Detection of Polyomavirus BK and JC in kidney Transplant Recipients

الكشف عن فيروس تورامي نوع BK و JC عند المرضى زارعي الكلى

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الخلاصة:

الهدف: هذه الدراسة صممت للكشف عن فيروس تورامي نوع (BK, JC) لدى المرضى الذين خضعوا لعملية زراعة الكلى وأ شخاص أصحاء كمجموعة سيطرة .

المنهجية: لقد اجريت هذه الدراسة للكشف عن الحامض النووي (DNA) بعينات من الإدرار التي جمعت من (97) شخصا ادخلوا ضمن هذه الدراسة وهم (72) مريض خضعوا لزراعة الكلى و (25) شخص معافى للفترة من ا يلول 2015 الى اذار 2016، وتم ايضا تصميم وتصنيع (new experimental indirect ELISA kit) يدويا للكشف عن الاجسام المضادة لفيروس JCV نوع (IgM,IgG). ولتقيم وظيفه الكلى تم قياس مستوى اليوريا و الكرياتنين لكل الاشخاص الذين ادخلوا ضمن مجموعتي الدراسة.

النتائج: كل العينات خضعت لفحص البلمرة التكراري (PCR) وقد أظهرت النتائج فروقات معنوية (P<0.01) الموجبة على النحو التالي 2 (8. 2%) في مجموعة المرضى الزارعين للكلى و 4(16.0%) في مجوعة الأشخاص الاصحاء. كما وكانت النتائج الموجبة لكشف فيروس(JCV) بواسطة Nested_PCR 6 من المجموع الكلي للعينات (2 مرضى / 4 اصحاء) (P <0.01)، بينما تم الكشف عن فيروس (BK) بواسطة Conventional_PCR ووقد ظهرت النتائج الإيجابية فقط عند اثنين من المرضى زارعين الكلى ولم يظهر عند اي من الاشخاص الاصحاء. وبينت النتائج فحص الاليزا وجود الجسم المضاد نوع IgM عند 25(25.8%) من مجموع 72 وايضا 8(8.2%) من اصل 25 اصحاء، وبالنسبة للجسم المضاد نوع (IgG) كانت النتائج الإيجابية عند 21(29.2%) من مجموع 72 مريض زارع للكلي ولم تظهر اي نتيجة موجبه عند مجموعة الاصحاء.

التوصيات: التشخيص الناجح يتطلب فحص دقيق مثل استخدام فحص البلمرة التكراري للتحري عن أدنى نسبه للفيروس عند الاشخاص المهيأين للتبرع بالكلى قبل قبولهم كمترعين، وذلك بسبب التأثير الضار لفيروس تورامي الذي قد يتسبب في خسارة الكلى المزروعة.

Abstract

objectives: To investigate the polyomaviruses (BK, JC) in asymptomatic kidney transplant recipients and healthy persons as control. It is one of the first reports on serological detection and molecular characterization that describes the circulation of polyomaviruses (BKV, JCV) have been done in Iraq recently.

Methodology: The present study was designed as prospective case control study was done during the period from November 2015 to August 2016. Total of 97 serum and urine samples were collected randomly from 25 healthy control person and 72 renal transplant recipients, attending Iraqi Renal Transplantation Society. “New experimental indirect ELISA kit" had been designed and synthesized manually for detection Anti-JCV IgM and IgG, Urea and creatinine level had been measured for total study groups for estimation of renal functions.

Results: All of the samples were submitted to a Nested-PCR. A significantly difference had been seen ($P < 0.01$) of PyV was found in renal transplant patients 2(2.8%) in comparison to the control group 4(16.0%). JCV was detected in.6 (6.2%) samples (2 patients/ 4 controls).by conventional PCR, BKV was detected in 2(2.8%) of renal transplant group and not detected with any one of control group. result for IgM revealed positivity for 25(25.8%) out of 72cases of renal transplant subject and 8(8.2%) person out of 25control groups. for IgG the result show that 21(29.2%) out of 72 cases of renal transplant Subjects have a positive result for IgG but this antibody was not detected in the control.

Recommendations: Success diagnosis required sensitive assay typically Nested-PCR to detect extremely low viral load present in potential kidney donor before access as a donor، Because of the potentially harmful effects of polyomavirus which may cause loss of the transplanted kidney

Key words: Polyomavirus, Kidney transplant recipients

Introduction

BK polyomavirus (BKV), JC polyomavirus (JCV), belong to family Polyomaviridae. In the last 10 years, improved immunosuppression drugs have decreased the rates of acute rejection in kidney transplantation but have also led to the emergence of polyomavirus-associated nephropathy (PVAN). Initially, lack of recognition or late diagnosis of PVAN resulted in rapid loss of graft function in more than 50% of patients [1].

