



**Sudan University of Science and Technology**  
**College of Graduate Studies**



**Molecular Detection of Human Polyomavirus (BKV) in  
Renal Transplant Recipients in El Gazera State, Sudan**

الكشف الجزيئي لفيروس البوليما البشري (البي كي في) لدى زارعي الكلى  
المستقبلين في ولاية الجزيرة- السودان

A dissertation submitted in partial fulfillment for the  
requirements of master degree in microbiology

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# الآية

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قال تعالى:

فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ <sup>قُلْ</sup> وَلَا تَعْجَلْ بِالْقُرْآنِ مِنْ قَبْلِ أَنْ  
يُقْضَىٰ <sup>أَوْ</sup> إِلَيْكَ وَحْيُهُ <sup>صَلِّ</sup> وَقُلْ رَبِّ زِدْنِي عِلْمًا

صدق الله العظيم

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## **DEDICATION**

**To my great parents ,my lovely sisters and all my  
friends and colleagues**

**I dedicated this thesis**

**Mohamed**

## **Abstract**

This study was carried out to detect human polyomavirus (BKV) in renal transplant recipients in E Gazera State, Sudan during the period between April to November 2015.

A total of 50 plasma and 50 urine samples were collected randomly from renal transplant recipients. The samples were subjected to Polymerase Chain Reaction (PCR) assay to detect viral DNA.

BK virus nucleic acid was detected in 14(28%) urine samples while only in 11(22%) of the plasma specimen were positive.

In conclusion establishing instant diagnosis of BKV may be of great value for monitoring the renal transplant recipients, who are at possible risk for development of BKV nephropathy. The results indicated that the routine testing of urine and plasma samples by PCR is a useful tool for the rapid and sensitive detection of reactivated BKV in asymptomatic Renal transplant recipients.

## الخلاصة

هذه الدراسة أجريت لكشف وجود فيروس البليوما (البي كي) على أوساط زارعين الكلى في ولاية الجزيرة, في فترة زمنية بين أبريل ونوفمبر لعام 2015 .

مجموع من 50 بلازما و 50 بول جمعت من خمسين مريض عشوائيا من أوساط زارعي الكلى المستقبليين , وأجري لها كشف لتقصي وجود دي ان اي فيروس البي كي بواسطة البي سي آر.

الحمض النووي لفيروس البي كي كان قد كشف عنه في 14 (28%) عينة بول و 11 (22%) عينة بلازما كانت موجبة.

في الخاتمة تأسيس فحص فوري لفيروس البي كي قد يكون له قيمة عالية للتحكم في زارعي الكلى المستقبليين الذين في احتمالية خطر لتطوير علة النيفرون المتعلقة بالبي كي, النتائج تدل على أن الفحص الروتيني لعينات البول والبلازما بواسطة البي سي آر هي أداة مفيدة للتحري السريع والدقيق لفيروس البي كي المعاود لنشاطه في زارعي الكلى المستقبليين الذين لا تظهر عليهم الأعراض.

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# **CHAPTER ONE**

## **1.INTRODUCTION AND OBJECTIVES**

### **1.1 Introduction**

BK virus belongs to the family polyomaviridae which includes several animal viruses (wide spread in rabbit), include simian vacuolating virus40 (SV40) and two human viruses JC virus and BK virus (Chakraborty, 2013).

Polyomaviruses are a groups of small non enveloped DNA viruses with icosahedral capsid of 40 – 44 nm in diameter and the viral genome within the capsid are circular double stranded DNA of 5300 base pair, coated by host cell histones that encode regulatory and structural protein. The primary infection can be followed by latency in the urinary tract epithelium as well as lymphoid cells and central nervous system (khaled, 2004).

The viral capsid is composed of the structural virion proteins (VP) 1, 2 and 3, and accommodates the viral minichromosome. Pentameres of VP1, arranged in an icosahedral lattice from the outer capsid. On the inside, the VP1 pentameres have a central groove in which VP2 or VP3 is inserted.

All three structural proteins contain DNA-binding motifs ( Aalderen *et al*, 2012).

Polyomaviruses are ubiquitous infectious agents found in over 90% of the general population , the polyoma virus hominis commonly known as BKV is a causative agent of human diseases, such as haemorrhagic cystitis , ureteric stenosis, pneumonitis, vasculopathy, and even multi organ failure, Primary infection is usually asymptomatic and reactivation of the virus results because of severe immunosuppression or altered cellular immunity as occur in kidney transplant patients , and this can be detected in plasma and urine of patient by PCR (khaled, 2004).

Renal failure in Sudan shows a continuous increase. This is generally indicated by the increase of haemodialysis centers and the attendees (Aboud, and Odsman, 1989). The transplantation of kidney allograft has become a standard management for end stage renal disease (Frohn *et al*, 2001).

