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Molecular Identification of JC Virus in Blood Samples of Iraqi Patients Diagnosed Clinically and Radiologically as having PML

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Abstract

Background: John Cunningham (JC) virus belongs to Polyomaviridae family as a member of Polyomaviruses genus that includes in addition BK virus and SV40. The first case of demyelinating disease described with the term PML was found in a patient with chronic lymphocytic leukemia (CLL) and Hodgkin's lymphoma in 1958. The usual diagnostic method is viral detection in CSF sample.

Objective: Molecular identificantion of JC virus in blood samples obtained from patients already diagnosed with PML.

Patients and methods: The present cross sectional study included 61 Iraqi patients, diagnosed as having multifocal leukoencephalopathy according to clinical and radiologic evidences. Their age range was from 16 to 65 years and they were 10 males and 51 females. Blood samples were obtained from all patients and subjected to PCR analysis for viral DNA detection and to serologic IL-17 estimation.

Results: Blood Real time PCR in patients with PML was positive in only 8.2 % of cases and there was no significant difference in mean IL-17 between those who were positive and those who were negative according to real time PCR results.

Conclusion: Blood sample real time PCR provides low sensitivity tool for identification of JC virus in patients having PML and that further development for blood sample collection and processing to optimize results are needed in the future.

Key words: JC virus, real time PCR

INTRODUCTION

John Cunningham (JC) virus belongs to Polyomaviridae family as a member of Polyomaviruses genus that includes in addition BK virus and SV40 [1]. The first case of demyelinating disease described with the term PML was found in a patient with chronic lymphocytic leukemia (CLL) and Hodgkin's lymphoma in 1958. These cases are all consistent with the pathology of PML, including the development of multiple white matter plaques in the brain stem, basal ganglia and thalamus, cerebral hemispheres, and cerebellum [2]. A viral cause of PML was proposed in 1959 following observations of inclusion bodies inside nuclei of damaged oligodendrocytes and the hypothesis that the distribution of lesions could be explained by an atypical viral infection [2]. The nuclei of oligodendrocytes with inclusion bodies were discovered by electron microscopy to harbor particles similar to the well known polyomaviruses [3]. The causative agent of PML was not identified until 1971, when the virus was isolated from a culture of glial cells and was named JC virus (JCV), after the initials of the patient [4].

The structure of the virus is described as non-enveloped icosahedral DNA viruses with 5 kb genomes. The genome is classified into 3 active regions; the first region is the early region that encodes for 2 non-structural proteins (large and small tumor antigens); the second region is the late region which encodes the 3 viral capsid proteins (VP1, VP2 and VP3); and the third region is a non-coding region that functions as a regulatory segment. Polyomaviruses BKV and JCV has 72% DNA sequence homology [1].

The name polyoma refers to the ability of the virus to induce tumors in many different organs [5]. JCV can be subcategorised into 14 subtypes that are found in different ethnic human population groups. For instance, types 3 and 6 are found in Africans, type7A in Southeast Asians, and types 1 and 4 in Europeans [6].

Primary infection is encountered somewhat later in childhood and around 75% of the worldwide population has antibody directed toward the virus and lifelong persistence is established. In persons who are infected, the virus is frequently shed in urine throughout life and sheding rate becomes more frequent during pregnancy or when there is immune suppression. JC virus can result in a lethal disease; progressive multi focal leukoencephalopathy (PML); which is a complication of an immune suppressant condition such as advanced disseminuteated malignant conditions such as Hodgkin’s disease or chronic lymphocytic leukemia. JCV may also is seen in association with primary or secondary immunodeficiency syndrome particulary AIDS, or following immune compromization in persons subjected to organ transplantation [7].