Serological evidence of past BKV exposure has been seen to reach 90% in adolescents and adults around the world and seems not to have changed since the first discovery of BKV in the 1970s. Similar to earlier studies, Hirsch et al., [2] found 80% seropositivity in a prospective study of patients with kidney transplantation.

Fecal-oral, oral and respiratory routes of transmission have been proposed for different human polyomaviruses.

Primary infections with BKV and JCV are typically subclinical or linked to mild respiratory illness and are followed by viral dissemination to the sites of lifelong persistent infection. The major sites of persistence for both BKV and JCV are the cells of the kidney and urinary tract [3]. Following primary infection, the virus can usually persist in the uroepithelial cells, oli-godendrocytes, and blood mononuclear cells lifelong [4].

Since all viruses can be detected at increased frequencies in blood and lymphoid tissues during host immunosuppression [5], it is likely that hematolymphoid cells can carry or harbor polyomaviruses. JCV and BKV establish persistent infections in renal tissue and virus is shed into the urine. Reactivation of JCV and BKV, as reflected by increased viruria, occurs during immunosuppression, but only BKV levels correlate with the degree of immunosuppression [6].The tissue tropism and mechanism of viral latency and persistence remains poorly defined, but likely varies within the viral family, partially accounting for the differences in disease observed upon reactivation [7]. Infection occurs in 2/3 of renal transplant recipients in the first year after transplantation and is associated with morbidity, mortality and graft-loss after transplantation [8]. Too little immunosuppression is associated with rejection and graft
loss and too much immunosuppression is associated with infection and malignancy. Thus, a delicate and immeasurable balance exists between these two extremes [9].

Methodology

Study Population:

This study was conducted on healthy volunteer subjects with no symptoms of urinary disease, whom did not undergo renal transplantation or any transplantation surgery and were considered as the control group included a twenty fife (25) volunteers of both males and females adults and (72) seventy tow asymptomatic renal post transplanted recipients, visiting the Department of kidney Transplantation in “AL-Kheial Hospital”, Baghdad, Iraq for routine checkup for renal function tests or immunosuppressive drugs

monitoring, patients' age were ranged from (16 to 62) years old, These patients were evaluated for relevant clinical data including age, gender, date of transplantation and type of drugs.

Specimen Collection:

Second morning urine samples and serum were collected from asymptomatic renal transplanted recipients and healthy volunteers. Urine specimens (20 ml) were collected using sterile wide mouth urine container, and then centrifuged at 3000 rpm for 15 minutes. All specimens of urine and serum were stored at -20 C°.

Methods:

- **DNA Extraction from Urine Samples:**

primers display nucleotide sequences that avoid annealing with other polyomaviruses than BKPyV and JCPyV. PCR was carried out based on Bofill-Mas *et al.* [11]. Figure (1) Show DNA bands on gel by electrophoresis.

In this study the manual method for extraction of DNA from urine was used according to [10].

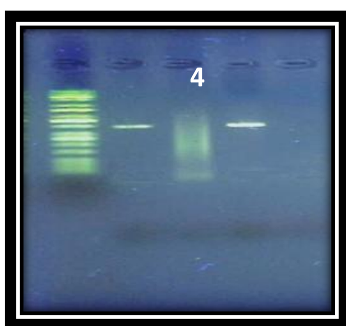
- **Estimation of Concentration and purification of viral DNA:**
The concentration of DNA was estimated by using Nano drop because the purity of the extracted DNA was based on the ratio A260/A280 nm.

- **Polymerase Chain Reaction:**

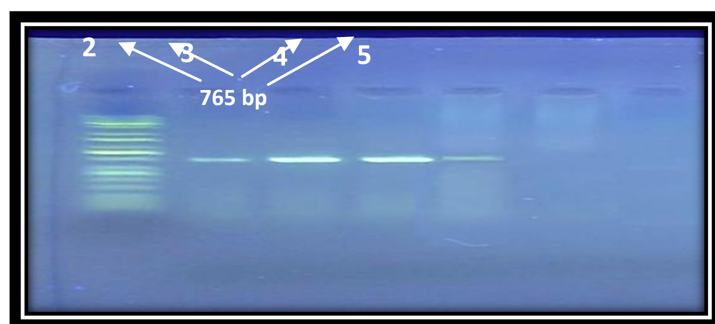
1. **Nested-PCR Assay:**
   - **First reaction to Detect JCV/ BKV polyomavirus**

Single set of primers was used to amplify both BKV and JCV **VP** gene. The primer sequences used as follows: **BJ2** (PF50-AACATTTTCYCCTCCTG-30) and **BJ1**(PR 50-TATTGCMCCAGGAGGT-30). Both

The reactions were performed under the following cycle conditions: 5 min at 94°C followed by 35 cycles of 1 min at 94°C, 1 min at 41°C, and 1.5 min at 72°C. A final elongation period of 5 min at 72°C was applied.



