J Korean Med Sci 2000; 15: 337-42
ISSN 1011-8934

Congenital Cytomegalovirus Infection
: Three Autopsy Case Reports

We report three autopsy cases of congenital cytomegalovirus (CMV) infection in fetuses with a review of literature. The clinical manifestations in these cases of congenital CMV infection include intrauterine fetal death, hydrops fetalis, and CMV pneumonia associated with cardiovascular defect. The pathological characteristics were as follows: 1) the kidney was the most frequently involved organ, followed by lung and liver, 2) CMV inclusions were found predominantly in epithelial cells and to a lesser degree in endothelial cells, 3) intrahepatic bile duct epithelial cells were frequently involved, and 4) inflammatory reaction around CMV inclusions was not prominent in the early stage of pregnancy. Diagnostic confirmation was obtained by in situ hybridization (ISH) using a biotinylated CMV-DNA probe, which demonstrated intranuclear inclusions and sometimes recognized cells that did not show intranuclear inclusion.

Key Words: *Autopsy; Cytomegalovirus; Inclusion Bodies; In Situ Hybridization*

Hyang Mi Ko*, Kyung Soo Kim†, Jae Woo Park, Young Jik Lee, Min Young Lee, Min Cheol Lee, Chang Soo Park, Sang Woo Juhng, Chan Choi

Department of Pathology*, Kwangju Veterans Hospital, Department of Pathology†, Kwangju Christian Hospital, and Department of Pathology, Chonnam National University Medical School, Kwangju, Korea

Received: 10 September 1999
Accepted: 13 October 1999

Address for correspondence
Chan Choi, M.D.
Department of Pathology, Chonnam National University Medical School, 8 Hak-dong, Dong-gu, Kwangju 501-190, Korea
Tel: +82.62-232-1246, Fax: +82.62-227-3429
E-mail: cchoi@chonnam.chonnam.ac.kr

INTRODUCTION

Cytomegalovirus (CMV) is the most common viral infection in the fetus and neonate. Fetal CMV infection may be caused by maternal viremia during which CMV reaches the fetus through the blood stream. Primary maternal infection is more likely to result in fetal infection than recurrent maternal disease. The incidence of congenital infection ranges from 0.2 to 2.4% of all live births (1). Approximately 20% of the offsprings infected in utero are damaged, infrequently with generalized disease (2). The severely affected infant may show growth retardation, microcephaly, hydrocephalus with periventricular calcification, chorioretinitis, thrombocytopenia, purpura and hepatitis. We report three autopsy cases of congenital CMV infection and discuss the pathologic features with a brief review of literature.

CASE REPORT

Case 1

A male fetus at 30 weeks gestation was delivered because of unexplained intrauterine fetal death. The fetus weighed 1,700 g and had a crown heel length of 39 cm. The fetus was severely macerated and internal organs showed autolysis. Microscopically, a diagnosis of CMV infection was made based on the presence of cytomegalic inclusion bodies (CIBs). The intranuclear amphophilic inclusion bodies surrounded by a clear halo, or basophilic granular cytoplasmic inclusion bodies were considered as CIB. CIBs were noted in the thymus, lungs, and kidneys. In the autolysed tissue, the nuclear inclusions lost their usual amphophilia, but cytoplasmic inclusions usually retained basophilia. In the thymus, a few degenerated CIBs were seen in thymic epithelial cells (Fig. 1). In the lung, the majority of CIBs lay free in the terminal air spaces and originated from pneumocytes. In the kidney, large numbers of degenerative CIBs were noted in dilated tubules of the cortex and they were not accompanied by inflammatory infiltrate.