Transplant rejection occurs when a transplanted organ or tissue is not accepted by the body of the transplant recipient. Transplant rejection can be reduced through serotyping to determine the most appropriate donor-recipient match and through the use of immunosuppressant drugs (Frohn *et al*, 2001).

BKV associated nephropathy (BVAN) has emerged as a leading viral cause of early kidney transplant failure with prevalence rates of 1–10% (Bechert *et al*, 2010).. BKVN occurs in 1- 10% of kidney transplant recipients, usually manifesting in the first year following transplantation and leading to graft loss in 15- 80% BKVN cases within 5 years (Bechert *et al*, 2010).

Laboratory diagnosis of BKV was achieved by PCR. Several studies have shown that the PCR for BKV DNA in plasma and urine is useful in the rapid diagnosis of BKV (Hirsch *et al*, 2002).

## **1 . 2. Objectives**

### **1 .2 .1 General objective**

To determine the incidence of BKV in renal transplant recipients in EL Gazera State, Sudan .

### **1 .2 .2 Specific objectives**

1-To detect BKV DNA in renal transplant recipients using PCR.

2- To compare between the urine and plasma samples in BK virus detection using PCR.

## **CHAPTER TWO**

### **2. LITERATURE REVIEW**

#### **2.1 Definition**

BK virus belong to polyomaviridae which is one of the medically significant nonenveloped DNA viruses which include : Adenoviridae, papillomaviridae , polyomaviridae and parvoviridae. All of them have relatively simple structure and genome organisation , although disease produced by them differ ranging from upper respiratory infection to tumor ( Chakraborty, 2013).

#### **2.2 History of BK VIRUS**

The first case of BKV allograft nephropathy was isolated from urine of the renal transplant recipient patient who developed ureteral stenosis after transplantation of allograft kidney at the university of Maryland in 1971 since then more than 100 cases have been identified(Aalderen *et al*, 2012) .

And since they isolated the BKV from this patient postoperatively they determined the BKV infection are widespread and asymptomatic in the majority of individual , BKV infection usually acquired in the childhood



and by the adulthood 60- 100% of the population are seropositive and the symptoms that appear and associated with primary infection noted as fever and respiratory infection after primary infection BKV enter the latency in the human host most likely in the urogenital tract , asymptomatic reactivation and intermittent shedding of the virus in urine occur spontaneously in immunocompetent persons and happened more among those with altered cellular immunity such as HIV1 or those undergoing immunosuppressive therapy (Hirsch *et al* , 2002).

### **2.3. Virus structure and genome organization**

#### **2.3.1 BKV Taxonomy**

BK virus belong to polyomaviridae and Polyomaviruses include several animal viruses (wide spread in rabbit population), including simian, vacuolating virus 40 (SV 40) and two human viruses JC virus(JCV) and BK virus (BKV). All polyomaviruses have the capacity to transform normal cell in culture and to induce tumors in species other than their own, However polyomavirus has not been shown to introduce tumours in human, these are wide spread in rabbit animal polyomavirus produce a variety of malignant tumours in newborn mice, hamster and guinea pig of malignant tumours in newborn mice, hamster and guinea pig, hence

the name polyoma (poly: many , oma: tumour) The simian vacuolating virus (SV 40) was isolated from uninoculated kidney tissue culture of rhesus and cynomolgus monkeys . The virus did not produce any disease or cytopathic effect in the original cultures but when fluid from such cultures was inoculated into kidney cell culture from other simian species (Africa green monkey) the virus produced cytopathic effect consisting of cytoplasmic vacuolation.SV 40 is oncogenic in newborn hamster . this is an example of a monkey virus crossing species barrier . This has got medical importance in that when viral vaccine are to be manufactured only in monkey kidney tissue culture .the tissue culture should be tested and found free from SV 40 before inoculating of vaccine strain (Chakraborty, 2013).

### **Human polyomavirus**

JC virus were recovered from brain of a child with Hodgkin's disease and progressive multifocal leucoencephalopathy (PML). Isolation from similar strain from PML cases were also reported from UK. france and united states , BK virus was isolated in 1971 from the urine of renal transplant recipient the JC and BK are derived from the initials of the persons. The two viruses are antigenically distinct but both of them encode a T antigen which is related to SV 40 T antigen. Both the viruses

can transform rodent cells as well as induce tumours in newborn hamster. replication of polyomavirus follows the basic pattern of DNA virus genome replication and gene expression in the nucleus . productive cycle lead to viral multiplication and ultimately death of host cell (Chakraborty, 2013).

