Journal of NeuroVirology, 8: 447–451, 2002
 ISSN 1355–0284/02 $12.00+.00
DOI: 10.1080/13550280290100824

High incidence of JC viruria in JC-seropositive older individuals

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The prevalence of the human JC virus (JCV) in the general population at various ages was investigated. Polymerase chain reaction was employed to detect viral DNA in the urine. The results showed that the incidence of JC viruria was low in the young population, but it was high in the elderly. Hemagglutination inhibition assay was performed for JCV seroprevalence study. The results showed that the seropositive rate of JCV was lower in children than that in adults. The ratio of viruria to seropositive for JCV increased with age and reached 79.7% for those older than 70 years. The results indicated that aging immunity may correlate with JCV reactivation. *Journal of NeuroVirology* (2002) **8,** 447–451.

Keywords: immunosuppression; JC virus; seroprevalence; viruria

Human polyomavirus, a subfamily of thePolyomaviridae, is a DNA virus containing a closed, circular, double-stranded DNA genome that is separated into early and late coding sequences by the viral regulatory region (Frisque *et al*, 1984; Cole, 1996). Two polyomaviruses, JC virus (JCV) and BK virus (BKV), which infect humans, were reported in 1971 (Padgett *et al*, 1971; Gardner *et al*, 1971). JCV is the etiological agent of progressive multifocal leukoencephalopathy (PML), which is a fatal, subacute, and demyelinating disease in humans (Padgett *et al*, 1971). BKV is associated with hemorrhagic cystitis (Arthur *et al*, 1986; Apperley *et al*, 1987; Bedi *et al*, 1995) and interstitial nephritis in immunocompromised patients (Purighalla *et al*, 1995; Pappo *et al*, 1996; Mathur *et al*, 1997; Nickeleit *et al*, 1999; Randhawa *et al*, 1999; Boubenider *et al*, 1999; Chen *et al*, 2001). Polyomavirus infection is usually asymptomatic. After primary infection, the virus causes lifelong latency in the kidneys and replicates the progeny excreted into the urine via an unknown reactivated mechanism (Jung *et al*, 1975; Yogo *et al*, 1990; Tominaga *et al*, 1992; Dorries, 1997).

Epidemiological studies have shown that polyomavirus infection is widespread in the human population (Walker and Frisque, 1986). The serological surveys revealed that the JCV-specific serum antibody with peak seroconversion was up to 80% in adulthood (Padgett and Walker, 1973; Brown and Gaidusek, 1975; Taquchi *et al*, 1982; Chesters *et al*, 1983; Dorries, 1997). The incidence of polyomavirus viruria in healthy individuals ranged from 20% to 29% for JCV and 0% to 17.6% for BKV (Kitamura *et al*, 1990; Markowitz *et al*, 1993; Sundsfjord *et al*, 1994).

The incidence of polyomavirus viruria in transient immunocompromised pregnant women and immunosuppressed patients with autoimmune diseases in Taiwan has been previously reported (Chang *et al*, 1996a, 1996b). The average detection rate of the JCV viruria was 26% and 37.5% in pregnant women and patients with autoimmune diseases, respectively. However, the incidence of the polyomavirus viruria in the general population at various ages in Taiwan has not been studied. In addition, the seroprevalence

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This work was supported by the National Health Research Institute (NHRI-EX91-8917SL) and the National Science Council (NSC90-2314-B-040-194-001), Taiwan.

Received 17 May 2002; revised 5 July 2002; accepted 2 August 2002.

of the human JCV in Taiwan has not been investigated yet. To extend our previous studies, the incidence of viruria and seroprevalence of JCV in the general population in Taiwan were investigated.

To investigate human polyomavirus shedding in the general population at various ages, urine samples were collected from 1012 healthy individuals including 104 from day care centers, 216 from elementary schools, 261 from high schools, 75 from colleges, and 356 from the Physical Examination Centers at the Chung Shan Medical University Hospital and Chia-Yi Christian Hospital. There were 532 male and 480 female subjects. Their ages ranged from 3 to 84 years. The samples were divided into eight groups including 135 samples from subjects who were aged 3 to 7 years old, 209 were 8 to 13 years old, 237 were 14 to 19 years old, 81 were 20 to 30 years old, 58 were 31 to 40 years old, 55 were 41 to 50 years old, 62 were 51 to 60 years old, 91 were 61 to 70 years old, and 94 were older than 70 years (Table 1).

