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Full Length Research Paper

Detection of viruses in the peritoneum of the Iranian patients on peritoneal dialysis

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Despite reductions in overall infectious peritonitis rate due to improvement in peritoneal dialysis (PD) systems, peritonitis is still a major complication of PD and a major cause of failure of this method of dialysis. In this context, viral infections have rarely been investigated, although up to 20% of cases remain culture negative (non-bacterial, non-fungal). The present study aimed to screen stored PD effluent samples for viral infection to assess the incidence in a cross-sectional cohort of patients on PD. Nucleic acid extracted from PD samples (n=205) were analysed using either conventional polymerase chain reaction (PCR) or real-time PCR for polyomaviruses (JCV, BKV, KIV, WUV, MCV, SV40, and LPV), human cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes simplex viruses (HSV-1 and 2), varicella-zoster virus, adenoviruses, enteroviruses, parechovirus and human norovirus. Of the 205 PD effluent samples analysed, 10 (4.8%) were found to be positive for viral infections (BKV: 2.9%; CMV: 1.9%). One out of ten positive patients were clinically well at the time of PD effluent collection although five patients who tested positively for CMV or BKV was diagnosed with sterile peritonitis. This study has described that BKV and CMV may be present in the peritoneum of patients on PD. BKV and CMV should be considered as a potential cause of aseptic peritonitis in patients on PD.

Key words: Peritoneal dialysis (PD), peritonitis, polymerase chain reaction (PCR), BK polyomavirus, cytomegalovirus (CMV).

INTRODUCTION

Peritonitis is a common complication in peritoneal dialysis (PD) patients (both continuous ambulatory peritoneal dialysis [CAPD] and automatic peritoneal dialysis [APD]). Despite reductions in overall infectious peritonitis rate due to improvements in PD systems (Dasgupta, 2000), peritonitis is still a major complication of PD and a major cause of failure of this method of dialysis. Encapsulating peritoneal sclerosis (EPS) is a rare but potentially fatal complication in patients on PD. Episodes of peritonitis may damage the peritoneum leading to pathological changes and EPS. Infectious agents may play a part in the development of this condition.

Bacterial and fungal peritonitis is one of the most common causes of morbidity in PD patients. However, up to 20% of peritonitis cases are culture negative (non-fungal and non-bacterial) (Peterson et al., 1987).

Viral infections have rarely been investigated in the context of peritoneal fibrosis and the impact of viral infection on membrane function and peritoneal morphology is unknown. Little is known about the frequency of viral

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frequency of viral peritonitis. Cytomegalovirus (CMV) DNA was identified in peritoneal fluid of kidney transplant recipients treated with PD (Shulman et al., 1992; Meier et al., 2005). Herpes simplex virus (HSV) peritonitis was described in a patient undergoing PD (Yakulis and Babinchak, 1995). HIV has been recovered from peritoneal dialysis effluent (PDE) using a culture system (Scheel et al., 1995). Hepatitis C virus RNA was detected in PD fluid of patients with viremia (Cusumano et al., 2005). HSV-2 has been reported in a 60 year-old woman on CAPD whom bacterial or fungal culture remained negative (Liesker et al., 2006).

Polyomaviruses, herpesviruses, adenovirus, enterovirus, parechovirus, and norovirus are among the most common of human viruses. Most viral infections are in apparent, but they may cause a wide spectrum of acute diseases including enteritis with diarrhoea and respiratory disease (Parechovirus), mild upper respiratory illness, febrile rash, aseptic meningitis, pleurodynia, encephalitis, and paralytic poliomyelitis (enteroviruses), gastroenteritis (noroviruses), respiratory, ocular, or gastrointestinal disease (adenoviruses), genital, nervous system, ocular, skin, lymphoproliferative and gastro-intestinal tract disorders (herpesviruses), nephropathy, ureteric stenosis and haemorrhagic cystitis (BK polyomavirus), progressive multifocal leukoencephalopathy (PML) and haemorrhagic cystitis (JC polyomavirus). However, other aspects of infection with these groups of viruses are poorly defined in both immunocompetent and immunocompromised patients. These viruses are significant pathogens complicating the post-transplant course of organ recipients. In addition, over the past decade, new viruses such as human metapneumovirus (hMPV), new coronaviruses (NL63 and HKU1), human bocavirus (HBoV) and new human polyomaviruses (KIV, WUV, and MCV) have been identified. The clinical impact of these new virus infections is not clear. To date, none of the three recently discovered human polyomaviruses, KIV, WUV or MCV have been linked with a specific disease or symptoms. Moreover, despite the presence of SV40 and LPV in humans, the role of these two non-human polyomaviruses in human diseases is not known.