The polymerase chain reaction (PCR) constitutes the best advance in molecular biology dated back to th advent of recombinant DNA technology. It makes possible for a single copy of any gene sequence to be amplified in vitro to at least a million times within short period of time. Hence, viral DNA obtained from a very small number of virions or

infected cells can be augmented to the degree where it makes direct identification of the virus [8]. The epidemiology of JCV transmission is still contraversial. The virus may have been described in the tonsil stromal cells and viral transmission via the respiratory droplets has been proposed, the virus has also been seen in the gastro-intestinal tract and in the raw urban sewage suggesting a possible oral/fecal route as a mode of transmission. Also the virus has been isolated in B-lymphocytes that suggests the risk of transmission through blood transfusion. Kidney transplantation has resulted in continued growing number of patients living with a functioning kidney allograft and may be another source of infection [7, 9].

PATIENTS AND METHODS

The present cross sectional study included 61 Iraqi patients, diagnosed as having multifocal leukoencephalopathy according to clinical and radiologic evidences. Their age range was from 16 to 65 years and they were 10 males and 51 females. Blood samples were obtained from all patients and subjected to PCR analysis for viral DNA detection and to serologic IL-17 estimation. Real-Time PCR was performed for detection of JC polyomavirus by using the specific primers and probe for large T antigen (LTA) gene. The primers and probe were designed in this study by using the complete sequence of John Cunningham virus (JC virus)large T antigen (LTA) gene, complete cds (GenBank: AF015527.1) using NCBI-Genbank and Primer3 plus design, and these primers and probe were used in Real-Time PCR assay for rapid detection of JC virus in human blood. Also another PCR primer for VP1 gene was used in PCR amplification that used in DNA sequence assay and phylogenetic tree analysis. The primers were provided by (Bioneer. Company, Korea). Human IL-7 ELISA Kit was used in this study for quantitative determination of IL-7 concentrations in serum of human blood samples and done according to company instruction. Statistical analysis was carried out using SPSS version 23. Variables were expressed as number, percentage, mean, standard deviation (SD), range, median and inter-quartile range (IQR). Mann Whitney U test was performed to compare the difference in IL-17 level between positive and negative PCR patients. The level of significance was considered at $P<0.05$.

RESULTS

This cross sectional study included 61 patients with clinical and radiological evidence of progressive multifocal leukoencephalopathy, suggesting a diagnosis of JC viral demyelination pathology. The demographic characteristics of the study group are shown in table 1. Mean age of all patients enrolled in the present study was 37.41 ±9.76 years and ranged from 16-65 years. The study included 10 male patients (16.4%) and 51 female patients (83.6%). Twenty three patients (37.7%) were either employed or have private job and 38 patients (62.3%) were either retired or housewives. Majority of cases were reported in Baghdad with small proportion from other Iraqi provinces.

Real time PCR analysis of blood samples obtained from patients showed a positive detection rate of 5 out of 61 (8.2%), figure (1). Mean serum IL-17 was 72.56 ± 28.19 ng/ml in all patients and there was no significant difference in mean serum Il-17 between patients with positive PCR and patients with negative PCR results (P= 0.207), despite being lower in positive cases, table (2) and figure (2).

Table 1: Demographic characteristic of the study group

Characteristic	Value
Number of cases	61
Age	
Mean ±SD (years)	37.41 ±9.76
Range (years)	16-65
Gender	
Male n (%)	10 (16.4%)
Female n (%)	51 (83.6%)
Occupation	
Having job or student	23 (37.7%)
Retired or housewife	38 (62.3%)
Province	
Baghdad	52 (85.2%)
Basra	5 (8.2%)
Al-Qadissiyah	3 (4.9%)
Wassit	1 (1.7%)

Table 2: Statistics regarding serum IL-17

Mean	Median	SD	Minimum	Maximum	Range	IQR
72.56	72.72	28.19	30.14	135.52	105.38	37.54