(A)



(B)

Figure (1): (A) Detection of polyomavirus DNA by Nested- PCR in urine samples of renal transplant subjects. Lane1 (10000bp) DNA-marker, Lanes 2, 4 (amplification of polyomavirus DNA).
(B) Detection of polyomavirus DNA by Nested- PCR in urine samples of control persons. Lane1 (10000bp) DNA-marker, Lanes 2-5 (amplification of polyomavirus DNA).

- **Second Reaction to Discriminate BKV and JCV:**

Two specific PCRs were developed using the product of the first PCR as a template and primers pairs previously described by Bofill-Mas *et al.* [(11).] The JCPyV specific DNA primers were: **JLP16** (PF 50-TAAAGCCTCCCCCCCAACAGAAA-30) and **JLP15** (PR 50 ACAGTGTGGCCAGAATTCCACTA CC-30).

The first cycle of denaturation was carried out for 4 min at 94°C.The conditions for the 29-cycle amplification were as follows: denaturing at 92°C for 60 s, annealing at 45°C for 60 s, and extension at 72°C for 75s. All amplifications were completed with a 4-min, 72°C extension period.

2. **Conventional PCR Assay to amplifying BKV LT-Ag:**

The third PCR reaction was carried out by using the primers that designed by NCBI/ primer blast External forward primer

BKLTF$_1$:5′AAACAAAGCAGGTGTC TTG 3′ External reverse primer **BKLTR1:**5′TTCCAGTACAGCATTT CCA 3′**.**

The reactions were performed under the following cycle conditions: 5 min at 94°C followed by 40 cycles of 1 minute at 94°C, 1 minute at 45°C, and 1 minute and 15 second at 72°C. A final elongation period of 5 minute at 72°C was applied.

The amplified PCR products were analyzed on 1.5% agarose gel electrophoresis, and visualized using UV translators (MismajorSince \Germany)

ELISA:

New Experimental indirect ELISA kit synthesized manually in research unit and manufacturing of alternatives in health and medical collage/Baghdad for detection anti-JCV IgM and IgG, by fixation VP1 surface antigen which is JCV Polyomavirus Major Capsid VP1 full length protein (abcom \USA).

- **Determination of Urea and Creatinine concentration:**

Urea and Creatinine levels were measured for total study groups in this study 97(100%). By using accent 200 auto analyzer, ACCENT-200 UREA diagnostic kit for determination of urea concentration. And using accent 200 auto analyzer, ACCENT-200 CREATININE diagnostic kit for determination of Creatinine concentration.

Results:

Table (1): Association of JCV Presence with Studied Groups

Study Groups	JCV Nested PCR - Result		Total
	Positive	Negative	
Cases	2 2.8%	70 97.2%	72 100%
Controls	4 16.0%	21 84.0%	25 100%
Total	6 6.2%	91 93.8%	97 100%
(*) C.C.= 0.233 with P.value = 0.018 < 0.05 (S) (Control : Cases) – Odds Ratio (Pos.) (1 : 0.15) With 95% C.I. (0.026 - 0.877)			

(*) S: Sig. at P<0.05; C.C.: Contingency Coefficient , CI: confidence Intevval, %:percent, P:probability level

JCV: JC- PolyomaVirus , PCR: Poly Chain Reaction

By Nested-PCR positive result for JCV **vp1** gene were detected at 6(6.2%) samples (2 patients \ 4 control). The results revealed significant difference between studied groups at (P<0.05) with weak positive responses in cases group compared with controls group, and that are proved throughout an odds ratio.