In order to investigate if polyomaviruses (BKV, JCV, KIV, WUV, MCV, SV40 and LPV), CMV, adenovirus, herpes simplex virus (HSV-1 and HSV-2), varicella zoster virus (VZV), Epstein-Barr virus, enterovirus, parechovirus and human norovirus were present in PD samples, this study was conducted on PD patients.

MATERIALS AND METHODS

Specimen collection and processing

A total of 205 PD effluent specimens were obtained from a random selection of patients who had undergone PD during a period of ten years from September 2000 to September 2010. These samples had been collected and stored after full consent of the patients concerned had been given for these samples to be used for research purposes. Tehran University of Medical Science (TUMS) research ethics committee approval was granted in June 2011 to test these samples for the presence of viruses by polymerase chain reaction (PCR) as part of a wider study into the aetiology and pathogenesis of encapsulating peritoneal sclerosis.

Conventional PCR assay

Nucleic acid was extracted from PD effluent samples according to the manufacturer's instructions using the BioRobot MDx and Blood BioRobot MDx Kit (Qiagen Ltd, USA) and stored at -20˚C until use. Samples were analysed by conventional PCR using virus specific primers designed based on the highly conserved regions identified for each virus (Table 1) for five human polyomaviruses (BKV, JCV, KIV, WUV, MCV) and two non-human polyomaviruses (SV40 and LPV). Amplification was performed as described previously (Abedi Kiasari et al., 2008).

Real time PCR assay

Nucleic acids extracted were analysed using virus specific primers designed based on the highly conserved regions identified for each virus and probes (Table 1) for CMV, adenovirus, herpes simplex virus (HSV-1 and HSV-2), Varicella-Zoster virus, Epstein-Barr virus, enterovirus and norovirus. Amplification was performed on the ABI 7500 real-time PCR 'fast' system using virus specific thermal profile.

Sequencing

PCR amplified fragments were sequenced using the Big Dye Terminator Cycle Sequencing kit (Applied Biosystems UK Ltd) on an ABI 3100 Genetic Analyzer. Contiguous sequences were assembled using Sequencher software version 4.6 (Gen Codes Corporation, Ann Arbor, Michigan). To identify virus specific sequences, a Blast search (NCBI) was performed upon GenBank.

RESULTS

Patient characteristics

PD effluent samples taken from 205 patients on PD in the period September 2000 to September 2010 were included in the study. The median age of the 205 patients was 65 years (range 20 to 88 years; mean=57 years), and the gender ratio (M/F) was 1.08:1.

Polyomavirus prevalence and clinical findings

PD effluent samples (n=205) from patients on PD were tested by PCR using PYVF/PYVR primers targeting large T antigen region of BKV, JCV, and SV40 genomes. Of the 205 samples analysed, 6 (2.9%) were positive for BKV/JCV/SV40 in conventional PCR assays using

Table 1. Primers and probes used in PCR assays.

Virus	Type of primer/probe	Sequence (5'-3')
JCV/BKV/SV40	PYVF	TAGGTGCCAACCTATGGAACAGA
	PYVR	GGAAAGTCTTTAGGGTCTTCTACC
JCV/BKV	PEP-1	AGTCTTTAGGGTCTTCTACC
	PEP-2	GGTGCCAACCTATGGAACAG
KIV	POLVP139F	AAGGCCAAGAAGTCAAGTTTC
	POLVP1363R	ACACTCACTAACTTGATTTGG
WUV	AG0044F	TGTTACAAATAGCTGCAGGTCAA
	AG0045R	GCTGCATAATGGGGAGTACC
SV40	T3	ACCACAACTAGAATGCAGTGAAAAAA
	T4	GAAGACAGCCAGGAAAATGCTGATAA
	TA1	GACCTGTGGCTGAGTTTGCTCA
	TA2	GCTTTATTTGTAACCATTATAAG
	LA1	GGGTGTTGGGCCCTTGTGCAAAGC
	LA2	CATGTCTGGATCCCCAGGAAGCTC
MCV	LT3F	TTTGCCAGCTTACAGTGTGG
	LT3R	TGGATCTAGGCCCTGATTTTT
LPV	LPV1	AGGGCCTGATGCTATTACCC
	LPV2	CCCACATCAAAATGGTGTCA
Adenovirus	Forward	CACATGACTTTCGAGGTCGATCCCATGGA
	Reverse	CCGGCTGAGGAGAAGGGTGTGCGCAGGTA
	Probe	CACCGCGGCGTCAT
CMV	Forward	CTGCGTGATATGAACGTGAAGG
	Reverse	ACTGCACGTACGAGCTGTTGG
	Probe	CGCCAGGACGCTGCTACTCACGA
HSV	Forward	GACAGCGAATTCGAGATGCTG
	Reverse	ATGTTGTACCCGGTCACGAACT
	HSV1 probe	CATGACCCTTGTGAAACA
	HSV2 probe	TGACCTTCGTCAAGCAG
VZV	Forward	GCGCGGTAGTAACAGAGAATTTC
	Reverse	TGGGCACATCTTCATCTAAACATT
	Probe	ACCATGTCATCGTTTCAA
Enterovirus	Forward	CCCCTGAATGCGGCTAATC
	Reverse	ATTGTCACCATAAGCAGCCA
	Probe	CGGAACCGACTACTTTGGGTGTCCGT
Parechovirus	Forward	CACTAGTTGTAAGGCCCACGAA
	Reverse	GGCCCCAGATCAGATCCA
	Probe	CAGTGTCTCTTACCTGCGGGTACCTTCT

