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Detection of Bk Polyomavirus Antigen in Patients with Acute Lymphoblastic Leukemia

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Abstract

Background: BK polyomavirus that is acquired in early childhood as BKPyV is near ubiquitous in adults with a seroprevalence of >80%, it has been found as the main cause of hemorrhagic cystitis in hematopoietic stem cell transplantation patients, due to immunosuppression regimen that lead to the activation of the virus from the latency status and lead to increased viral shedding in urine (viuria).

Objective: To investigate the frequency of BK Polyomavirus antigen excretion in urine of children with acute lymphoblastic leukemia with and without chemotherapy and compare it with normal controls.

Patients and Methods: A case-control study conducted from December 2021 to May 2022 in Baghdad, Iraq on leukemia patients of Central Pediatrics Hospital (Al-Eskan). Urine samples and urine sediment smears were collected from 60 acute lymphoblastic leukemia patients. And compared with 60 apparently healthy age and sex-matched children, BK polyomavirus antigen in urine was detected using Enzyme-linked Immunosorbent Assay and urine cytology were Pap stained for the detection of decoy cells (DCs).

Results: Positive BKPyV antigen in urine was seen in 55 (91.7%) of Acute lymphoblastic leukemia patients and 39 (65.0%) in controls (p=<0.001) and all the patients were decoy negative. There was no significant effect of the positivity of antigenuria on neither leukocytes level nor on the occurance of relapse in leukemia patients.

Conclusion: The very high frequency of BKPyV in the urine signifies the importance of reactivation of this virus in ALL patients with and without chemotherapy.

Keywords: Acute lymphoblastic leukemia, BK Polyomavirus, Antigenuria

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Introduction

Polyomavirus BK virus (BKPyV), a small, non-enveloped, double-stranded DNA virus that is ubiquitous and species specific. It is a member of the polyomaviridae family [1]. How BKPyV transmitted is unknown however the urine-oral route appears sensible given that it is mostly detectable in urine and the kidney [2]. BKPyV binding to cellular receptors such as polysialylated gangliosides and BKPyV hemagglutination of human type

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O red blood cells]3-5[as it has been proposed that multiple BKPyV subtypes/serotypes bind to a different spectrum of cell surface receptors, resulting in different cellular tropisms [6].

Based to reports, between the ages of 5 and 10 years old, up to 90% of the normal population might become seropositive for BKPyV]7[. The BK virus infects the renal epithelium and stays latent until immunosuppression causes reactivation [8]. By using techniques now used in the clinical laboratory, BKPyV can be detected in the urine after reactivation [8]. The occurrence of renal diseases such hemorrhagic cystitis, ureteral stenosis, and nephropathy can be related to BKPyV viruria [8]. Under immunocompromised circumstances, such as solid or bone marrow organ transplantation and using immunosuppressive therapies, latent BKPyV infection may become active. In contrast to kidney transplant recipients, who are more likely to experience renal failure, individuals who have undergone bone marrow transplants are more likely to get hemorrhagic cystitis (HC) [1].

In childhood acute lymphoblastic leukemia (ALL), an aggressive form of cancer, an excessive number of immature lymphocytes (a type of white blood cell) are produced by the bone marrow. In a healthy child, the bone marrow makes blood stem cells (immature cells) that become mature blood cells over time. The quantity of stem cells that develop into lymphoblasts, B lymphocytes, or T lymphocytes in a child with ALL is overwhelming. They are also referred to as leukemia cells. Infection-fighting abilities of these leukemia cells are less effective than those of healthy lymphocytes [9].

In immunocompromised patients, this virus is known to develop decoy cells, which may be detected in a urine sample utilizing Papanicolaou staining [10]. Clinically significant decoy cells can be employed as a prognostic marker for pathologies such polyomavirus BK-induced nephropathy and hemorrhagic cystitis in any circumstance when immunosuppression is present [11,10]. Given the scarcity of data about the prevalence of BKPyV infection in children with ALL [10]. In order to investigate into the urine excretion of BKPyV and its potential connection to the disease or as a result of chemotherapy-induced immunosuppression, which may worsen ALL, we conducted this study on children with ALL [11,12].

Regarding BKPyV several Iraqi studies have conducted on this virus in renal transplant recipient patients (immunocommpromised) such as “Detection of BK polyomavirus using real time pcr and urine cytology in 99 renal transplant recipients” [13] in the objective to detect the virus in renal transplant patients using molecular and cytological parameters and “Molecular and Immunological Study for Detection of BK Human Polyoma Virus in Patient with Hemorrhagic Cystitis in some Iraq People ” [14] in objective using molecular parameter for detection of the virus and immunological parameter to detect CD4, CD8 levels in patients with Hemorrhagic Cystitis.

