Rate of Polyoma Virus Allograft Nephropathy among Iraqi Kidney Transplant Patients

A Single center study

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ABSTRACT

Background: Polyoma virus is a ubiquitous human virus with a peak incidence of primary infection in children 2 to 5 years of age and a seroprevalence rate of more than 60% to 90% among the adult population worldwide.

Objectives: To study the rate of polyoma virus allograft nephropathy among Iraqi kidney transplant patients.

Methods: All recruited patients were considered from nephrology and transplant outpatients' Baghdad Clinic Medical City, from January-December 2015. The patients with graft dysfunction were recorded on an already prepared data sheet for the type of induction therapy antithymocyte globulin or basiliximab, type of immunosuppressive regimens, renal function test by estimation of Glomerular filtration rate by the Chronic Kidney Disease Epidemiology Collaboration equation, renal Doppler ultrasound, urine for decoy cell and renal graft biopsy for light microscopy and immunohistochemistry stain.

Results: This cohort study enrolled 162 cases, 97 males and 65 females, patients with the age ranged from 20 to 60 years. There was high incidence of polyoma virus allograft nephropathy among patients receiving antithymocyte globulin (28.6%) as compared to the basiliximab group (3%). There were increasing incidence of BK virus nephropathy among patients taking calcineurin inhibitors + steroid + mycophenolate mofetil. The difference was statistically significant (p=0.012). There was an increasing incidence of decoy cells in the urine of patients with polyoma virus allograft nephropathy (100%).

Conclusions: There was a high incidence of polyoma virus allograft nephropathy among transplant recipient's patients. Histological features of polyoma virus allograft nephropathy were reliable diagnostic tools and should be considered in every renal transplant patient.

Keywords: Graft rejection, Kidney transplantation, Polyomavirus.

Iraqi Medical Journal Vol. 68, No. 2, July-Dec 2022; p. 102-108.

Polyoma virus is a DNA virus that is a member of the polyomavirus family. It shares >70% homology to the other polyomaviruses such as John Cunningham virus (JC)[1].

Polyoma virus hominis, also known as (BK virus nephropathy) is currently a major cause of allograft failure in renal transplant recipients[2]. After primary infection, polyoma virus preferentially establishes latency within the genitourinary tract and frequently is reactivated in the setting of immunosuppression[3].

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In renal transplant recipients, polyoma virus is associated with a range of clinical syndromes including asymptomatic viruria with or without viremia, ureteral stenosis and obstruction, interstitial nephritis, and polyoma virus allograft nephropathy[4]. During the last decade, BK nephropathy has emerged as an important cause of allograft dysfunction after renal transplantation[5].

The highest prevalence of polyoma viruria and viremia occurs at 2 to 3 months and 3 to 6 months, respectively[6]. The risk for development of polyoma viremia increases when urine viral load is greater than 10^4 copies/ml, whereas polyoma virus allograft nephropathy is unusual in the absence of polyoma viremia[7].

Polyoma virus associated nephropathy (PVAN) commonly presents with asymptomatic rise in serum creatinine during the first post transplantation year. However, BK nephropathy may occur as early as the first week (where it resembles delayed graft function DGF in the first week)[8] to as late as 6 years after transplantation[7].

Polyoma viral inclusions in renal tubular cell nuclei and occasionally in glomerular parietal epithelium[9]. There are variable degrees of interstitial mononuclear inflammation, often with plasma cells, degenerative changes in tubules, and focal tubulitis, which may mimic acute rejection[10].

Polyoma virus associated nephropathy often is associated with very focal and sharply demarcated areas of tubulointerstitial inflammation, corresponding to foci of viral infection[11-13].

In late PVAN, few characteristic intranuclear inclusions are seen, and the histologic changes may be indistinguishable from chronic rejection[14].

A histological classification system for PVAN based on the degree of active inflammation, acute tubular injury, and tubulointerstitial scarring may have prognostic significance[15].

Urine cytology for decoy cells and quantitative determinations are surrogate markers for the diagnosis of PVAN[16-18].

The detected virus could originate anywhere along the urinary tract[19]. Therefore, transplant kidney biopsy remains the gold standard for diagnosing PVAN[20]. However, in renal biopsy specimens it is often difficult to differentiate between the tissue effects of viral pathology and changes caused by acute cellular rejection[21].