Table 1. Contd.

	COG 1F	CGYTGGATGCGNTTYCATGA
	COG 1R	CTTAGACGCCATCATCATTYAC
	RING 1A	AGATYGCGATCYCCTGTCCA
Norovirus	RING 1B	AGATCGCGGTCTCCTGTCCA
	COG 2F	CARGARBCNATGTTYYAGRTGGATGAG
	COG 2R	TCGACGCCATCTTCATTCACA
	RING 2	TGGGAGGGCGATGGCAATCT

Table 2. C_t values and quantitative values for positive samples in real-time PCR assay.

Sample	C_t value	Quantitative value (copies/ml)	Log value
CMV positive 1	39	265	2.66
CMV positive 2	44.5	19	1.21
CMV positive 3	34.24	182	2.41
CMV positive 4	38	188	2.53

PYVF/PYVR primers. Of these, all positive samples (n=6) could be amplified by the BK/JC PCR using the BKV and JCV specific primers (PEP1/PEP2) and no sample could be amplified in the SV40 assay using the SV40 specific primers (TA1/TA2, T3/T4, LA1/LA2). Therefore, of the 205 samples analysed, 6 (2.9%) were confirmed to be positive for BKV/JCV by conventional PCR assay using PEP1/PEP2 primers. Six positive samples were further investigated through bidirectional sequencing of their LT assay amplification product. The sequences of the 6 samples showed high homology to the previously described BKV strain sequences including the new isolates (AB211369, AB211371, AB211378-91, AB269848).

Most of the positive patients (n=5) were aged >60 years, and one patient was 48 years old. BKV was detected in 3 female and 3 male patients. The clinical implications of these infections are unknown. The cause of the end stage renal failure (ESRF) of these patients was recorded to be hypertension, light chain deposits, unilateral small kidneys and glomerulonephritis (GN).

PD effluent samples (n=205) from patients on PD were analysed by PCR using POLVP1-39F/POLVP1-363R primers for KIV, AG0044/AG0045 primers for WUV, LT3F/LT3R primers for MCV and LPV1/LPV2 primers for LPV. No KIV, WUV, MCV, and LPV polyomavirus DNA could be detected in any of the samples tested by virus specific PCR assay. The negative and positive controls gave the expected result in all PCR assays.

Viral prevalence and clinical findings in real time PCR assay

PD effluent samples (n=205) from patients on PD were analysed by real-time PCR for CMV, adenovirus, herpes simplex virus, Varicella-Zoster virus, Epstein-Barr virus, enterovirus and norovirus. No false positive results were identified from the amplification plots. All positive controls worked as expected and the negative controls showed no amplification. Of the 205 samples analysed, 4 (1.9%) were positive for CMV. Ct and quantitative values for positive samples are shown in Table 2.

MCV positive patients (n=4) were of ages 51 to 60 years. Three MCV positive patients were female and one was male. The cause of the ESRF of MCV positive patients was recorded to be nephropathy and chronic tubulo-intestinal nephritis. None of the samples examined by real-time PCR were found to be positive for adenovirus, herpes simplex virus, Varicella-Zoster virus, Epstein-Barr virus, enterovirus and norovirus.

DISCUSSION

A total of 205 PD effluent samples were screened for viral infection by either conventional PCR or real-time PCR to assess the incidence of polyomaviruses, CMV, adenovirus, herpes simplex virus, Varicella-Zoster virus, Epstein-Barr virus, enterovirus, parechovirus and norovirus in a cross-sectional cohort of patients on PD. Of the 205 samples analysed, six (2.9%) and four (1.9%) were positive for BK polyomavirus and CMV, respectively yielding an overall case prevalence of viral infection of 4.8%. Further, as several other viruses including rotavirus, coronavirus, astroviruses and sapoviruses were not tested in this study, the proportion of viral infections is possibly even higher. The positive samples were further investigated through bidirectional sequencing of their

assay amplification product. The sequence data showed 98 to 100% homology to the previously described virus strain sequences in GenBank.