While The objectives of this study is to investigate the frequency of BK Polyomavirus excretion in the urine of children with acute lymphoblastic leukemia with and without chemotherapy and compare

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it with normal controls. And to compare viral antigen level in urine, patients' leukocytes levels and relapses.

Patients and Methods

A case-control study conducted In Central Pediatrics Hospital (Al-Eskan) during the period from from December 2021 to May 2022, urine samples were collected from a total of sixty 60 ALL children (20 children newly diagnosed with ALL and from 40 children on chemotherapy for ALL) (group 1) and (60) apparently healthy age and sex-matched children (from colleagues' and relatives' children) were enrolled in this study as controls (group 2). An informed consent was obtained from the parents of all the patients and normal controls enrolled in this study.

Urine cytology: For decoy cell screening, 10 ml of urine was centrifuged in white cap tubes for 10 minutes at 3000 rpm. After discarding the supernatant, the sediment was re-suspended in the remaining urine. Two slides were prepared and stained using the Papanicolaou procedure for each patient, and they were then looked at under a light microscope. A cut-off level of 10 DCs/slide is regarded to be a decoy positive for DCs quantification [15].

Sandwich-ELISA technique was used for detection of human polyomavirus BK antigen (Cat. No. SL2698Hu Sunlong Biotech, China) in urine samples.

Statistical Analysis

Microsoft Excel 2016 and the statistical package for social sciences (SPSS) 20.0 were used to perform the statistical analysis for this case-controlled prospective study. The median and 5–955th percentile of the numerical statistics were given. Count and percentage were used to describe categorical data. To calculate the level of association between variables, the chi-square test was used. While the Kruskal-Wallis and Mann-Whitney U tests are used to compare numerical data between two or three groups, respectively. The minimum threshold of statistical significance that is generally recognized is below or equal to 0.05. [16,17].

Results

The current study showed that (91.7%) of leukemia patients were positive for BKPyV antigenuria as compared to (65%) positive cases in control (p=<0.001) Table (1), and there is significantly higher number of positive cases for BKPyV antigen in urine in ALL patients who were under the age of 9 years (p= 0.001) Table (2) and who were males ALL patients (p=0.001) Table (3). While BKPyV antigen in urine positivity had no significant effect on leukocyte levels of the patients (p=0.9) Table (4), there was no significant correlation between BKPyV positivity and frequency of relapses (p=0.366) Table (5).

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Table (1): Number of cases positive for BKPyV antigen in study groups

		Study groups		**P value**
		Leukemia	Control	
BKPyV antigen	**Positive**	55 (91.7%)	39 (65.0%)	**<0.001****
	Negative	5 (8.3%)	21 (35.0%)	
Total		60	60	
		100.0%	100.0%	

Table (2): The relation between the number of positive cases of BKPyV antigen and age of patients

Age groups			Study groups		Total	P value
			Leukemia	Control		
< 9 years	BKPyV antigen	Positive	47 90.40%	31 62.00%	78 76.50%	**0.001**
		Negative	5 9.60%	19 38.00%	24 23.50%	
	Total		52 100.00%	50 100.00%	102 100.00%	
≥ 9 years	BKPyV antigen	Positive	8 100.00%	8 80.00%	16 88.90%	**0.294**
		Negative	0 0.00%	2 20.00%	2 11.10%	
	Total		8	10	18	
			100.00%	100.00%	100.00%	

Table (3): The relation between the number of positive cases of BKPyV antigen and sex of patients

Sex			Study groups		Total	P value
			Leukemia	Control		
Females	BKPyV antigen	Positive	13 81.20%	12 75.00%	25 78.10%	**0.5**
		Negative	3 18.80%	4 25.00%	7 21.90%	
	Total		16 100.00%	16 100.00%	32 100.00%	
Males	BKPyV antigen	Positive	42 95.50%	27 61.40%	69 78.40%	**<0.001**
		Negative	2 4.50%	17 38.60%	19 21.60%	
	Total		44 100.00%	44 100.00%	88 100.00%	

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Table (4): BKPyV antigen in urine positivity effect on leukocytes level