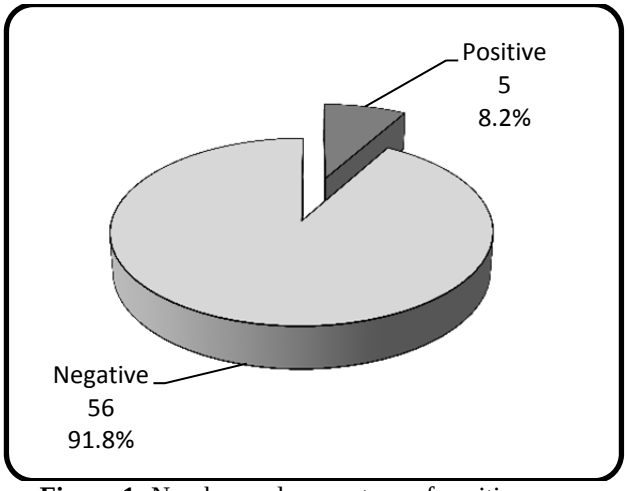

Figure 1: Number and percentage of positive cases according to blood sample real time PCR

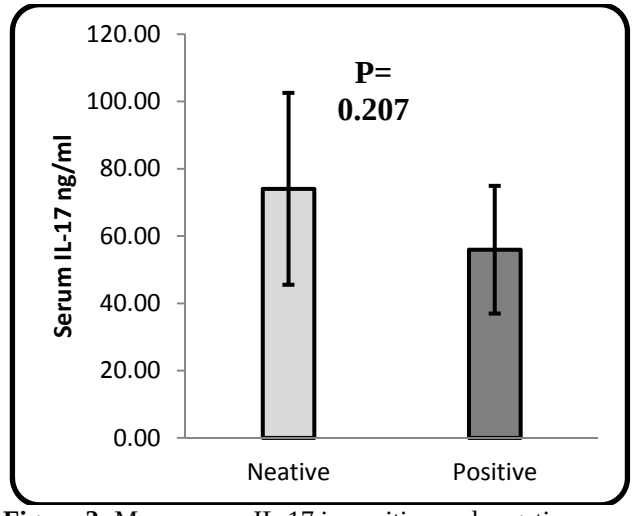

Figure 2: Mean serum IL-17 in positive and negative cases

DISCUSSION

When PML is taken into consideration, there is a problem regarding difficulties encountered in the diagnosis and the lack of useful biomarkers for assessment of disease progression. Future research may be directed toward a better understanding of the virology and life cycle of JCV and the pathogenesis and immunopathology of PML. A lot of studies have been done for detection and quantification of JC virus in blood and serum samples obtained from patients, already diagnosed to have the virus by CSF examination, for various reasons. In one study, JCV DNA was detected by QPCR in plasma from 14 (17%) of 83 cases and 28 (12%) of 230 controls and the median detectable level of JCV DNA was 168 copy numbers per mL of plasma (interquartile range, 108–364) [(10)]. In another study, done on patients with multiple sclerosis (MS) who received monthly infusions of natalizumab, the results were as following: Overall, 17 of the 49 patients (35%) had viremia at some time. they analyzed plasma samples for the presence of JC virus antibodies and viral DNA [(11)].

The availability of clinical samples from PML patients at the time of laboratory diagnosis and follow up for extended periods of time has revealed viral persistence that can occur for years. This observation might be important if not critical when evaluating new clinical episodes of neurological deficits and may warrant periodic assessment for viral genome in the CSF as well as in the plasma [(12)]*. It is not known whether the origin of CSF viral DNA during persistence is derived from the brains of PML patients or virus periodically enters the brain from the periphery [(13)].

In addition, Variable reports of viral load ranging from 13.6% up to 46.1% was registered by Delbue *et al*., in 2007 in a study included patients with multiple sclerosis [(14)].

In a study conducted in Sudan the qualitative PCR procedure was used to detect JCV DNA in plasma and urine samples. Specimens were obtained from 50 renal transplant patients. The results showed the superiority of plasma samples (48%) over urine samples (28%) for the detection of JCV. This is considered unusu al result for plasma samples [(15)].