Table (2): Association of BKV Presence with Study Groups

Study Groups	BKV PCR -Results		Total
	Positive	Negative	
Cases	2 2.8%	70 97.2%	72 100%
Controls	0 0.0%	25 100%	25 100%
Total	2 2.1%	95 97.9%	97 100%
(*) C.C.= 0.085 with P.value = 0.401 > 0.05 (NS) For cohort groups = Cases Value=(1.357) With 95% C.I. (1.203 - 1.530)			

(*) NS: Non Sig. at P>0.05; C.C.: Contingency Coefficient, CI: confidence Intevval, %:percent, P:probability level

BKV: BK- PolyomaVirus , PCR: Poly Chain Reaction

By conventional PCR method The L-TAg of BKV was detected in 2(2.8%) out of 72(74.2%) renal transplant group.

Table (3): Association of IgM Detection with Study Groups

Study Groups	IgM ELISA Levels		Total
	Cases	Controls	
Positive	25 34.7%	8 32.0%	33 34.0%
Negative	47 65.3%	17 68.0%	64 66.0%
Total	72 100%	25 100%	97 100%
(*) C.C.= 0.025 with P.value = 0.805 > 0.05 (NS) (Control : Cases) – Odds Ratio (Pos.) (1 : 1.130) With 95% C.I. (0.428 - 2.983)			

(*) NS: Not Significant. at P>0.05; C.C.: Contingency Coefficient, CI: confidence Intevval, %:percent, P:probability level

IgM: Immunoglobulin M, ELISA: Enzyme linked immune sorbent assay.

Serum samples were analyzed for detection anti-JCV IgM antibodies by ELISA. Table (4-6) demonstrates that results.

Table (4): Association of IgG Detection with Study Groups

Study Groups	IgG ELISA Levels		Total
	Cases	Controls	
Positive	21 29.2%	0 0.00%	21 21.6%
Negative	51 70.8%	25 100%	76 78.4%
Total	72 100%	25 100%	97 100%
(*) C.C.= 0.296 with P.value = 0.002 < 0.01 (HS) For cohort groups = Cases Value=(0.671) With 95% C.I. (0.573 - 0.785) IgG: Immunoglobulin G ELISA: Enzyme linked Immune Sorbent Assay.			

Serum samples were analyzed for detection anti-JCV IgG antibodies by ELISA. Table (4-8) demonstrates that results.

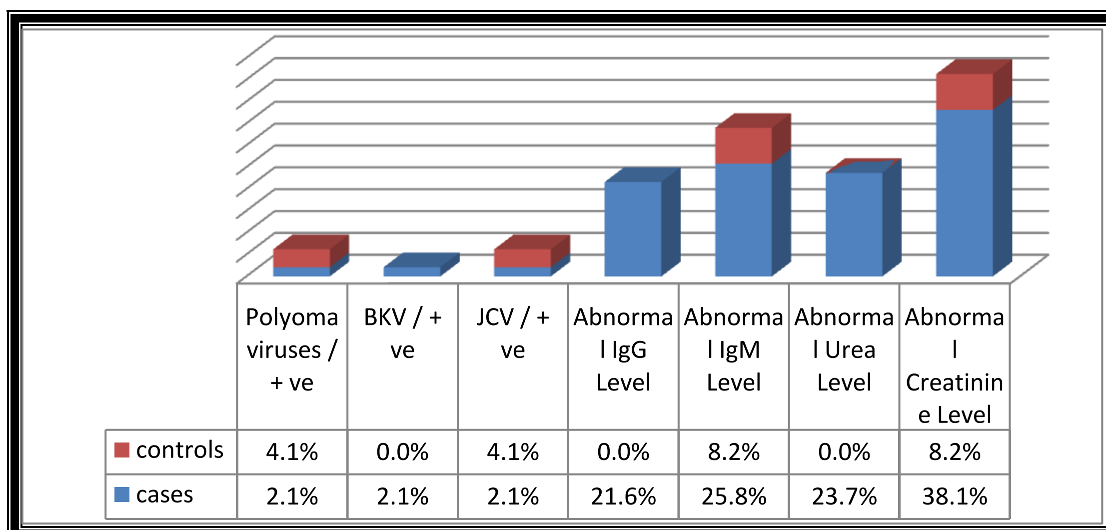


Figure (2): The positive and abnormal levels of studied Clinico - patholological factors distribution among study groups (Cases and Controls)

Discussion:

In Iraq, active kidney transplantation program was started in 1973 at Al-Rasheed Military Hospital; and since then, renal transplantation (RT) is being successfully done at several centers in Iraq [12]. Very few studies are conducted for the detection of viral infections or reactivation in Iraqi (RT) recipients using PCR [13].

Post-renal transplantation, immunosuppressant toxicity, and infection are the principal worries for both patients and doctors. One of the foremost reasons of graft injury post renal transplantation is polyomavirus infection [14].