Despite the use of sensitive PCR assays, none of the samples were found to be positive for adenovirus, herpes simplex virus, Varicella-Zoster virus, enterovirus, norovirus, JCV, SV40, MCV, KIV, WUV, and LPV polyomaviruses. Extraction efficiencies and inhibition were not evaluated, leading to a possible underestimation of DNA load and detection, however, amplification of positive samples suggest efficient extraction of DNA and the lack of polymerase inhibitors in the samples.

BKV infection was demonstrated to be present in the peritoneum of patients on PD (2.9%). The age of four positive patients were >60 years, and one patient was 48 year old and all patients were severely immunocompromised. BKV was detected in 3 female and 3 male patients. Two patients were diagnosed with sterile peritonitis. The cause of the ESRF of these patients was recorded to be hypertension, light chain deposits, unilateral small kidneys and glomerulonephritis. BKV infection in immunocompromised patients leading to severe organ involvement such as nephropathy in renal transplantation patients and haemorrhagic cystitis (HC) in bone marrow transplanted (BMT) patients. In renal transplantation, symptom less BKV replication is found in 10 to 68% of patients. In some of these patients, BKV infection leads to ureteric stenosis, transient allograft dysfunction, and irreversible failure due to polyomavirus nephropathy (Hirsch, 2002; Hogan et al., 1980). Detection of BKV in PD samples in this study may suggest a possible role for BKV in aseptic peritonitis.

Four patients (1.9%) were found to be positive for CMV. The age of three MCV positive patients were 51 to 60 years, and all four patients were severely immunocompromised. Three CMV positive patients were diagnosed with sterile peritonitis. The cause of the end stage renal failure of these patients was nephropathy, chronic tubulu-intestinal nephritis and pyelonephritis. The presence of CMV in the peritoneum of patients on dialysis has been previously reported (Meier et al., 2005; Ohtani et al., 2000). Our finding supports previous conclusion that CMV should be considered a potential diagnosis in cases of peritonitis where no other cause can be found.

This study could not determine whether BKV and MCV have a role in the aetiology of EPS as none of the ten positive patients nor the other individuals tested are yet to develop EPS, clinical followup is ongoing in all of these cases. The clinical implications of the viral infections are unknown and further investigation in longitudinal studies are needed to assess whether these viruses, which may become latent over time, may cause damage to the peritoneum, influence membrane function and indeed be a source of infection post transplantation. BKV and CMV are significant pathogen complicating the post-transplant course of organ recipients. They cause a variety of problems in immunocompromised individuals. Their presence in the peritoneum may be as a result of either a primary infection or reactivation. The primary infection may affects the peritoneum leading to the more sever local reaction than a reactivations of a virus affecting the peritoneum. Frequent reactivation of virus in the peritoneum may also create conditions facilitating the fibrosis.

This study showed that viruses can be found in the peritoneum of patients on PD. However, the presence of a virus in the peritoneum does not necessarily associate with overt clinical symptoms. These viruses may either actively replicated in the peritoneum or passed into the area passively, perhaps through a damaged peritoneal membrane. To elucidate this, additional information maybe required such as serological data, an assessment of the peritoneal membrane function and possibly also histological evidence of infection in the peritoneum. Also, with the advent of the rapid results generated by PCR, there may be an argument that viral PCRs should be considered part of the standard investigations of a PD patient presenting with peritonitis. In support, Pauwels et al. (2012) reported a case of viral peritonitis caused by coxsackievirus B1 in a 79 year-old patient on CAPD suggesting that viral diagnostic tests is necessary to avoid unnecessary treatments (Pauwels et al., 2012).

Several other viruses such as rotavirus, coronavirus, astroviruses and sapoviruses that may have a role to play in peritoneal pathology were not tested in this study. Viruses such as astroviruses and sapoviruses have been associated with gastrointestinal disorders and only limited information is available on their full aspects. Coronaviruses has a wide spectrum of symptoms from respiratory infections to diarrhoeal illnesses. It is noteworthy that a coronavirus (FIPV) causes a condition called feline infectious peritonitis (FIP) in cats (Soma and Ishi, 2004) indicating that a coronavirus has this affiliation for causing peritoneal disease, although the pathology of FIP (accumulation of ascites followed by multiple pyogranulomatous lesions) is clearly different from the fibrosis seen in EPS. In the past decade, a large number of viruses including coronaviruses have been discovered and therefore further studies are needed to shed light on the viral peritonitis.

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