### **2.3.2 Genome and Nucleocapsid**

BK viruses (BKV) represent a discrete species within the genus Polyomavirus of the Polyomaviridae, a family of nonenveloped viruses with icosahedral capsids. The capsid encloses a circular double-stranded DNA genome of approximately 5100 nucleotides that is coated by host-cell histones. All polyomaviruses display a similar genome organization . The genome can be functionally divided into three regions with those coding for the early and late proteins being separated by a non-coding region (NCR) that contains the origin of replication and promoters The early region consists of two open reading frames (ORFs), both of which encode the non-structural proteins designated T and t-antigen (T-Ag, t-Ag). The two early transcripts are produced by alternative splicing from a common pre-mRNA prior to viral replication. Because both T-Ag and t-Ag use the same start codon, the N-terminal amino acids of these two proteins are identical. The late region of the BKV genome codes for the

structural proteins VP1, VP2, and VP3 as well as for the agnoprotein (VPx). As with the early mRNAs, late transcripts are generated from a common pre-mRNA by alternative splicing. The major capsid protein VP1 is responsible for the antigenic variability among BKV isolates. In contrast to the generally high degree of similarity of the entire coding region of VP1 (>95% similarity across all BKVs), similarity between amino acid residues 61 to 83 is only 61 to 70%. These latter amino acids probably constitute the epitope responsible for serological subtyping. Four BKV serogroups I–IV are distinguishable that correlate with the division

of BKVs into four major subtypes based on genotyping of this subgenomic region (.Krumbholz, 2008).

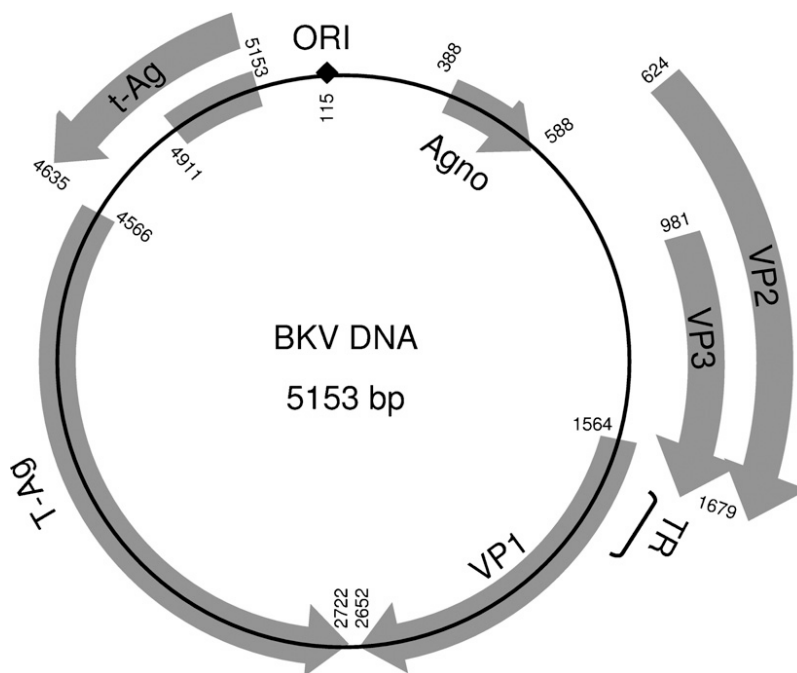


Figure (1) BKV Genome and nucleocapsid (.Krumbholz, 2008).

## **2.4 Virus replication**

The lifecycle of BKV, started by the binding of VP1 to certain sialic acid motifs on N-linked glycoproteins and/or to gangliosides GD1b and GT1b on the cell membrane. After attachment, BKV traverses the cell membrane by caveolae-mediated endocytosis. Caveolae arise from lipid rafts, plasma membrane regions enriched in cholesterol and the aforementioned gangliosides. Next, BKV is transported towards the endoplasmic reticulum via microtubules. Disassembly of the outer capsid is essential for the exposure of VP2 and VP3 which mediate entry into the nucleus via importins. The precise mechanism of capsid disassembly is not known but seems to involve an early acidification step and ultimately leads to cleavage of VP1 molecules and capsid rearrangement. The viral minichromosome consists of circular double-stranded DNA wound around histones. The BKV genome can roughly be divided into three regions depicted schematically: the non-coding control region (NCCR), which contains the origin of replication, a bidirectional promoter-enhancer region and binding sites for host transcription factors; the early region, containing genes coding for the tumour antigen (TA<sub>g</sub>) proteins; and the late region, which contains genes coding for agnoprotein and

VP1, 2 and 3. Counter-clockwise transcription of the early region is the first step of replication and leads to the production of the TAg proteins. Three T antigen proteins are produced: large TAg (LTAg), truncated TAg (TruncTAg), and the small T antigen (stAg). Multiple LTAg molecules form a dodecameric complex that binds to the viral origin of DNA replication. Here it acts like a helicase by opening up and unwinding the DNA to initiate clockwise transcription of the late regions. LTAg was also demonstrated to bind to heat shock proteins, members of the retinoblastoma protein family, and p53, as such driving the cell into the S phase and preventing cell cycle arrest. Knowledge on the function of BKV stAg is limited. In mice, stAg of murine polyomavirus (mPyv) complements LTAg in driving cell cycle progression by several mechanisms, such as activation of the promoter of the proto-oncogene c-myc. TruncTAg results from alternative splicing of the LTAg transcript and its functions remain to be elucidated. Transcription of the late region genes leads to the production of the structural VP1, VP2 and VP3 proteins, as well as the non-structural agnoprotein. Little is known on the functions of agnoprotein but it seems to mediate assembly of BKV virions. JCV agnoprotein was found to inhibit double-stranded DNA repair and may as such increase the production rate of more virulent

mutant viruses. Intranuclear accumulation of daughter virions ultimately results in rupture of cell membranes, thereby releasing virus progeny into the extracellular space. (Van Aalderen *et al*, 2012).