Case 2

A female fetus at 24 weeks gestation was delivered because of hydrops fetalis. The fetus died at 1 min of age. The fetus weighed 640 g and had a crown heel length of 29.5 cm. The head was enlarged and measured 21 cm in circumference. There were no skin lesions and

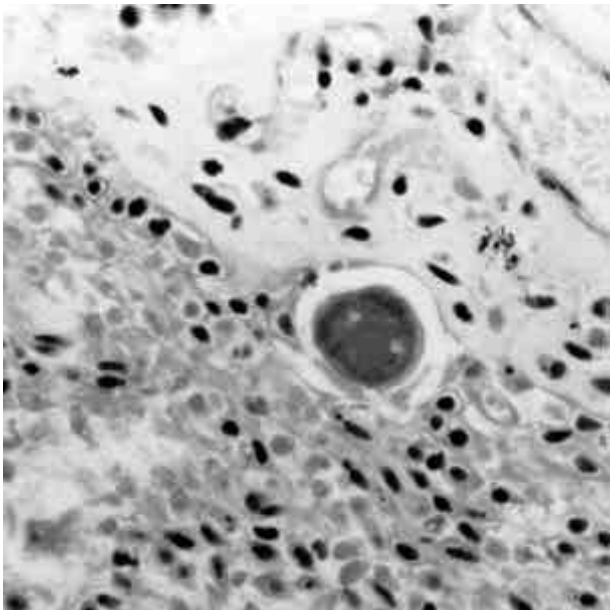

Fig. 1. A relatively well-preserved CIB is seen in the autolyzed thymic epithelial cell (case 1) (H&E, ×400).

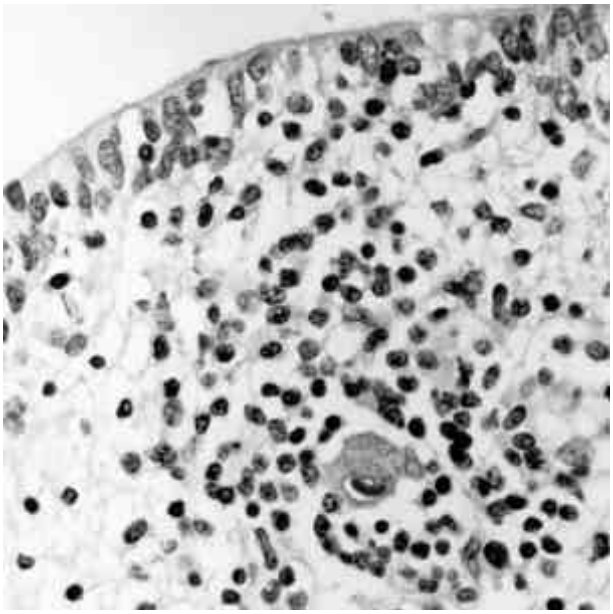

Fig. 2. Note the periventricular CIB bearing neuron and the mononuclear cell infiltration in the periventricular area (case 2) (H&E, ×400).

no remarkable changes on the body surface except for abdominal distension. The brain weighed 25.4 g and showed underdevelopment of gyri. Coronal sections of the cerebral hemisphere showed cystic dilatation of ventricles. Both lungs showed increased consistency and moderate atelectasis. The liver weighed 50 g with tense and smooth surface. Each kidney weighed 1.3 g with smooth cortical surface. The spleen weighed 5 g. All other organs were grossly unremarkable. Microscopically,

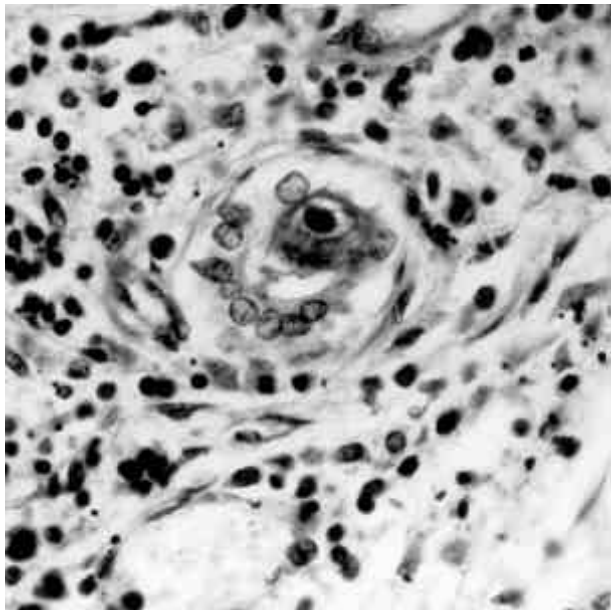

Fig. 3. A CIB is seen in the bile duct epithelium (case 2) (H&E, ×400).