The urine samples were examined by polymerase chain reaction (PCR) (Chang *et al*, 1996b) using JCV- and BKV-specific primers, JBR1 (5′-CCTCCACGCCCTTACTACTTCTGAG-3′) and JBR2 (5′-GTGACAGCTGGCGAAGAACCATGGC-3′). The primers annealed at the conserved regulatory regions of both JCV and BKV (White *et al*, 1992). After amplification, the DNA fragments flanking the regulatory region of the virus was expected to be approximately 334 base pairs as analyzed using agarose electrophoresis. For genotyping, restriction fragment length polymorphism (RFLP) analysis (Chang *et al*, 1999) was performed. The DNA fragments of PCR products were digested with *Bst* N1 restriction enzyme and then analyzed using acrylamide gel electrophoresis. The results showed that 193 of the examined urine samples were PCR positive. Of the PCR-positive urine samples, 55% were Taiwan-1 (TW-1) JCV (Chang *et al*, 1996b) and 45% were archetype (CY) JCV (Yogo *et al*, 1990). There is a pentanucleotide (GGGAA) deletion within the regulatory region (nucleotides 218 to 222) of TW-1 when compared to CY JCV. TW-1 and CY JCVs were the predominant genotypes in Taiwan as demonstrated in current and previous studies (Chang *et al*, 1996a, 1996b; Tsai *et al*, 1997). BKV DNA was not detected in the examined samples. Furthermore, the PCR results also showed that the incidence of JCV urinary shedding was 2.2% for the group of subjects who were 3 to 7 years old, 2.8% for those 8 to 13 years old, 6.7% for those 14 to 19 years old, 12.3% for those 20 to 30 years old, 27.6% for those 31 to 40 years old, 32.7% for those 41 to 50 years old, 41.9% for those 51 to 60 years old, 47.3% for those 61 to 70 years old, and 65.5% for those older than 70 years. It was evident that the incidence of JCV viruria increased with age and the highest incidence occurred for those older than 70 years (Table 1).

To understand seroprevalence of JCV in Taiwan, 1938 human sera were collected from the Newborn Center, Department of Pediatrics, and Physical Examination Center at the Chung Shan Medical University Hospital. The ages of the subjects ranged from 0 to 94 years old. The ratio of males to females was 1.1 to 1. The subjects were divided into nine groups including 99 samples from subjects who were aged 0 to 2 years old, 472 were 3 to 7 years old, 226 were 8 to 13 years old, 78 were 14 to 19 years old, 293 were 20 to 30 years old, 159 were 31 to 40 years old, 144 were 41 to 50 years old, 139 were 51 to 60 years old, 185 were 61 to 70 years old, and 143 were older than 70 years (Table 1). Hemaggultination inhibition (HAI) assay (Ou *et al*, 1999) was performed for serological determination by using recombinant JCV virus-like particles (VLPs) purified from yeast cells (Chen *et al*, 2001). It has been demonstrated that JCV antibody is species specific and not recognizing BKV or SV40 (Hamilton *et al*, 2000). The results showed that the JCV seropositive rate for under 2 years old was 29.3%, 50% seropositive for those 3 to 7 years old, 58.8% for those 8 to 13 years old and 65.4% for those 14 to 19 years old. Approximately 73% of all subjects older than 20 years were seropositive (Table 1). These results indicated that JCV seroprevalence initiated during childhood and reached 73% in the adult population.

Table 1 Summary of incidence of JC viruria and seroprevalence in various age groups

Age group (year)	*Sample*		*Viruria*		*Seropositive*		*Ratio of viruria/ seropositive (%)*
	Urine	*Serum*	*No.*	*(%)**	*No.*	*(%)*	
0–2	—	99	—	—	29	(29.3)	—
3–7	135	472	3	(2.2)	236	(50.0)	(4.4)
8–13	209	226	6	(2.8)	133	(58.8)	(4.8)
14–19	237	78	16	(6.7)	51	(65.4)	(10.2)
20–30	81	293	10	(12.3)	214	(73.0)	(16.9)
31–40	58	159	16	(27.6)	116	(72.9)	(37.9)
41–50	55	144	18	(32.7)	106	(73.6)	(44.4)
51–60	62	139	26	(41.9)	102	(73.4)	(57.1)
61–70	91	185	43	(47.3)	136	(73.5)	(64.4)
>70	84	143	55	(65.5)	105	(73.4)	(79.7)
Total	1012	1938	193	(19.1)	1228	(63.4)	(30.1)

*Regression analysis is used to identify the association between the incidence of JC viruria and various ages ($r = .9$, $P < .05$).

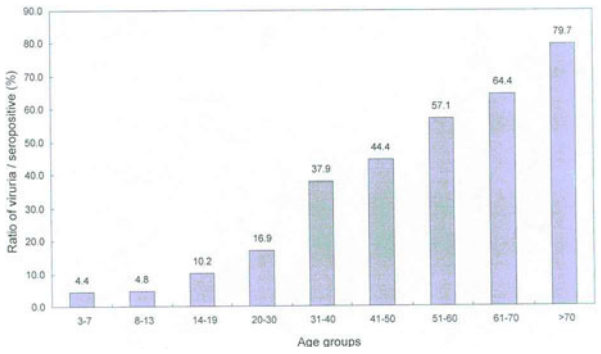

Figure 1 Ratio of JC viruria to seropositive in various age groups.