The lack of specific targeted therapies has prompted a preemptive active surveillance strategy with routine screening intervals post transplantation for viral replication using polymerase chain reaction assays[22].

Saturation of the IL-2R α subunit persists for up to 120 days after daclizumab induction and 25 to 35 days after treatment with basiliximab. No major side effects have been associated with anti-CD25 therapy[23].

Antithymocyte globulin (ATG) is a potent immunosuppressive agent, the lack of specificity coupled with marked immunosuppression increases [24].

The aim of this study is to study the rate of polyoma virus allograft nephropathy among Iraqi kidney transplant patients.

Methods

The study was conducted in the nephrology and renal transplant center, Medical City in Baghdad. The period of data collection was from January-December 2015.

This cohort study enrolled 162 transplant recipient patients within the first year post renal transplantation presented to the center with renal dysfunction. All recruited patients had their ages, gender and case histories recorded on an already prepared data sheets.

The patients were recorded on an already prepared data sheet for the type of induction therapy (ATG or basiliximab), type of immunosuppressive regimens, renal function test by estimation of glomerular filtration rate (GFR) by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, renal doppler ultrasound, urine for decoy cell and renal graft biopsy for light microscopy and immunohistochemistry stain. After following up the patients during one year we categorize the patients into two groups. Group A; all patients with graft dysfunction with evidence of PVAN by histopathological examination. Group B; all patients with graft dysfunction without evidence PVAN by histopathological examination.

Inclusion criteria for patients: All patients with graft dysfunction and candidate for graft biopsy.

The CKD-EPI equation for estimation creatinine clearance is more accurate than

the Modification of Diet in Renal Disease (MDRD) study equation across a wide range of characteristics, including age, sex, race, body mass index, and presence or absence of diabetes or history of organ transplantation. With the CKD-EPI equation, it is now possible to report estimated GFR across the entire range of values without substantial bias[6].

The drugs of the patients were recorded and defined as following: Induction therapy: Basiliximab versus ATG.

Regimens that use in patients and control cases in the transplant center:
Group 1: Tacrolimus 0.1 mg/kg / mycophenolate mofetil 1-2 gm/day / prednisone 1-0.25 mg /kg. Cyclosporine 4 mg/kg/ mycophenolate mofetil 1-2 gm/day/ prednisone 1-0.25 mg/kg.
Group 2: mTOR inhibitors (sirolimus tab) 0.1 mg/kg / prednisone 1-0.25 mg/kg/ mycophenolate mofetil 1-2 gm/day.
Group 3: Calcineurin inhibitors (tacrolimus, cyclosporine)/ prednisone /azathioprine 1-2 mg/kg.

All patients were sent for decoy cells. It can be identified by urine cytology by using a specific stain which is Papanicolaou stain[25,26]. The Papanicolaou stain includes three steps: 1; hematoxylin for nuclear staining. 2; orange stain for keratin and 3; eosin for cytoplasm[26].

Graft biopsy: The aim is to identify acute rejection, and therefore the diagnosis can be made on a formalin-fixed sample alone for light microscopy. If vascular rejection is suspected, a snap frozen sample for C4d immunostaining should also be obtained[6]. The characteristic intranuclear polyomavirus inclusions tubulointerstitial nephritis is suggestive of BK nephropathy[27].

Protocol for pathological examination: Histological samples obtained through kidney biopsy were analyzed by optical microscopy (OM), immunofluorescence. The samples (only one biopsy fragment per patient) have been harvested with GBL 16 G guillotine needles, rapidly placed in saline, and divided as follows: 2 mm of tissue ends were separates with a sharp razor blade (IF) and placed in 4% buffered glutaraldehyde, while the Middle part was placed in a cryostat for frozen sections.

The histological stages of polyomavirus nephropathy: Stage A (Early); Viral activation in cortex and /or medulla with intranuclear inclusion and/or positive immunohistochemistry or in situ hybridization, Minimal tubular epithelial cell lysis. No denudation of tubular basement membrane (TBM). Stage B (Florid); Marked viral activation in cortex and/or medulla. Marked virus induced tubular epithelial cell necrosis/lysis and associated denudation of TBM. Interstitial inflammation (mild to marked). Interstitial fibrosis and tubular atrophy (minimal to moderate ≤50%. Stage C (late); Viral activation in cortex and /or medulla. Interstitial fibrosis and tubular atrophy>50%. Tubular epithelial cell necrosis/lysis and tubular basement membrane denudation Interstitial inflammation (mild to marked).