Some others have reported uncommon transient, and low viremia [(16)]. However in another study led on 20 patients, 25% demonstrated JCV viremia [(17)]. Every one of these studies was completed by utilizing quantitative continuous PCR. In this study, the use of plasma samples rather than whole blood specimen to detect JCV DNA might have increased the sensitivity of JCV detection and could explain why current results are different from those of other studies. However, the reports that JCV viremia is more commonly detected among renal transplant recipients than viremia may be due to the fact the virions may enter the circulation through peri-tubular capillaries following tubular damage. Such patients could be at potential risk of developing JCV nephropathy, thus detection of JCV DNA by PCR in urine is currently concurrently used to detect viral replication in renal transplant patients. Detection of JCV DNA by PCR in plasma specimens is not efficient in patients with low viral replication in the urinary tract. For this reason JCV viremia is only used to confirm JCV after first screening urine [(17)].

A feature that is common among the human polyomaviruses is that the incidence of virus-associated disease in the population is very low and yet a large percentage of people has antibodies to the virus indicating widespread infection. For example, most people become seropositive to JCV and BKV in childhood but very rarely virus can be detected in the blood (viremia) and usually only in patients with PML and PVAN respectively. Viruria (virus in the urine) can occur somewhat more often and tends to be episodic and at low levels in normal people [(18)].

It is likely that mixtures of multiple human polyomaviruses may be present in biological samples from clinical sources such as blood and urine and so assays for JC viral load must be highly specific. The development of quantitative polymerase chain reaction (qPCR) assays provides a specific, sensitive and quantitative method to measure JC viral load [(19)]. Pal et al designed sets of primer/probes for BKV, JCV and SV40 specific for both the early and late coding regions of each virus. These were characterized and it was shown that they could detect between 1–10 copies of their cognate genomes while failing to react with as many as 10^8 copies of DNA from the other viruses [(19)]. Thus qPCR assays have the capacity to enumerate small numbers of viral genomes in the presence of large amounts of unrelated DNA. The reliability of any given set of primer/probes should be evaluated by generating standard curves at a broad range of dilutions and in the presence and absence of other viruses. It is important to note that the number of PCR cycles can affect both the sensitivity and specificity of the assay [(19)]. Another factor that needs to be considered is sequence divergence within the viral isolate. For example in the case of BKV, there is substantial diversity of the genomes of viral isolates [(20, 21)]. Bayliss et al [(22)] reported that sequence analysis of the early region of JCV in four immunosuppressed patients revealed eight novel single nucleotide polymorphisms. However, Dumoulin and Hirsch reevaluated and optimized JCV and BKV qPCR assays to detect rare sequence polymorphisms and updated the BKV assay for the occurrence of polymorphisms. They concluded that their original JCV and updated BKV qPCR assays were robust and able to detect rare variants routinely encountered in the clinic [(23)].

CONCLUSION:

Blood sample real time PCR provides low sensitivity tool for identification of JC virus in patients having PML and that further development for blood sample collection and processing to optimize results are needed in the future.

REFERENCES

[1] Kwak, E.J., Vilchez, R.A., Randhawa, P., Shapiro, R., Janet, S., et al. Pathogenesis and management of polyoma virus infectiuon in transplant recipients. *Clin Infect Dis*. 2002, 35,1081-1107.

[2] Ferenczy, M.W., Marshall, L.J., Nelson, C.D., et al. Molecular Biology, Epidemiology, and Pathogenesis of Progressive Multifocal Leukoencephalopathy, the JC Virus-Induced Demyelinating Disease of the Human Brain. *Clinical Microbiology Reviews*. 2012,25(3),471-506.

[3] Tan, C.S., Koralnik, I.J. Beyond progressive multifocal leukoencephalopathy: expanded pathogenesis of JC virus infection in the central nervous system. *Lancet neurology*. 2010,9(4), 425-437.