CIBs were noted in the brain, thymus, heart, lungs, liver, and kidneys. In the brain, single scattered CIBs were mostly found in periventricular glial cells surrounded by mild infiltration chronic inflammatory cells (Fig. 2). Ependymal cells lining the ventricles had no CIBs and were severely damaged by inflammation. There were foci of necrosis accompanied by microscopic calcification in the lateral part of the frontal lobe. In the thymus, single scattered CIBs were noted in thymic epithelial cells. In the heart, only one CIB was noted in the vascular endothelial cell of the epicardium. In the lung, CIBs were noted in alveolar epithelium, attached to alveoli, and occasionally floating in alveolar space. Inflammatory cells were not observed. In the liver, increased extramedullary hematopoiesis, focal hepatocellular necrosis and cholestasis were seen in the parenchyma. CIBs were found both in hepatocytes and bile duct epithelial cells, and they were observed throughout the entire lobule (Fig. 3). There was little inflammatory reaction. The spleen showed increased extramedullary hematopoiesis. In the kidney, CIBs were accompanied with mild inflammatory cell infiltration, and they were more frequently noted in distal tubules than in proximal tubules. All other organs did not exhibit remarkable changes.

Case 3

A female infant was delivered at 38 weeks gestation by Caesarian section. The infant was lethargic and cyanotic. Delivery was complicated by meconium passage at birth. Apgar score was 3 at 1 min and 6 at 5 min. Ven-

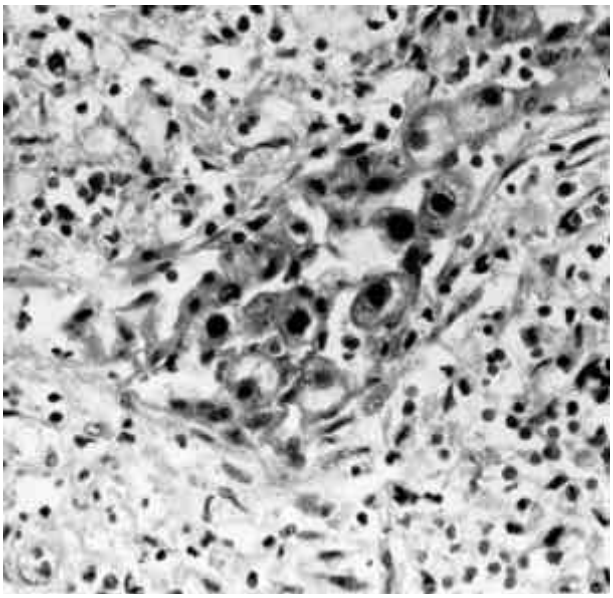

Fig. 4. Many CIBs are noted in alveolar epithelial cells. The adjacent alveoli are filled with inflammatory exudate (case 3) (H&E, ×400).

tilator care was provided. Echocardiogram revealed coarctation of the aorta (COA), large patent ductus arteriosus (PDA) and pulmonary hypertension. The infant showed hepatomegaly, oliguria, and signs of congestive heart failure. Treatment with fluid restriction and diuretics was performed. The infant remained thrombocytopenic despite the platelet transfusion. On the 3rd day after birth, cardiac arrest developed and was expired. The infant weighed 2,220 g and had a crown heel length of 48 cm. The heart showed preductal COA and PDA with cardiomegaly. The lungs were bilaterally heavy, voluminous, congested, and firm. Cut surface was pinkish and diffusely consolidated. The spleen measured 6×3×2 cm in dimension. All other organs appeared grossly unremarkable. The brain was not examined. Microscopically, CIBs were noted in the lung, kidney, and liver. The lung alveoli contained extensive exudate, and alveolar walls were thick with infiltration of lymphocytes. Hyaline membranes were formed. CIBs were infrequently observed. Most of them were found in pneumocytes (Fig. 4). In the kidney, CIBs were noted in proximal and distal tubular epithelial cells as well as in glomerular endothelial cells (Fig. 5). Moderate degrees of inflammatory cell infiltrate and foci of necrosis were noted in the interstitium. In the liver, bile duct epithelial cells contained CIBs, but hepatocytes did not contain CIBs. Inflammatory cells were not observed. The spleen showed increased extramedullary hematopoiesis. All other organs did not exhibit remarkable changes.