To elucidate the trend of JCV shedding in urine in seropositive individuals, the incidence of viruria was compared with that of seropositive in various age groups. The results showed that the ratio of viruria to seropositive increased with age. The ratio was low, less than 5%, in children and gradually increased with ages (Table 1 and Figure 1). The ratio of viruria to seropositive reached about 80% for those older than 70 years (Table 1 and Figure 1). These findings suggest that JCV is more reactivated in the seropositive elderly than in other seropositive children and young adults.

The purpose of this study was to understand the prevalence of human polyomavirus in the general Taiwanese population. Therefore, we examined urine and serum samples collected from day care centers, schools, and physical examination centers in order to minimize the samples from individuals with immunosuppression as much as possible. The current serological results showed that the JCV seropositive rate increased with age during childhood from new born (29.3%) to adolescence (58.8%), and it reached the maximum (73%) in young adults. It is not known why a certain percentage, approximately 20% to 30%, in adult population was JCV seronegative. Our serological findings in this study are similar with those of previous reports (Padgett and Walker, 1973; Daniel *et al*, 1981; Taguchi *et al*, 1982).

Serological evidence indicates that primary infection of the human polyomavirus may occur during childhood. The virus is latent in the kidney throughout the individual's lifetime (Chesters *et al*, 1983; Dorries and ter Meulen, 1983; Dorries, 1984). Although the virus is occasionally found in urine, it is still not clear what physiological conditions trigger virus reactivation and what causes virus progeny to shed into the urinary tract. Conditions of immunosuppression or immunodeficiency usually result in virus shedding into the urine (Coleman *et al*, 1980; Myers *et al*, 1989; Markowitz *et al*, 1993; Kitamura *et al*, 1994; Chang *et al*, 1996a, 1996b). In addition, viral pathology, such as PML caused by JCV (Padgett *et al*, 1971; Houff *et al*, 1988; Major *et al*, 1992) and nephritis caused by BKV (Purighalla *et al*, 1995; Pappo *et al*, 1996; Mathur *et al*, 1997; Nickeleit *et al*, 1999; Randhawa *et al*, 1999; Chen *et al*, 2001), usually occurred in patients with immunodeficiency or immunosuppression. Therefore, immunity should correlate with JCV reactivation. In PML patients, lymphocyte proliferation was blunted in response to mitogenic stimulation indicating that the cell-mediated immunity was impaired in the PML patients (Willoughby *et al*, 1980). DNA replication of JCV in glial cells was inhibited by nuclear factors secreted by the activated T cells (Chang *et al*, 1996). Treatment with cytotoxic immunosuppression agents increased urinary excretion of JCV in patients with autoimmune diseases (Wang *et al*, 2000). More recently, Weber *et al* (2001) demonstrated that T cells from PML patients reduced interferon-gamma production and elevated interleukin-10 production after being treated with the JCV VLPs. These results indicate that Th1-type T-helper cells were defective in PML patients. Therefore, these findings suggested that immunodeficiency or immunosuppression, especially in cell-mediated immunity, were associated with JCV reactivation.

In this study, the incidence of JC viruria in the general population aged from 3 to 84 years was investigated. The results clearly showed that the incidence of JC viruria increased with age, although the JC seropositive rate reached the maximum at the age group of 20 years old and was about the same for other older adults. To illustrate a more specific profile of JC viruria in JCV-infected individuals, the ratio of viruria to seropositive in various age groups were compared. The results showed that the incidence of JC viruria in JCV-seropositive children was low (less than 5%). The ratio of JC viruria to seropositive increased with age and was up to about 80% for the individuals older than 70 years. It is still not clear why the incidence of viruria was also low (16.9%) in young adults, although their seropositive rates (73%) were almost the same as that of the elderly. Therefore, these findings of the ratio of viruria to seropositive suggested that reactivation of the JCV more commonly occurred in the elderly than in children or young adults.

Aging immunity is most affected in the adaptive immunity comprised of T and B lymphocytes (Globerson and Effros, 2000). The alterations of T-cell function were relatively predominant. There was a shift of decreased numbers of naïve T cells with a concomitant increase of aged CD4 memory T cells, which were hyporesponsive and appeared to be nonfunctional or anergic with time in elderly immunity. The characteristic shift contributed to the immunodeficiency in old age (Linton *et al*, 1997). It is known that T lymphocytes play an important role in the fight against pathogens. The decline of the function of T lymphocytes is consistent with the increase of infection and virus reactivation in the elderly. Although the number and responsiveness of peripheral B cells in aged mice was found to be intact, maturation of B cells was blocked (Riley *et al*,

1991). Therefore, the humoral immunity was subsequently affected by the dysfunction of T-lymphocytes (Klinman and Kline, 1997). The substantial changes due to aging immunity may explain why the reactivation of JCV is more common in the elderly. Recently, Liu *et al* at Chang Gung Memorial Hospital, Taiwan, verified a PML case, which occurred in an old patient without obvious immunodeficiency (personal communication). Taken together, these findings may bring up our attention on the relationships between JCV infection and neurological disorders in the elderly.

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