BKV very disseminated in population with a primary infection that appear as pulmonary tract infection or fever then the BKV disseminated to kidney or urinary tract and became latent and the BKV after suppression of immunity and reactivation can lead to a severe disease and the kidney is the major site of BKV associated disease ,BKV replicate in the urogenital tract and present as hematuria , hemorrhagic cystitis , uretic stenosis and interstitial nephritis, progressive replication of BKV in recipient kidney can cause progressive dysfunction and eventually loss of allograft transplanted kidney (Hirsch *et al*, 2002).

## **2.5 Epidemiology and transmission**

BK virus an ubiquitous virus and it infects humans at adulthood, It is not known how this virus is transmitted. It is known, however, that the virus is spread from person to person, and not from an animal source. It has been suggested that this virus may be transmitted through respiratory fluids or urine, since infected individuals periodically excrete virus in the urine. A survey of 400 healthy blood donors was reported as showing that

82% were positive for IgG against BK virus, And with increasing prevalence of allograft rejection and allograft renal dysfunction in recipients has been correlated with new immunosuppressive agents with decline in acute rejection rates in recent years ( Chakraborty, 2013).

## **2.6 Pathogenesis**

polyomavirus (BK and JC) infection are ubiquitous. It is believed that primary infection with these viruses commonly occurs in childhood . Antibodies to one or both viruses are found in sera of about 75% of adults .Human polyomavirus (BK and JC virus) Human polyomavirus BKV and JCV are transmitted by droplets from the upper respiratory tract of the infected persons . Each polyomavirus is limited to specific hosts and cell type within that host. Thus JC and BK viruses are exclusively human viruses which probably enter the respiratory tract and then infect lymphocytes after asymptomatic primary infection, the BK virus establishes latent infection in the kidney in B cells and in monocyte-lineage cells, they may persist in the tubular epithelium of kidney and lymphoid tissue of healthy individuals after primary infection . Both of them establish latent infection and replication is blocked in immunocompetent people, The productive cycle leads to viral



multiplication that ultimately results in death of the host cells , The viruses are also reactivated during pregnancy , although they have got no effect on fetus (Chakraborty, 2013).And in renal transplanted patients BKV one of the important causes of interstitial nephritis and (BKVAN) BK virus associated nephropathy among kidney recipients, BK virus nephritis one of the important cause of renal failure,BK viremia and nephritis are serious problem in renal recipients ,but the exact causes are remain understood (Braham *et al* , 2005).

## **2.7. Human BK virus infections in renal transplant patients**

### **2.7.1 Clinical manifestations**

BK virus appears to be associated with untreated stenosis observed in renal transplant , and also in hemorrhagic cystitis observed in bone marrow transplants(Chakraborty, 2013). The clinical manifestation in renal transplanted varies from viremia asymptomatic state and renal stenosis and nephritis to clinical renal dysfunction and in previous studies show that up to 5% of renal allograft recipients can be affected about 40 weeks post transplantation with decreasing in viral load once they reduce the immunosuppressive drugs(Hirsch *et al*, 2002).

### **2.7.2 About nephropathy associated with polyomaviruses**

Since it was first reported in 1995, nephropathy associated with the polyomavirus type BK (BKV) has emerged as an important cause of allograft failure in renal-transplant recipients. BKV is closely related to another human polyomavirus, JC virus (JCV), which causes progressive multifocal leukoencephalopathy in immunocompromised patients. Infection with either polyomavirus is widespread, as indicated by seroprevalence rates of up to 90 percent worldwide. The risk factors for BKV nephropathy in renal-transplant recipients are not known, but most patients with BKV nephropathy have received newer immunosuppressive drugs such as tacrolimus or mycophenolate mofetil. Because BKV persists in the kidney, the transplantation of organs from seropositive donors into seronegative recipients may lead to BKV nephropathy. According to retrospective studies, BKV nephropathy develops in 1 to 5 percent of renal-transplant recipients, with loss of allograft function occurring in about half the cases. BKV-specific antiviral therapy is not available, but in some cases, BKV replication may be controlled by reducing the level of maintenance immunosuppression, though this change may result in an increased risk of

