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Molecular and Immunological Study for Detection of BK Human Polyoma Virus in Patient with Hemorrhagic Cystitis in some Iraq People

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Abstract

Hemorrhagic cystitis (HC) is considering one of the important complications in lower urinary tract hematuria, hemorrhage and dysuria are most common symptom .In hemorrhagic cystitis, short-term hematuria can also be seen in bladder infections as a result of viral infection. Viral cystitis represents another form of non-bacterial urinary tract infection affected adult and children. Human polyomavirus BK type I and V, Adenovirus types 21 and 11 and CMV viruses also can be cause of hemorrhagic cystitis. The aim of the present study work was to find out the relation of polyoma virus BKVS with acute syndrome consists of hematuria and studying the sequence and molecular genotyping of such virus with certain immunological factors among infected patients. This study was performed on two hundred and thirty bloody urine and blood specimens were collected from patients that were selected to eliminate other causes of hematuria, the patients group comprised 170 male and 60 female age group 6 -65 years. Patient samples (Urine and serum) were collected for detection of Human polyoma virus (BKVS) by using conventional PCR technique and immunological parameters. All bloody urine cultured on ordinary media to differentiate between viral and bacterial infection. The result of current study was found that 154 samples (67%) have positive bacterial culture which excluded and the other 76 samples(33%) give negative bacterial culture were included as suspected a viral cause of hematuria. All negative culture cases was classified as 4(5.26%) patients with glomerulonephritis ,15 (19.73%) chronic renal failure,14(18.42%)urinary tract infection ,27(35.5%) kidney transplantation ,16(21.5%) cystitis. The age range distribution mostly in age group more than 50 years at 21 (27.6 %) as higher percent in comparison with other age groups as well as the male percentage higher than female. All urine patients 76 with 25 urines samples of control were analyzed by Convention PCR for detection BVS. It was found only 9(11.8%) as a positive result. There were different immunological parameters used to evaluate function immune system such as IFN-α, IFN-ɤ, TNF-α, CD4 and CD8, to maintain the disease status. The result of IFN-α IFN-ɤ, CD4, CD8 showed a higher significant level in comparison with control samples at P value (< 0.05), while the ‘result of TNF-α showed no significant difference in comparison with control. It is noted that the result of CD4 and CD8 in relation to age group showed that the age > 50 years have higher level of infection than other. The percentage of BKVS is more dominant in adult age in both sexes, mostly at age more than 50 years. Bioinformatics was used for specific primers designed from the data base sequence

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information, for capsid protein genes (vp1, and agno) genes in (NCBI) and Sequencing of DNA And Sequencing analysis were used to diagnosis genes of BKVS .

Conclusion: This study showed that the detection of Human polyoma virus in bloody urine could be applicable in hospitals laboratories by rapid decony cell. It reported the application of qPCR assay for rapid specific and highly sensitive for confirmative detection of BKVS as causative agents in hemorrhagic cystitis. The elevation level of immunological factors(CD4,CD8,TNF-α,IFN-α and IFN-ɤ) act as monitor the immune response to effectiveness of BK virus on Hemorrhagic cystitis patients by ELISA assay. Sequencing and phylogenetic analysis reported that Baghdad and Al-Najaf population are infected with hemorrhagic cystitis induced by BKVS is more predominantly with newly genotype.