In situ hybridization

CIBs were confirmed by in situ hybridization (ISH), using biotinylated specific probes for CMV-DNA (Research Genetics, Alabama, U.S.A.). The detection system was streptavidin-conjugated alkaline phophatase with Fast Red TR chromogen. ISH showed positive reactions corresponding to intranuclear inclusions, granular cytoplasmic inclusions, and even the cells without apparent intranuclear inclusions (Fig. 6).

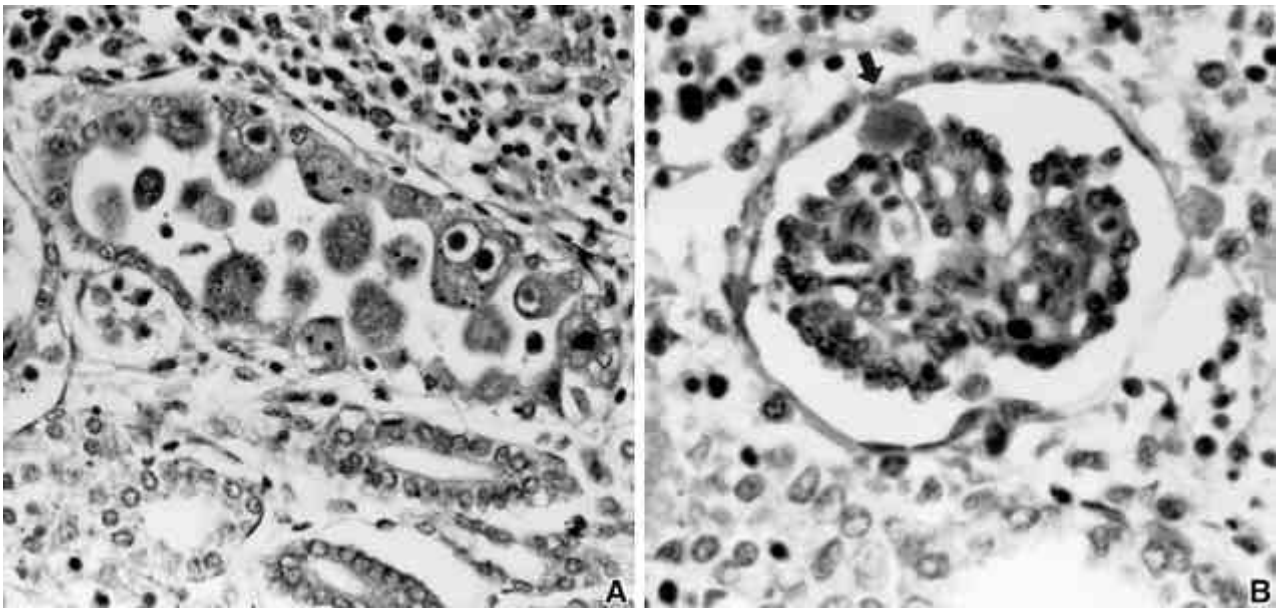

Fig. 5. A: A dilated tubule is lined by CIB bearing cells (H&E, ×400). B: A basophilic cytoplasmic inclusion body is noted in a glomerulus (case 3) (H&E, ×400).