subsequent rejection. Progression to BKV nephropathy occurs without clinical signs or symptoms, except for increasing serum creatinine concentrations over a period of weeks. Diagnosis of BKV nephropathy is based on the histopathological demonstration of viral alterations that are distinct from signs of rejection in specimens from allograft biopsies. Cells in the urine that have viral inclusions, known as “decoy cells,” are a sign of BKV replication in the renourinary tract but are not a specific marker of BKV nephropathy. BKV replication in the allograft has been correlated with the detection of BKV DNA in plasma by polymerase-chain-reaction (PCR) assay. BKV DNA may serve as a quantifiable surrogate marker of the course of the infection. To determine the association between BKV replication and nephropathy, They conducted a prospective, single-center study of renal-transplant recipients who were receiving immunosuppressive therapy that included tacrolimus or mycophenolate mofeti (Chakraborty, 2013).

## **2.8 BKV and renal transplanted**

The incidence of BKAN has increased from 1% for patients transplanted in 1997 to 5.8% for patients transplanted in 2001. BKAN is an important cause of premature kidney graft loss at the University of Maryland Transplant Program. One-third of the patients diagnosed with BKAN

since 1997 have already lost graft function, and a third of the remaining patients have creatinine levels over 3 mg/dl. We could not determine that a specific immunosuppressive drug increased the incidence of BKAN. Older patients had an increased risk of developing the disease. The histological diagnosis of BKAN was made at a mean time of 14.4 months after transplantation (range 1.2-53 months). BKAN occurred in 4.3% of all patients biopsied during the period described. The diagnosis of BK allograft nephropathy was based on a combination of renal biopsy to demonstrate viral cytopathic changes, urine cytology and quantitative viral load in plasma. A threshold of >10,000 copies of BK virus per ml of plasma is proposed as an indication of BKAN. Following diagnosis of BKAN, patients on a single immunosuppressive drug (FK506, CsA, sirolimus or MMF) in addition to prednisone had less graft loss and higher viral clearance in comparison to patients on prednisone and 2 immunosuppressant drugs (FK506, CsA or sirolimus and MMF). There was no difference in the rate of acute allograft rejection among different immunosuppression reduction protocols. Three patients who lost their grafts to BKAN were retransplanted. For these patients there has not yet been evidence of recurrence of BKAN. After reduction of immunosuppression, the course of BKAN in most patients followed one

of 2 pathways: 1) Clearance of the infection and disappearance of the viral cytopathic changes in biopsies and urine (20%); 2) Persistence of viral replication with continuous associated tubular damage (70%). Renal transplant patients should be routinely screened with urine cytology. The presence of decoy cells in the urine is an indication for quantitative measurement of viral load in plasma. Patients with any evidence of BK viral reactivation should be followed closely. In patients biopsied early due to persistence of BK virus-infected cells in urine, there is a higher rate of conversion from positive to negative urine cytology after reduction of immunosuppression, in other prospective study of polyomavirus type BK replication and nephropathy in renal transplant recipients (Ramos *et al*, 2002)

In a prospective, single-center study, they followed 78 renal-transplant recipients who were receiving immunosuppressive therapy that included tacrolimus (37 patients) or mycophenolate mofetil (41 patients). Urine was tested for the presence of decoy cells at routine visits. BKV DNA was measured 3, 6, and 12 months after transplantation and whenever decoy cells were detected. The viral load in plasma was quantified with the use of a real-time polymerase-chain-reaction method. Renal biopsy was performed if allograft function deteriorated they found that Twenty-three

patients had decoy-cell shedding a median of 16 weeks after transplantation (range, 2 to 69), 10 patients had BKV viremia at a median of 23 weeks (range, 4 to 73), and 5 had BKV nephropathy at a median of 28 weeks (range, 8 to 86). Kaplan–Meier estimates of the probability of decoy-cell shedding, viremia, and nephropathy were 30 percent (95 percent confidence interval, 20 to 40 percent), 13 percent (95 percent confidence interval, 5 to 21 percent), and 8 percent (95 percent confidence interval, 1 to 15 percent), respectively. Antirejection treatment, particularly with corticosteroids, was associated with BKV replication and nephropathy. The viral load in plasma was higher in patients with BKV nephropathy than in those without nephropathy ( $P < 0.001$  by the Mann–Whitney test). BKV antibodies were detected in 77 percent of the 78 patients before transplantation, including 4 of 5 with BKV nephropathy and they concluded that BKV nephropathy in renal-transplant recipients represents a secondary infection associated with rejection and its treatment in most cases possible and could be monitored by measuring the viral load in plasma (Hans *et al*, 2002).

## **2.9 Laboratory diagnosis of BKV**

Electron microscopy , PCR or DNA probe analysis can be done to detect JCV in brain tissue ,Urine cytological test can reveal presence of JC or

BK virus infection both can agglutinate erythrocyte of guinea pig and chicken .JC virus grown only in human fetal cell culture where as BK virus can grow in a wide range in primary and continuous cell culture (Chakraborty, 2013).