Key words : BK viruse,Haemorrhagic cystitis, PCR and Immunological parameter

Introduction

Hematuria and irritative voiding considered featurestic symptoms for lower urinary tract Hemorrhagic cystitis. viral infection is recognized by the entity of distinguishable viral cause with infectious manifestation .Hemorrhagic cystitis occur due to injury of the vessels and bladder's urothelium by microorganism , medication, rays, disorder or poison (1,10).It is defined by injury and hemorrhage from the urothelium, which lead to hematuria and dysuria as a consequence for certain radiotherapy drugs typically cyclophosphamide or ifosfamide cause damage to the bladder, a clotting disorder that leads to bleeding. It can also be a late effect occurring years after therapy of pelvic radiation (12, 13). The symptoms of lower urinary tract infection include hematuria, genital or lower abdominal pain, urgency, frequency (secondary to inflammatory response and irritation of the bladder wall), pyuria and hematospermia. In rare instances of prostatic abscess, the obstructive voiding symptom and urinary retention can be found (14). Viral cystitis represents another form of non-bacterial urinary tract infection affecting adult and children. Polyoma virus BK, Adenovirus types 11 and 21, Cytomegalo virus, and herpes simplex viruses. Immunosuppressed patient undergoing kidney or bone marrow transplantation, or those receiving chemotherapy are especially susceptible to viral cystitis, including those caused by BK polyomavirus Cytomegalovirus and Adenoviruses 21, 35, and 11 are associated with glomerulonephritis and pyelonephritis patients (21). The occurrence of HC considered the dependent variable and the following factors were entered as covariates: age of the patients, the source of renal(donor),duration of disease and State of disease (acute or chronic).It is a distinct clinical disease that has been associated with various predisposing factors such as drug, environmental toxins, radiation, infections(bacterial, viral) and other systemic conditions(18).In particular HC is linked to direct bladder mucosal injury caused by irradiation and chemotherapy during treatment for cancers and autoimmune diseases. It is also recognized as an important complication after hematopoietic stem cell transplantation (HSCT) and renal transplantation (16). The diagnosis of HC is based on the clinical history, physical examination and the exclusion of alternative causes of painful hematuria. Its manifestations vary from microscopic hematuria (mild) to severe bladder hemorrhage leading to clot retention and urinary tract obstruction (3). The pathogenesis of HC has not been elucidated. This is especially true for HC that occurs after renal transplantation. In particular BK (HBKVS) during renal transplantation is the commonest and most

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consistent risk factor for HC, as it is almost in variably present in the urine of patients with this disorder. However, the virus can also be identified in the urine of patients without HC as well as in healthy immunocompetent individual (8). The pathogenesis link between HBKVs and HC remains enigmatic and as a result, BKPyV is set to remain an important source of morbidity in These patient populations, since no specific antiviral treatment is available for BKPyV infection there is difficult to treat complication for this disease(4). BK polyomavirus (BKPyV) are a significant cause of morbidity and can cause mortality in all age groups. BKPyV infected and replicate number of different cell types including Human renal epithelial cells, human fibroblasts, fetal neuronal cell lines, endothelial cells, epithelial cells from sub-mandibular and parotid salivary glands (9). Hemorrhagic cystitis is a serious complication and the presence of blood in the urine in patients receiving these medications requires prompt treatment. Your care team will take steps during treatment with these medications to protect the bladder (5).Lower urinary tract infections (UTIs) are common among the general population and are most often caused by bacterial pathogens.Viruses are an uncommon cause of UTIs in an immunocompetent host; however, viruses are increasingly recognized as the cause of lower UTI, especially hemorrhagic cystitis, among immunocompromised patients. BK virus, adenovirus, and cytomegalovirus are predominant pathogens involved in hemorrhagic cystitis after stem cell and solid organ transplantation (8, 19). BK polyoma virus (BKPyV) is a significant cause of morbidity and can cause mortality in all age groups. It is difficult to treat complication for this disease BKPyV infected and replicate in several body cell (6, 11, 13).It is difficult to observe and detect the viruses even with the best light microscope due to the small size of virus. In addition, it requires a long duration that range from several weeks to one month. It is considered useless to give patients medication to control the viral infection (12). The most preferred method for BK virus detection is by urinary PYV aggregates ("polyoma virus-haufen" test), urinary decoy cells, serological, genomic amplification (quantitative or qualitative) by molecular biology and Immunohistochemistry on renal biopsies biology (5, 7, 18).Diagnosis of viral UTI is more challenging because viruses are small organisms, and they cannot be visualized with even the best optical microscope. The culture of viruses may take up to 14 to 28 days, and often it is too late to treat a patient with disseminated multi organ viral infections at that time thus, molecular and immunofluorescence techniques are used more commonly. The reliability of diagnosis depends On adequate technique, obtaining and transporting the specimen as well as technique of detection (4). Clinicians should be familiar with commonly used methods of virus detection by culture, urinary PyV aggregates("polyomavirus-haufen" test), urinary decoy cells, serologic no commercial reagents exist for the detection of circulating BKPyV-specific antibodies genomic amplification (quantitative or qualitative) by molecular biology and Immunohistochemistry on renal biopsies biology and (13,23).BKPyV genomic DNA has been detected in many different tissues renal epithelia,urothelium, prostate gland, salivary glands brain, and lymphocyte although renal epithelial cells appear to constitute the main reservoir of persistently infected cells. (15).