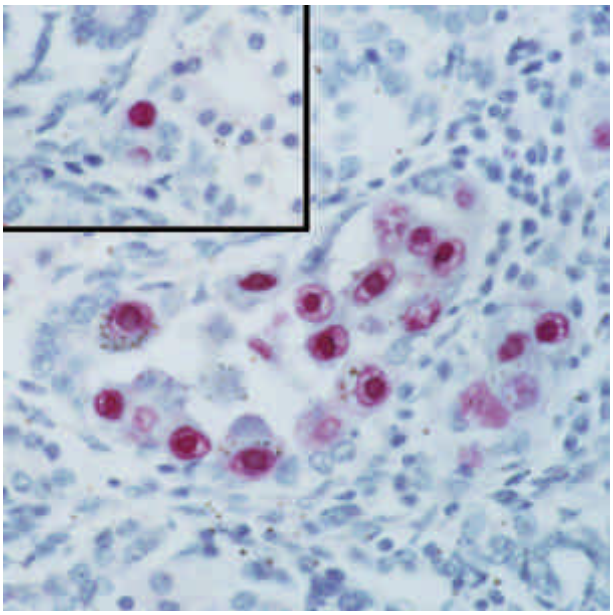

Fig. 6. In situ hybridization reveals strong positive reaction in the nuclei and cytoplasm of inclusion-bearing cells of the kidney. Inset: note the positive reaction in a cell without apparent intranuclear inclusion (case 2) (in situ hybridization, ×400).

DISCUSSION

Intrauterine CMV infection is usually hematogenous, transplacental infection and rarely direct infection from the cervix after amniotic membrane rupture. In all three instances, there was no infection history, which the mother was recognizable during pregnancy, or no amniotic membrane rupture. Thus, all of these cases could be regarded as a congenital CMV infection.

The congenital CMV infection vary in the clinical manifestation. Although 90-95% of infants with congenital CMV infection are normal at birth, it can prove to be fatal. Congenital CMV infection is a cause of death in 0.3% of all stillbirths and 0.5% of all neonatal deaths (3). In the present study, Case 1 showed intrauterine fetal death (IUFD). It is uncertain whether there is a direct relationship between CMV infection and IUFD. In light of the absence of any other identifiable cause of death, it is suggested that CMV may be a cause of IUFD. Case 3 revealed congenital cardiovascular defects. A variety of congenital malformations have been observed in infants with congenital CMV infection in previous reports (4, 5). Several investigators have reported an association between congenital cardiovascular defects and CMV infection (3-5). However, besides the rarity and diversity of these lesions, there is no evidence that CMV is capable of affecting the development of the cardiovascualr system.

Differing intrinsic susceptibilities of tissue may influence the frequency of the involved organs during systemic CMV infection (3). We observed CIBs in the kidneys, lungs, liver, thymus, brain and heart, in descending order of frequency. We reviewed the cases of congenital

Table 1. Reported cases of congenital cytomegalovirus infection in Korea

Case	Authors	Age at death (gestational age)	Sex	Associated findings	Involved organ								
					Lung	Kidney	Liver	Brain	Thymus	Pancreas	Adrenal	Thyroid	Others
1	Han et al. (22)	23 days	M	–	+	+	+	–	–	+	+	+	Pituitary
2	Han et al. (22)	76 days (28 wks)	M	–	+	–	–	–	–	–	–	–	-
3	Paik et al. (23)	9 hr (36 wks)	M	Prematurity	+	+	+	–	–	–	–	–	-
4	Jung et al. (24)	27 days (38 wks)	F	LBW	–	+	+	–	–	+	+	+	Spleen pituitary
5	Yu et al. (25)	2 hr (38 wks)	M	Massive periventricular calcification	–	+	–	–	–	–	–	–	-
6	Lee et al. (26)	22 days (39 wks)	M	LBW, PDA, Foramen ovale	+	+	+	–	+	+	–	–	Testis epididymis
7	Cho et al. (27)	at birth (21 wks)	M	Hydrocephalus	+	+	+	+	–	+	–	–	Intestine
8	Present case 1	at birth (30 wks)	M	IUFD	+	+	–	–	+	NE	–	–	–
9	Present case 2	at birth (24 wks)	F	Hydrocephalus	+	+	+	+	+	NE	–	–	Heart
10	Present case 3	3 days (38 wks)	F	COA, PDA	+	+	+	NE	–	NE	–	–	–

LBW, low birth weight; PDA, patent ductus arteriosus; IUFD, intrauterine fetal death; COA, coarctation of aorta; NE, not examined

CMV infection reported in Korea and summarized them in Table 1. Nine of the ten cases were disseminated infection. The kidney is the most commonly involved organ in congenital CMV infection in Korea. Previous reports in other countries described that the lung is the most common site in congenital CMV infection (3, 6, 7). Others described that salivary gland is frequently involved (4), but none of the cases reported in Korea were consistent with this finding.

