascular endothelial cells in vitro. *Chongqing Med University* 2006; 35: 512-514.

## \*Correspondence to

Lechi Zhang  
Department of Rehabilitation  
Suzhou Municipal Hospital  
PR China