The two human polyomavirus species **BK** and **JC**, can cause interstitial nephritis and lead to graft failure in renal transplant recipients [15]. The prevalence of polyomavirus BK load was more elevated in the first year compared with the second year post-transplantation follow-up visits [16].

In current study the results on the finding of JCV and BKV DNA from the urine samples of both healthy persons group (n =25) and renal transplant recipients RTRs patients (n =72) were of the 97(100%) urine samples examined, 6(6.2%) and 2(2.8%) were positive for JCV and BKV DNA, respectively.

Studied groups in present study were classified according to gender. Out of 72 renal transplant group, 55 (76.4%) patients were males and 17 (23.6%) were female with male to female ratio (3.2:1). For the control group 16 (64.0%) females. with male to female ratio (1.7:1). according to sex there was no significant difference (P> 0.05).

Human polyomavirus is associated to different clinical manifestations among (RTRs) patients. The repetitive use of urine cytology and PCR technique on urine and plasma is a valuable tool for the rapid and sensitive detection of reactivated BKV in asymptomatic recipients [17].

Like BK, JC polyomavirus infection continues hidden in the urinary tract. BKV and JCV-DNA Are often noticed in urine samples. [18] A revealed the prevalence rates of BK and JC viral infections in kidney transplant recipients are between 10% and 30% [18]. Studies displayed that (1-7%) of (RTRs) undergoing (PVAN), and the incidence seems to be rising [19]. Discriminating BKPyV from JCPyV is vital clinically because JCV infrequently causes (PVAN) and is not correlated with loss of transplanted kidney [18], In immunosuppressed renal transplant receivers, the incidence of the secretion of the BKV polyomavirus in urine is elevated, and is found in 23 to 57% of patients [20]. And large forthcoming studies probing the communication of JCV with BKV reactivation and serology in renal transplant recipients are missing.

The low prevalence of **BKV** and **JCV** in samples from the renal transplant group was probably due to the small number of samples size, and conclusions about these results are premature. The low rates of Polyomavirus infection reported here may represent an epidemiological feature of the virus in Iraq, which may be related to the population density and environmental conditions, which are of paramount importance for JCV and BKV transmission. JC reactivation in renal transplant recipients is less expected to be donor derived but, as is the case for BK infection, recipient JCV sero-positivity does not defend against JCV reactivation. Based on many studies, The result recommend that JC viruria in the nonappearance of JC viremia marks acceptable however not over immunosuppression, possibly resulting in to a lesser acute rejection rate. Screening for polyomavirus viruria with viremia might deliver a way to screen and regulate immunesuppression [21]. Qualitative PCR technique is supposed to remain delicate sufficient to sense all those patients with positive who result are at risk of developing (PVAN) [22].

The Frequency of JCV in present study 6(6.2%) (2 patients of 72 \ 4 of 25 control) was positive result for JCV **vp1** gene by Nested PCR The results revealed significant difference between study groups (P<0.05). This result similar to that reported in [23]. JCV was detected in seven patients (6.8 %) (31/1487 whole blood samples) at a median time of 35-139 days post-transplant.

Results revealed the prevalence of JCV was 3.9% (4/102) in the Chronic Renal Disease group, 3.4% (2/59) in transplant recipients, and 20.1% (27/134) for the control group[24].

These differences in rate of virus could be attributable to different study designs and follow-up sampling timings also different methodology and small sample size may be the possible reasons.

In current study only two (2.8%) renal transplant recipient were have positive DNA-uria for both BKV and JCV as a Co-infection. Previous studies report the occurrence of co-infection being (4%) in all renal transplant recipients studied [25].

One case of combined BKV and JCV PVAN has been histologically documented and reported [26].

From the above we can see there is wide variability in the incidence of studied groups this is may be as a result to the very low viral-load in possible renal donors. Other probable variables involve the "transient" nature of infection, the occurrence of plasma "inhibitors", the short life-span of infected (mononuclear cells), and variances in "susceptibility" of variety mononuclear fractions [27].

In healthy immunocompetent persons, JCV shedding in the urine can be detected with the rate growing with age [28]. Like BK, JC polyomavirus after primary infection stay dormant in renal tissues [29]. And B-lymphocytes [30].

Recommendations: Success diagnosis required sensitive assay typically Nested-PCR to detect extremely low viral load present in potential kidney donor before access as a donor. Donor's samples should have been interesting to analyze beside to recipient's samples because of the potentially harmful effects of polyomavirus which may cause loss of the transplanted kidney.

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