Although all the present cases exhibited generalized infection, most CIBs were found in epithelial cells, and very few in endothelial cells. In the present cases, most of cells bearing CIBs in the kidney were tubular epithelium, and only a small number of CIBs were noted in glomerular endothelial cells. These findings are consistent with others that renal inclusion bodies is found predominantly in tubular epithelium, with only a small minority occurring in glomeruli (8, 9). However, a previous study of adult immunocompromised patients with CMV infection showed that glomerular endothelial cells were more commonly infected than tubular epithelial cells (10). CIBs in the liver were more frequently found in bile duct epithelium than in hepatocytes, as shown in Cases 1 and 2. Previous reports have shown that bile duct epithelium involvement is frequent in infants (11-13), but rare in adult immunocompromised hosts (14). From these findings, it is presumed that immature fetus cells differ from mature adult cells in susceptibility to CMV infection.

Inflammatory cell infiltration indicates an immune reaction to CMV infection. Cases 1 and 2 showed more frequent CIBs and less inflammatory reaction than Case 3. These findings indicate that cellular damage of the former is caused by a direct cytopathic effect of CMV rather than immunologic attack. Cases 1 and 2 expired at earlier gestational ages than case 3. They expired at times when their immune system would have been less developed than that of Case 3. Cellular immune reactions, particularly those mediated by T-lymphocytes and involving macrophages, are essential for the elimination of CMV infection (15, 16). In general, T cell development is well advanced by the end of the first trimester of pregnancy (17). The absence of inflammatory lesions in early fetuses has been attributed to infections that precede the development of the cellular immune reactivity, which at later gestational age, would initiate an inflammatory reaction and the destructive disease (18). The direct cytopathic effect of CMV is more common than immunologic attack in early gestational infection. Among the cases presented here, it is suspected that the fetuses of Cases 1 and 2 were infected at an early gestational age.

The morphologic diagnosis of CMV infection is straightforward, when large inclusion bearing cells are present. However, to improve the sensitivity of histopathologic methods and to confirm diagnosis, ISH was attempted in sections of paraffin-embedded tissue. In our three cases, all CIBs-containing cells revealed positive reactions in ISH. Sometimes ISH recognized CMV in cells that did not show typical CMV inclusion. Several studies have attested the ability of ISH to label CMV-infected cells that lack typical inclusion bodies (19-21). These cells may be CMV-infected cells that have not yet begin to actively replicate or may be latent CMV infection.