Study groups			Leukocyte Level			Total	**P value**
			Leukopenia	normal	leukocytosis		
Leukemia	BKPyV antigen	Positive	24 92.30%	7 87.50%	24 92.30%	55 91.70%	**0.900**
		Negative	2 7.70%	1 12.50%	2 7.70%	5 8.30%	
	Total		26	8	26	60	
			100.00%	100.00%	100.00%	100.00%	

Table (5): The relation between the number of positive cases of BKPyV antigen and relapse

		Relapse		**P value**
		Yes	No	
BKPyV antigen	Positive	5 100.0%	30 85.7%	**0.366**^{NS}
	Negative	0 0.0%	5 14.3%	

Discussion

To the best of our knowledge, this is the first research detecting urine BKPyV excretion in children in Iraq receiving chemotherapy and newly diagnosed with acute lymphoblastic leukemia (ALL) as very limited studies has been done regarding this virus in ALL patients.

The result of viral antigen detection by ELISA was higher among patients with ALL for the first group as compared with normal controls second group (p=0.001).

This is supported by the observation of having a BKPyV urine viral load peak is significantly higher in ALL patients than in controls [18, 19]. As in controls (second group) levels of BKPyV loads are detected in 33 subjects (55.0%), this is supported by the fact that it is widespread in the general population with a seropositivity rate of >90% by early school-age years [20]. Also, in otherwise healthy individuals, HPyVs can reactivate and cause asymptomatic viruria [21,22]. Urban sewage thus typically becomes contaminated and provides higher virus titres [23].

As for the antigenuria of BKPyV, the results of this study showed that 55 leukemic patients out of 60 (91.7%) were positive which is significantly higher than controls as only 39 subjects (65.0%) were positive (P<0.001). As compared to older studies using dot ELISA technique was applied for direct detection of BK virus in clinical urine samples. The polyethylene glycol precipitated urine samples that were free of cell debris were spread on nitrocellulose paper during the assay. A monoclonal antibody against the BK virus was used to identify it, and immunoperoxidase staining was used to identify the complex. Well-defined dark blue spots suggested a positive response. The 110 urine samples analyzed yielded 31 positive dot ELISA results and 79 negative ones [24].

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BK Polyomavirus was investigated for its potential role to trigger cancer in humans by the International Agency for Research on Cancer (IARC) in 2012, BKPyV was found to really be "possibly carcinogenic to humans" [25]. Also, in a research involving 43 children with acute lymphoblastic leukemia who were hospitalized to the pediatric oncology unit in Egypt, Polyoma BK was the virus that was most frequently found (51.2%)]26[. The large T-antigen (T Ag) and small t-antigen (tAg), which are the two viral oncoproteins that produce them, have the ability to modify the normal cell cycle, which ultimately leads to cell immortalization and neoplastic transformation [27].

In vitro and in neonatal hamsters, rats, and mice, the oncoprotein Tag is assumed to be the motivating factor behind the transformation of BKPyV laboratory infection, resulting in carcinogenesis or transformation [28-30]. Only BKPyV and JCPyV are recognized as being likely to cause human malignancies and are categorized as 2B carcinogens among the known HPyV members (other than MCPyV) (possibly carcinogenic to humans). It is obvious that thorough research is required to understand the molecular processes underlying viral oncogenic activity and identify a virus as the etiological cause [31]. BK Polyomavirus has been associated in several case studies for bone and brain cancer, Kaposi sarcoma, and bladder and prostrate carcinomas [32-35].

Or since the viral load is significantly higher than normal controls so the virus activation could be a result of the disease development as ALL patients are Immunocompromised or with disturbed immunity that cause the reactivation of the virus and its' shedding in urine [36, 37]. Since BKPyV nephropathy often affects people with impaired immune systems. Chermotherapy may also suppress the immune system. Similar to the most recent case in an adult with CLL, which was the first documented case of native kidney BK virus nephropathy, there is a reported case of native kidney BK virus nephropathy in a child with ALL without any form of transplant [38].

The results of this study showed significantly higher number of positive cases for BKPyV antigen in urine in ALL patients who were under the age of 9 years (p value = 0.001) table (2) and whom where males (p value = 0.001) table (3). As it has been established that urinary excretion of BKPyV is more in children under the age of 9 years, this is supported by the results of a study of "age-related Urinary excretion of BK Polyomavirus by nonimmunocompromised individuals" that in the 0- to 9-year-old group, the urinary detection rate for the BKPyV was relatively high (24%) but declined in the 10- to 19- and 20- to 29-year-old groups [39].