Analysis of data was carried out using the available statistical package of SPSS-20 (Statistical Packages for Social Sciences- version 20 Statistics) for determination of statistical significance among different variables. A descriptive statistic like mean together with analytic statistics, have been done when appropriate. A p-value of less than 0.05 was considered as significant and calculated by a method of Pearson Chi-square equation.

Results

This cohort study enrolled 162 kidney transplant recipients with renal dysfunction within the first-year post transplantation. Male patients were 97 while female patients were 65, the age ranges from 20 to 60 years, with male to female ratio of 1.4:1. The rate of BK virus nephropathy was 7% of total transplant patients in this study.

As seen in table 1, there were no statistically significant differences between male and female patients with BK virus nephropathy as compared to patients without BK virus nephropathy. At the same time there was no statistically significant

difference between transplanted patients older than 55 years as compared with those younger than 55 years, (p=0.9).

There was a high incidence of BK virus nephropathy among patients receiving ATG as compared to the basiliximab group; the difference was statistically significant (p=0.0001), (Table 2).

There were increasing incidence of BK virus nephropathy among patients taking (CNI+ Steroid + MMF) group (1) patients, the difference was statistically significant (p=0.012), (Table 3).

There was an increasing incidence of decoy cells in the urine of patients with BK virus nephropathy and the difference was statistically significant, (Table 4).

The histological features of BK virus nephropathy as viral inclusions was increasing incidence of among patients with BK virus nephropathy as compared with cases with tubulitis and interstitial and tubular atrophy the difference was statistically significant (p=0.001). There was no statistically significant difference among transplanted patients with tubulitis and IFTA cases, (p=0.2, 0.3 respectively), (Table 5).

Table 1: Age and gender distribution among patients with BK virus nephropathy groups and patients without BK virus nephropathy groups.

Variables	Patients with BK virus nephropathy		Patients without BK virus nephropathy		P value
	No.	Percentage	No.	Percentage	
Male	7	7.2	90	92.8	0.9
Female	5	7.7	60	92.3	
Age < 55 years	4	7.4	50	92.6	1
Age > 55 years	8	7.4	100	92.6	

Table 2: Induction therapy for renal transplant patients with BK virus nephropathy group and patients without BK virus nephropathy group.

Induction Therapy	Patients with BK virus nephropathy		Patients without BK virus nephropathy		Total		P value
	No.	Percentage	No.	Percentage	No.	Percentage	
Basiliximab	4	3	130	97	134	100	0.0001
ATG	8	28.60	20	71.40	28	100	
Total	12	7.40	150	92.60	162	100	

Table 3: Distribution of immunosuppressant drugs in studied patients with BK virus nephropathy groups and patients without BK virus nephropathy groups.

Immuno-suppressant drugs	Patients with BK virus nephropathy		Patients without BK virus nephropathy		Total		P value
	No.	Percentage	No.	Percentage	No.	Percentage	
Group 1 (CNI +steroid + MMF)	8	5.8	131	94.2	139	100	
Group 2 (mTOR +steroids MMF)	2	33.3	4	66.7	6	100	
Group 3 (CNI +steroid + AZA)	2	11.8	15	88.2	17	100	0.012

Table 4: Urinary Decoy cells distribution among patients with BK virus nephropathy group and patients without BK virus nephropathy group

Decoy cells	Patients with BK Virus nephropathy		Patients without BK virus nephropathy		Total		P value
	No.	Percentage	No.	Percentage	No.	Percentage	
No	8	5.10	149	94.9	157	100	0.0001
Yes	5	100	0	0	5	100	
Total	12	7.4	150	92.6	162	100	

Table 5: Histological features distribution between patients with BK virus nephropathy group and patients without BK virus nephropathy group.

Variables	Patients with BK virus nephropathy		P value
	No.	Percentage	
Viral inclusion	12	100	0.001
Tubulitis	10	10.4	0.2
IFTA	7	10	0.3

Discussion

The incidence coincides with Li RM, Mannon RB, Kleiner D et al[28], and coincide with Al-Obaidi et al, who found in their study that the incidence of biopsy proven PVAN polyomavirus allograft nephropathy was 5,1%[29].