IN (BKVAN) BKV DNA PCR analysis is the most widely used blood test for the laboratory diagnosis and the positive results for BKV DNA reflects the dynamics of the disease BKV DNA is typically detectable in urine prior to plasma and may indicate as an impending of BKVAN, in other study show that serum , plasma tissue biopsies and cerebrospinal fluid and urine can be used for detection of BKV for explaining that the persistent presence of BKV DNA in urine detected by PCR has been used to identify the basis of hemorrhagic cystitis in bone marrow transplant patients and definitive diagnosis of nephropathy among kidney transplant recipients has historically required the identification of BKV DNA in a renal biopsy but recently they discovers the detection of BKV in plasma or serum by PCR correlates with renal biopsy and proven the nephropathy (Hirsch *et al*, 2002).

## **2.10 Treatment , Prevention and control**

The reduction of immunosuppressive therapy we found in others study that researcher take a 41 cases of BKV nephritis from 1001 from renal /pancrease transplant recipients and divided the 41 cases to two groups, group 1 from 16 patients that are diagnosed based on renal biopsy alone from January 1996 to august 2001 and group 2 contain 25 patients who diagnosed by quantitative PCR of plasma and biopsy from 2001 to august 2003 after he measured the immunosuppressive drugs and requantitative the group two he found reduction in the viral load after reduction of immunosuupresive therapy (Krumbholz, 2008). And in other study we found he detect the BKV by detection of decoy cells in urine and histological exceed to blo od/ urine PCR detection and he found that infected patients varies from asymptomatic state of viremia and nephritis to clinical renal dysfunction and approximately 30 to 60 % of subjects of BKNV experienced irreversible graft failure and the routine detection of BKV post transplantation by screening of BK viruria and BK viremia prior to the occurrence of nephritis (BKNV) appear to be attractive approaches and they measure the viral load by Quantification of BKV DNA in plasma and serum has shown that BKV viral load is reduced in some patients following reduction in immunosuppression. These



discoveries suggest that a non-invasive procedure may be used for both diagnosing BK virus nephropathy and monitoring the patient's response to therapy. Focus Diagnostics offers both qualitative and quantitative PCR based tests for the detection and quantification of BK virus DNA. The BK Virus DNA assay (Test Code 48900) provides sensitive and specific detection BKV DNA in a variety of specimens including plasma, serum, whole blood and urine. The BK Virus DNA Quantitation assay (test code 47900) is able to quantify BKV DNA in the linear range of 500 to 39,000,000 copies/mL for each of the same specimen types. Although the sensitivity and specificity of PCR has made this methodology a very promising tool in clinical diagnosis, diagnosis of BKV infection should not rely solely upon the result of a PCR assay. A positive PCR result should be considered in conjunction with clinical presentation and additional established clinical tests. A negative PCR result indicates absence of BKV DNA in the sample tested and does not exclude the diagnosis of the infection.

Treatment with antiviral after detection of virus by PCR which is a non-invasive procedure that can be helpful in determining BK virus reactivation disease in bone marrow and renal transplant patients and in monitoring the results of therapy (Hirsch *et al*,2002). And in other study

they suggest that the infected persons treated by reduction of immunosuppressive therapy with antiviral treatment cidovofir and/ or leflunomide for alloimmune activation in renal graft or combination of both can solve this problem (Braham *et al* , 2005).

## **CHAPTER THREE**

### **3. MATERIALS AND METHODS**

#### **3 . 1 Study design**

This a descriptive cross sectional Hospital based study.

#### **3 . 2 Study area**

The study was conducted in EL Gazera Hospital for renal disease, Wad Madeni from April to November 2015 in patients who were suspected of acquiring polyomavirus infection after kidney transplantation were included in this study.

#### **3.3 Study population**

The study was done in adult asymptomatic renal transplanted who visted El Gazera Hospital for renal disease for routine checkup for renal function tests or immunosuppressive drugs monitoring.

#### **3.4 Ethical consideration**

The study was approved by the Ethical Review Committee (ERC) of the Ministry of Health , El Gazera , Sudan , patients were informed verbal consent were obtained from patients.

### **3.5 Data collection**

Through a structured questionnaire , information on age, gender , date of transplantation , date of sample collection , place of sample collection and type of immunosuppressive drugs were recorded.

### **3. 6 Sample collection**

Fifty blood (plasma) and fifty urine samples were collected from asymptomatic renal transplanted patients , collected samples were transported on ice box at the same day of collection to the Department of virology ,Central laboratory ,(The Ministry of Higher Education and Scientific Research).

Blood specimens (5ml) in EDTA, were collected from the cubital vein and then centrifuged at 4000 rpm for 5 minutes to obtain the plasma.

The clear plasma was taken immediately for DNA extraction or stored at -20° C until used .