[4] De Gascun, C.F., Carr, M.J. Human Polyomavirus Reactivation: Disease Pathogenesis and Treatment Approaches. *Clinical and Developmental Immunology*. 2013, 2013, 373579.
[5] Dalianis, T., Hirsch, H. H. Human polyomaviruses in disease and cancer. *Virology*. 2013, 437(2), 63-72.
[6] Taheri, S., Kafilzadeh, F., Shafa, M., Yaran, M., Mortazavi, M., et al. Comparison of polyoma virus (BK virus and JC viruses) viruria in renal transplant recipients with and without kidney dysfunction. *J Res Med Sci*. 2011, 16, 916-922.
[7] David, O.W., Frank, J.F. *Medical virology*; Fourth Edition. Albuquerque, New Mexico 1994, p: 303.
[8] Delbue, S., Ferraresso, M. JC polyoma virus infection in renal transplant patients. *J Transplant Technol Res*. 2012, 2, 3.
[9] Kayser, F.H, Bienz, K.A., Eckert, J., Zangernagel, R.M. Thieme Stuttgart; *Flexbook Medical Microbiology* 2005, p: 415.
[10] Viscidi, R.P., Khanna, N., Tan, C.S., et al. John Cunningham Virus Antibody and Viremia as Predictors of Progressive Multifocal Leukoencephalopathy in Human Immunodeficiency Virus-1–Infected Individuals. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*. 2011,53(7),711-715.
[11] Major, E.O., Frohman, E., Douek, D. JC Viremia in Natalizumab-Treated Patients with Multiple Sclerosis. *The New England journal of medicine*. 2013, 368(23), 2240-2241.
[12] Gorelik, L., Bixler, S., Lerner, M., et al. Evaluation of the incidence of anti-JCV antibodies in a cohort of natalizumab-treated MS patients. *American Academy of Neurology Annual Meeting*. 2010, S31,003.
[13] Ryschkewitsch, C.F., Jensen, P.N., Monaco, M.C., Major, E.O. JC Virus persistence following PML in MS patients treated with natalizumab 64 characters. *Annals of neurology*. 2010,68(3), 384-391.
[14] Delbue, S., Guerini, F.R., Mancuso, R., et al. JC virus viremia in interferon-beta -treated and untreated Italian multiple sclerosis patients and healthy controls. *J Neurovirol*. 2007,13(1),73-77.
[15] Badri, A.M., Janay, A., Jonas, H., Liban, A.H., El Fadol, A.S., et al. Molecular Detection of JCV in Urine and Blood Samples of Renal Transplant Patients in Khartoum State, Sudan. *Mol Biol*. 2017, 6, 178.
[16] Nishiguchi, M.K., DeSalle, R., Giribet, G., Wheeler ,W.C. *DNA isolation procedures techniques in molecular systematics and evolution* 2002, pp: 247-287.
[17] Drachenberg, C.B., Hirsch, H.H., Papadimitriou, J.C. Polyoma virus BK versus JC replication and nephropathy in renal transplant recipients: A prospective evaluation. *Transplantation*. 2007, 84, 323-330.
[18] White, M.K., Sariyer, I.K., Gordon, J., et al. Diagnostic Assays for Polyomavirus JC and Progressive Multifocal Leukoencephalopathy. *Reviews in medical virology*. 2016, 26(2), 102-114.
[19] Pal, A., Sirota, L., Maudru, T., et al. Real-time, quantitative PCR assays for the detection of virus-specific DNA in samples with mixed populations of polyomaviruses. *J Virol Methods*. 2006,135,32–42.
[20] Chen, Y., Sharp, P.M., Fowkes M, et al. Analysis of 15 novel full-length BK virus sequences from three individuals: evidence of a high intra-strain genetic diversity. *J Gen Virol*. 2004,85,2651–2663.
[21] Moens, U., Van Ghelue, M. Polymorphism in the genome of non-passaged human polyomavirus BK: implications for cell tropism and the pathological role of the virus. *Virology*. 2005,331,209–231.
[22] Bayliss, J., Karasoulos, T., McLean, C.A. Frequency and large T (LT) sequence of JC polyomavirus DNA in oligodendrocytes, astrocytes and granular cells in non-PML brain. *Brain Pathol*. 2012 ,22, 329–336.
[23] Dumoulin, A., Hirsch, H.H. Reevaluating and optimizing polyomavirus BK and JC real-time PCR assays to detect rare sequence polymorphisms. *J Clin Microbiol*. 2011, 49, 1382–1388.