Among the most important risk factors is the leukocyte level for the patients groups and its effect on the presence of antigenuria, however the results of this study indicates that there is no significant effect of BKPyV on the leukocyte level among the groups (p= 0.900).

Leukemia patients are immunocompromised either as a result of the disease, which involves the clonal expansion of undifferentiated, improperly functioning

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lymphoid progenitors. They invade extramedullary regions, peripheral blood, and bone marrow [40]. Or immunocompromised as a result of immune suppression caused by intensive chemotherapy. Patients with weakened immune systems are more prone to virus infections or viral reactivation [41], reactivation of latent viruses either alone or in combination can also have bad consequences [42]. Human herpes family and polyoma family viruses are the most prevalent latent viruses that can become activated in an immunocompromised status]43, 44[. When the host immunity is overly weakened, the virus reactivates, resulting in viral replication, tubular cell lysis, and viruria [45].

Conclusions

The very high urinary shedding of BKPyV antigen in ALL patients as compared to controls indicates a risk of BKPyV urinary infection among those patients, however the absence of decoy cells in the urine might be a good indicator of the benign effect of the virus especially in the absence of hematuria or renal impairment.

Recommendations

The level of BKPyV antigen could be checked by ELISA in the urine of ALL patients and the level of immune suppression in leukemic children on chemotherapy must be monitored to prevent the activation of BKPyV replication and subsequent infection.

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Ethical clearance: Ethical approval was obtained from the Institutional Review Board (IRB) of College of Medicine, Al-Nahrain University (No.22 in 17th November, 2021).

Conflict of interest: Nil

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الكشف عن مستضد فيروس بي كي التورامي في المرضى الذين يعانون من سرطان الدم الليمفاوي الحاد

صفا حسن علي[1] , سوسن ساطع عباس[2] , الاء حسين علي[3], اسماء باقر العبيدي[4]

الملخص

خلفية الدراسة: فيروس بي كي التورامي المكتسب في الطفولة المبكرة واسع الانتشار في كل مكان عند البالغين الذين لديهم معدل انتشار مصلي أكثر من 80٪ ، وقد وُجد على أنه السبب الرئيسي لالتهاب المثانة النزفي لدى مرضى زرع الخلايا الجذعية المكونة للدم ، بسبب نظام كبت المناعة الذي يؤدي إلى تنشيط الفيروس من حالة الكمون ويؤدي إلى زيادة إفراز الفيروس في البول (فيوريا).

اهداف الدراسة: لفحص وتيرة إفراز مستضد فيروس بي كي التورامي في بول الأطفال المصابين بابيضاض الدم الليمفاوي الحاد مع العلاج الكيميائي وبدونه ومقارنته بالاطفال الطبيعيين.

المرضى والطرائق: دراسة حالة وضبط أجريت في الفترة من كانون الأول (ديسمبر) 2021 إلى أيار (مايو) 2022 في بغداد ، العراق على مرضى سرطان الدم في مستشفى الأطفال المركزي (الإسكان). تم جمع عينات البول ومسحات الرواسب من 60 مريضاً بسرطان الدم الليمفاوي الحاد. وبالمقارنة مع 60 طفل يبدو أنهم يتمتعون بصحة جيدة (الأطفال الاصحاء يتطابقون مع الجنس والعمر مع المرضى)، تم اكتشاف مستضد فيروس بي كي التورامي في البول باستخدام الفحص المناعي المرتبط بالإنزيم وعلم الخلايا البولية تم تلوينها بالكشف عن خلايا الطعم. (DCs)

النتائج: شوهد مستضد BKPyV إيجابي في البول في 55 (91.7٪) من مرضى ابيضاض الدم الليمفاوي الحاد و 39 (65.0٪) في المجموعة الضابطة ($p = <0.001$) وكان جميع المرضى سلبيين من ناحية وجود خلايا الديكوي. لم يكن هناك تأثير معنوي لإيجابية البيلة المستضدية لا على مستوى الكريات البيض ولا على حدوث الانتكاس في مرضى اللوكيميا.

الاستنتاجات: إن التردد العالي جداً لـ فيروس بي كي التورامي في البول يدل على أهمية إعادة التنشيط لهذا الفيروس في جميع المرضى الذين يعانون من العلاج الكيميائي وبدونه.

الكلمات المفتاحية: ابيضاض الدم الليمفاوي الحاد ، فيروس بي كي التورامي ، انتجينيوريا.

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