Urine specimen (20ml) were collected from the patients using sterile wide mouth urine container, and then centrifuged at 3000 rpm for 15 minutes. The deposit was immediately prepared for DNA extraction.

### **3.7.1 DNA Extraction from plasma samples**

Commercial kit (Viral Gene spin) were used for DNA extraction according to the protocol of the manufacture (Viral Gene Spin ,South Korea), Briefly 150 µl of samples were added to 250 µl of the Viral Gene Spin<sup>TM</sup> lysis buffer and incubated at room temperature for 10 minutes then , subsequently resulting similar solution was applied to a column and centrifuged at 13000 rpm for 1 minute , A volume of 500 µl of washing buffer was added for washing and the nucleic acid and centrifuged at 13000 rpm for 1 minute then eluted with 60 µl RNase free water and centrifuged at 13000 rpm for 1 minute and the product stored at -20 until used.

### **3.7.2 DNA Extraction from urine samples**

Seven ml of urine samples were washed by using phosphate buffer saline (PBS), then the samples were centrifuged at 3000 rpm for 10 min to collect the precipitated pellet , Two ml lysis buffer 10 µl proteinase k were added, one ml guanidine chloride and 300 µl NH<sub>4</sub> acetate were added to the each tube and incubated at 37°C overnight .The sample were cooled to room temperature and then 2ml pre chilled chloroform was added, vortexed and then centrifuged at 3000rpm for 5 minutes . The upper layer was collected to a new tube and then 10ml cold absolute

ethanol was added gently shaken and kept at -20°C for overnight. The samples were centrifuged at 3000rpm for 15 minutes, Carefully The supernatant was drained and the tube were inverted on a tissue paper for 5min. The pellet was washed with 4 ml of 70% ethanol, centrifuged at 3000rpm for 15 minutes, The supernatant was poured off and the pellet was allowed to dry for 2 hrs. The pellet was eluted in 200 µl distilled water and stored at -20°C until used (Nishiguchi *et al*, 2002).

### **3.8 Polymerase Chain Reaction(PCR)**

The PCR was performed by processing the extracted DNA from plasma and urine samples with primers that are specific for the BKV (T-gene) gene. The primers used consisted of forward primer: 5'-AGT CTT TAG GGT CTT CTA CC3' (BKV genome1353-1377) and reverse primer: 3'GGT GCC CTA TGG AAC G-5' (BKV 1702-1681). The reaction was performed in 25 µl volume using Solis Bio dyne master mix (Intron, Korea). The volume included: 5 µl master mix, 1 µl forward primer, 1 µl reverse primer, 5 µl extracted DNA and 13 µl distilled water. The DNA was amplified in thermo- cycling conditions using PCR machine Techno (Japan) as follow: initial denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 2 min, with a final extension 72°C for 5 min.

### **3.9 Gel electrophoresis**

Seven  $\mu$ l of the amplified product was subjected to direct analysis by gel electrophoresis in 2% Agarose, the gel was prepared by adding 0.7 g of Agarose to 35 ml 5X Tris Borate EDTA buffer. The product was visualized by staining with 0.15% Ethidium bromide using UV gel documentation system INGeNius. The expected size of BKV(T-gene) amplicon was 176bp.

## CHAPTER FOUR

### 4. RESULTS

BK viral DNA was identified by PCR in 14 (28%) urine specimens and 11 (22%) plasma specimens (Table 1) .

All positive PCR Samples revealed abundant amplicons of 176bp indicate of Large T-gene of the BK virus genome (figure 2).

Cross tabulation of the results indicated no correlation between urine and plasma samples for PCR detection of BKV, P value (0.047) .

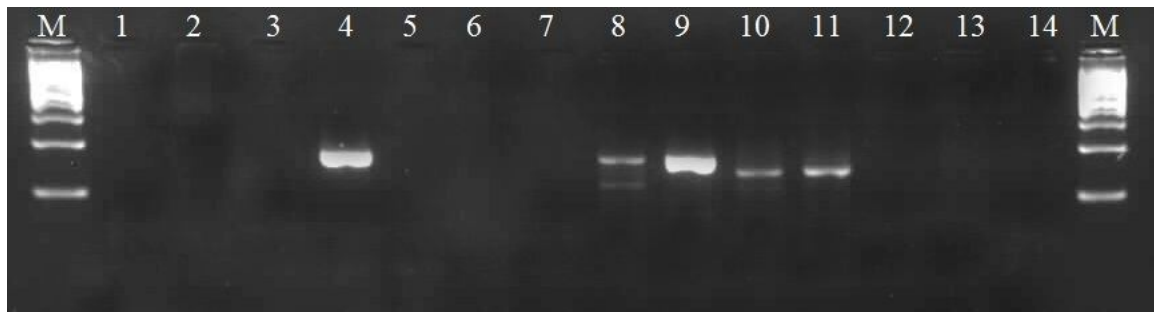
**Table(1) Detection of BKV in urine and plasma samples using PCR in Renal Transplant recipients, EL Gazera state, Sudan, 2015**