REFERENCES

1. Stago S. *Cytomegalovirus. In: Behrman RE, Kliegman RM, Arvin AM, eds. Nelson Textbook of Pediatrics. Philadelphia: WB Saunders, 1996; 895-6.*
2. O'Shea PA. *Congenital and acquired systemic infectious diseases. In: Stocker JT, Dehner LP. Pediatric pathology. Philadelphia: JB Lippincott, 1993; 208-11.*
3. Becroft DMO. *Prenatal cytomegalovirus infection: epidemiology, pathology and pathogenesis. Perspect Ped Pathol 1981; 6: 203-41.*
4. Overall JC Jr. *Viral infections of the fetus and neonate. In: Feigin RD, Cherry JD, eds. Textbook of Pediatric Infectious disease. Philadelphia: WB Saunders, 1987; 966-1007.*
5. Hanshaw JB. *Developmental abnormalities associated with congenital cytomegalovirus infection. Adv Teratol 1969; 4: 64-93.*
6. Wyatt JP, Saxton J, Lee RS, Pinkerton H. *Generalized cytomegalic inclusion disease. J Pediatr 1950; 36: 271-9.*
7. Gajl-Peczalska K. *Cytomegalic inclusion disease. Arch Dis Child 1967; 42: 14-9.*
8. Embil JA, Krause VW, Haldane EV, Easterbrook KB, Crosby JM. *Congenital cytomegalovirus infection: clinical, pathological and virological studies of two fetal cases. Can Med Assoc J 1969; 101: 32-40.*
9. Fetterman GH, Sherman FE, Fabrizio NS, Stundnicki FM. *Generalized cytomegalic inclusion disease of newborn. Localization of inclusions in the kidney. Arch Pahol 1968; 86: 86-94.*
10. Nadasdy T, Miller KW, Johnson LD, Hanson-Painton O, Debault LE, Burns DK, Hawkins E, Silva FG. *Is cytomegalovirus associated with renal disease in AIDS patients? Mod Pathol 1992; 5: 277-82.*
11. Kosai K, Kage M, Kojiro M. *Clinicopathologial study of liver involvement in cytomegalovirus infection in infant autopsy cases. J Gastroenterol Hepatol 1991; 6: 603-8.*
12. Sung SH, Park CI, Kim HG, Jung WH, Chung KS. *Histopathologic appearance of cytomegaloviral liver disease in neonates and infants. Korean J Pathol 1992; 26: 137-45.*
13. Griffiths PD. *CMV and the liver. Sem Liver Dis 1984; 4: 307-13.*

14. Sano N, Izumi K. *Hepatic cytomegalovirus involvement in autopsy cases. Acta pathol Jpn 1991; 41: 668-72.*
15. Starr SE, Allison AC. *Role of T lymphocytes in recovery from murine cytomegalovirus infection. Infect Immun 1977; 17: 458-62.*
16. Mims CA, Gould J. *The role of macrophages in mice infected with murine cytomegalovirus. J Gen Virol 1978; 41: 143-53.*
17. Lobach DF, Hensley LL, Ho W, Haynes BF. *Human T cell antigen expression during the early stages of fetal thymic maturation. J Immunol 1985; 135: 1752-9.*
18. Benirschke K, Mendoza GR, Bazeley PL. *Placental and fetal manifestations of cytomegalovirus infection. Virchows Arch B Cell Pathol 1974; 16: 121-39.*
19. Wolber RA, Lloyd RV. *Cytomegalovirus detection by non-isotopic in in situ DNA hybridization and viral antigen immunostaining using a two color technique. Human Pathol 1988; 19: 736-41.*
20. Gnann JW Jr, Ahlmen J, Svalander C, Olding L, Oldstone MBA, Nelson J. *Inflammatory cells in transplanted kidneys are infected by human cytomegalovirus. Am J Pathol 1988; 132: 239-48.*
21. Masih AS, Linder J, Shaw BW, Wood RP, Donovan JP, White R, Markin RS. *Rapid identification of cytomegalovirus in liver allograft biopsies by in situ hybridization. Am J Surg Pathol 1988; 12: 362-7.*
22. Han WS, Suh JH, Ro JY, Kim CW. *Two autopsy cases of cytomegalic inclusion disease. Korean Central J Med 1971; 21: 233-7.*
23. Paik OJ, Cho CC, Moon SK. *Generalized cytomegalic inclusion disease. Korean J Pathol 1977; 11: 231-5.*
24. Jung BY, Kim JH, Kim GC, Park WJ. *Congenital systemic cytomegalic inclusion disease. J Korean Ped Soc 1981; 24: 1197-201.*
25. Yu ES, Chi JG. *Congenital cytomegalovirus infection associated with massive periventricular calcification and probably in burn out phase. Korean J Pathol 1986; 20: 503-8.*
26. Lee DB, Kim DH, Min JS, Choi CH, Chi JG. *Congenital systemic cytomegalic inclusion disease. J Korean Ped Soc 1990; 33: 100-3.*
27. Cho KR, Yun CH, Kim SP, Park KK, Chang ES, Kim TH. *Congenital cytomegalic inclusion disease combined with hydrocephalus. Korean J Pathol 1994; 28: 439-41.*