There was no statistically significant difference among transplanted patients between males and females. Also, there was no statistically significant difference among transplanted patients with age older than 55 years as compared with younger than 55 years, (p=0.9). These results coincide with Fernando et al study[30], which show no statistically significant differences between the experimental and control groups for the sex ratio (p = 0.523), mean age (p = 0.648) and age distribution.

There was high incidence of polyomavirus virus nephropathy among patients receiving ATG as compare to basiliximab group[30], the difference was statistically significant (p=0.0001). These results coincide with Sonia C et al[31] and Brennan D et al and Binet I et al[32].

ATG is a potent immunosuppressive agent, act on T- and B-lymphocyte which lead to induces a rapid lymphocytopenia by several mechanisms including: complement dependent cytolysis, cell-dependent phagocytosis, and apoptosis. This marked immunosuppression increases the risk of polyoma virus infection[33-37].

That there was increasing incidence of polyomavirus nephropathy among patients taking (CNI + Steroid + MMF) group (1) patients, the difference was statistically significant (p=0.012). These results coincide with Hardinger Ketal study[38]. It hypothesized that tacrolimus-MMF create a permissive immunosuppressive environment for polyoma virus replication. Also, coincide with Mengel et al who found that use of tacrolimus in combination with MMF increased the risk of PVAN[39].

There was an increasing incidence of decoy cells in the urine of patients with PVAN and the difference was statistically

significant. This result coincides with Zeljko V et al[40]. This could be explained by the polyoma virus can proliferate within the nuclei of renal tubular and urothelial cells producing viral cytopathic effect manifested with nuclear enlargement and basophilic intranuclear inclusions that lead to formation of Decoy cells in urine[41,42].

The histological features of polyomavirus nephropathy in form of viral inclusions were relatively high in patients with polyomavirus nephropathy as compared with tubulitis and IFTA, the difference was statistically significant (p=0.001, 0.01) respectively. Biopsies showing lesser degrees of renal scarring at the time of diagnosis were associated with, more likely, resolution of the infection, in response to decreasing immunosuppression. Initial immunosuppression reduction consisted of a decrease in the target level of tacrolimus from 11-15 mg/ml to 5-7 mg/ml and cyclosporine A from 150-200mg/ml to 75-100mg/ml. dose of MMF was reduced to 1 gm/day, plus low dose prednisolone, in addition ciprofloxacin given to some patients with more advanced tubulointerstitial atrophy, active inflammation and higher creatinine level at diagnosis correlated with worse graft outcome. Due to the focal nature of PVAN, correlation of biopsy results with viruria and viremia are required for diagnosis. The type of inflammation in PVAN was almost mononuclear, consisting of plasma cells and lymphocytes.

There was no statistically significant difference among transplanted patients with tubulitis as compared with IFTA cases, (p=0.2).This result coincides with Daniel L. Bohl and Daniel C[43], also coincides with Drachenberg CB, et al[44]. The latter identified three patterns of histological injury: Pattern A with viral cytopathic changes and almost normal parenchyma, pattern B with viral cytopathic changes and significant inflammation and tubulitis with varying degrees of interstitial fibrosis and tubular atrophy, and pattern C with diffuse fibrosis and tubular atrophy associated with some inflammation and very little viral cytopathic changes. Pattern B was divided into B1, B2 and B3 based on the degree of interstitial fibrosis and tubular atrophy. In their evaluation, they noted that pattern A was associated with 15% risk of graft loss, pattern B was associated with 25-75% risk of graft loss and pattern C was associated with >80% risk of graft loss[45].

In conclusions; the incidence of BK virus nephropathy is significant. The histological feature of BK virus nephropathy is a reliable diagnostic tool and should be considered in every renal transplant patient. We should avoid routine use ATG drugs in low-risk patients. Decoy cells are a marker of BK virus nephropathy. Patients using drugs regimen including calcineurin inhibitors prednisone, mycophenolate mofetil is high risk for developing BK virus nephropathy. The pathological changes can be patchy in nature and a renal allograft biopsy can miss the diagnosis of PVAN.

Acknowledgments: The authors acknowledge the contribution and cooperation of the patients enrolled for the study.

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