<b>Technique</b>	<b>Positive</b>	<b>Total%</b>
PCR (urine)	14(28%)	50(100%)
PCR (plasma)	11 (22%)	50(100%)



**Table(2) Cross tabulation of positive and negative PCR results of the plasma and urine samples in EL Gazera state , Sudan 2015**

Samples		Plasma		Total
		Negative	Positive	
Urine	Negative	29	7	36
	Positive	10	4	14
Total		39	11	50



**Figure (2)** BKV DNA results (176 bp) on 2% agrose gel. Lane 1 shows negative control. Lane 4 shows positive control, lanes. 2,3,5,6,7,12,13,14 show Negative samples and lanes show positive samples 4,8,9,10,11: Positive samples M: 100bp DNA marker.

## **CHAPTER FIVE**

### **5. DISCUSSION, CONCLUSION AND RECOMMENDATION**

#### **5.1 DISCUSSION**

There has been limited investigation in Sudan regarding BKV reactivation in renal transplant recipients with asymptomatic nephritic syndrome . Given the association of BKV reactivation with the use of immune suppressive therapies, we sought to investigate whether BKV reactivation occurred in renal transplant recipients with asymptomatic nephritic syndrome .

Early detection of BKV reactivation in the urine and plasma is a powerful clinical tool for identifying patients at risk for developing BKVN and for monitoring response to therapy (Charles *et al*, 2010)

This study was aimed at establishing the screening diagnostic technique (PCR) for early detection of BKV viruria and viremia. The hypothesis was that BKV viruria is more common detection among asymptomatic renal transplant recipients than viremia.

In this study, 50 renal transplant recipients were investigated for the presence of BKV(50 blood samples and 50 urine samples). The BKV were identified in 14/50 (28%) urine specimens, 11/50 (22%) plasma specimens

using PCR. This finding agrees with the study by (Koukoulaki *et al*, 2008).

This finding can be explained by the fact that the augmented immunosuppressive can lead to continuous viral replication and shedding of the virus in urine, (Koukoulaki *et al*, 2008) . It is important to state that immediately after renal transplantation, there is commonly clinically silent BKV viruria, preceding the development of BKV nephropathy (Kalvatchev *et al*, 2007). .

The presence of circulating virus in plasma appears at late stage and this evidence by only 11 specimens positive in plasma samples. This is associated with active nephropathy because the virions enter the circulation through per tubular capillaries following tubular damage (Kalvatchev *et al*, 2007). Thus demonstrating BKV DNA in blood could be surrogate diagnostic marker to urine PCR for diagnosis confirmation and further patient's monitoring. The small percentage of plasma PCR positivity's, as compared to urine PCR results, shows that limited number of patients could be at potential risk at developing BKV nephritis. It seems that in most of the cases viruria is not linked to distinct pathologic entity, and testing for BKV DNA in blood combined with urine PCR has much higher clinical significance (Kalvatchev *et al*, 2007).

The mean age of our study group was 40 years. This age group is similar to previous work of Daniel in USA in 2005, the mean age of his group was 45 years. This may highlight association between this particular age group and renal transplantation(Daniel, 2005)

Early diagnosis of BKV may allow for medical interventions such a reduction of immunosuppressive therapy which can then cause a stabilization of nephropathy and an improvement of kidney function (Arza *et al*, 2014)

Screening is not cheap, but in cases of BKV nephropathies it is justified by the disease that can be contrasted only by an early diagnosis of viruria and viremia which must be monitored to avoid organ loss.

BKV screening by PCR assays may be clinically useful and non invasive test for detecting concurrent BKVN in renal allograft recipient, in Sudan.

Based on the results of our study and those of above previously studies, we believe that urine is a reliable and in invasive sample for PCR detection of BKV, so can use instead of plasma sample.

In this study, the use of PCR in the detection of BKV among renal transplant patients in Sudan was established. A system in Central Laboratory to monitor BKV infection in renal transplant patients . Such system will enable the prevention and/or minimization of the incidence of BKV infection in renal transplant patients. This study was geared to serve as a baseline for

future plans aiming at introducing the pre-emptive (anti-viral drugs) therapy against BKV in the Sudan.

## **5.2 CONCLUSION**

Presence of BKV was documented in Sudan through the detection of BKV DNA among renal transplant patients in EL Gazera Hospital for Renal Disease. The Results of present study indicated that urine sample can be used for PCR detection of BKV as plasma sample.

## **5.3 RECOMMENDATIONS**

- Further research should be carried out to study the prevalence of BKV in other groups. The risk factors associated with BKV infection should be determine.
- Further research work should be carried out to characterize BKV at molecular level.
- To study disease association with other opportunistic pathogens and the impact of BKV on graft survival, which might in the wider context define additional indication for treatment and monitoring, again data would be best be derived from multicentre studies.

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