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Materials and Methods

Patients:

This study was performed on two hundred thirty bloody urine and blood specimens were collected from 230 selected patients to eliminate other causes of hematuria depending on the diagnosis and opinion the Urologist doctor, the patients group comprised 170 male and 60 female. The age of the patients were ranged between 6 to 65 years, who attending to different hospitals in Baghdad and Al-najaf governorate include Baghdad Medical city Teaching Hospital, Al Sadr Teaching Hospital, during period between March 2016 to February 2017. Questionnaires were used to obtain information from the patients itself and parents or guardians accompanying the patients to hospital .Information included signs and symptoms of illness (hematuria slight pink to frank bright red blood with or without blood clots, dysuria (painful urination), burning with urination. Urinary frequency, urinary urgency, urinary incontinence (involuntary loss of urine, etc.) exclusion and induction criteria.

Collection of samples:

Two hundred thirty urine samples varied in volume from 25 ml to 60 ml per patient were collected in disposable sterile containers and centrifuged at 3500 rpm for 30 minutes then examined under light microscope to determination grade of hematuria (mild, moderate and sever) and divided to three parts and distributed in Eppendorf tubes to perform bacterial culture by Inoculation urine samples on to culture media (blood agar and MacConkey agar). Than incubated at 37ºC for 48 hours. After that, the negative bacterial culture urine samples were used molecular assay for detecting BK Human polyoma virus in urine patients and control by conventional PCR.

BK Extraction of DNA from urine

Viral DNA was extracted from frozen urine samples by using Accuprep ®Genomic DNA extraction kit (Bioneer. Korea) **.**Transferred 200µl of urine to sterile 1.5ml micro centrifuge tube and done according to the instruction of the manufacture company **.**The purified DNA was eluted in 100 ul elution buffer provided with kit and store at -20℃**.** The extracted DNA was checked by using Nano drop spectrophotometer.

BKV PCR primer

In this research, they are supplied in National Center Bio- Informatic Gene-Bank for Bioneer in South Korea as shown in the following Table (1).

Journal of University of Babylon for Pure and Applied Sciences, Vol. (28), No. (2): 2020
Online ISSN: 2312-8135, Print ISSN: 1992-0652

Table (1): BK virus detection by PCR primers (design primers)

Primer	Sequence	Size
FK1VP1F	GGGGGATCCAGATGATAACC	528
FK1VP1R	GGAACA TTTTCCCCTCCTGT	
Agno F	CCATGGTTCTGCGCCAGCTG	202
Agno R	CTAGGAGTCTTTTACAGAGTCT	

DNA from urine samples by using specific qPCR kit for BK virus. They are from Bioneer/ South Korea and supplied as lyophilized from which they were dissolved in Tris-EDTA(TE)to give concentration 100µM(stock) depending on manufacture instruction as stock solution (100µm).working solution Primer(10µM) was prepared by diluted the stock (10µm) with TE buffer (90µm).

BK PCR Mix preparation

Optimization of polymerase chain reaction was accomplished after several trials. Thus the following mixture was according to information of (Bioneer company/Korea) as in the following Table (2).

Mixture solution	Volume
Master mix	5 µL
DNA template	3.6µL
Forward primer	0.7µL
Reverse primer	0.7µL
Deionidied water(d d water)	10 µL
Final volume	20 µL

PCR master mix preparation

PCR master mix was prepared by using (AccuPower PCR PreMix Kit) and this master mix done according to company instructions as following table (3):

Journal of University of Babylon for Pure and Applied Sciences, Vol. (28), No. (2): 2020
Online ISSN: 2312-8135, Print ISSN: 1992-0652

Table 3: PCR master mix preparation

PCR master mix	Volume
DNA template	5 µl
PCR water	13 µl
VP1 & agno gene Reverse primer(10 p mol)	1 µl
VP1& agno gene Forward primer(10pmol)	1 µl
Total volume	20 µl

After that, these PCR master mix component that mentioned above placed in standard AccuPower PCR PreMix kit that containing all other components which needed to PCR reaction such as (Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0,KCl, MgCl2,stabilizer, and tracking dye). Then, all the PCR tubes transferred into Multi spin vortex centrifuge at 3000rpm for 3 minutes. Then placed in PCR thermocycler (Mygene . Bioneer . Korea).

PCR Thermocycler Conditions

Polymerase Chain Reaction thermocycler conditions by using conventional gradient PCR thermocycler system as following table (4):

Table 4: PCR thermocycler conditions

PCR step	Tem	Time	Repeat cycle
Initial	95 °C	4 min.	1
Denaturation	95 °C	30 sec.	40
Annealing	44 °C	30 sec.	
Extension	72 °C	45 sec.	
Final extension	72 °C	10 min.	1
Hold	4 °C	Forever	_
Total	42 cycle		

Journal of University of Babylon for Pure and Applied Sciences, Vol. (28), No. (2): 2020
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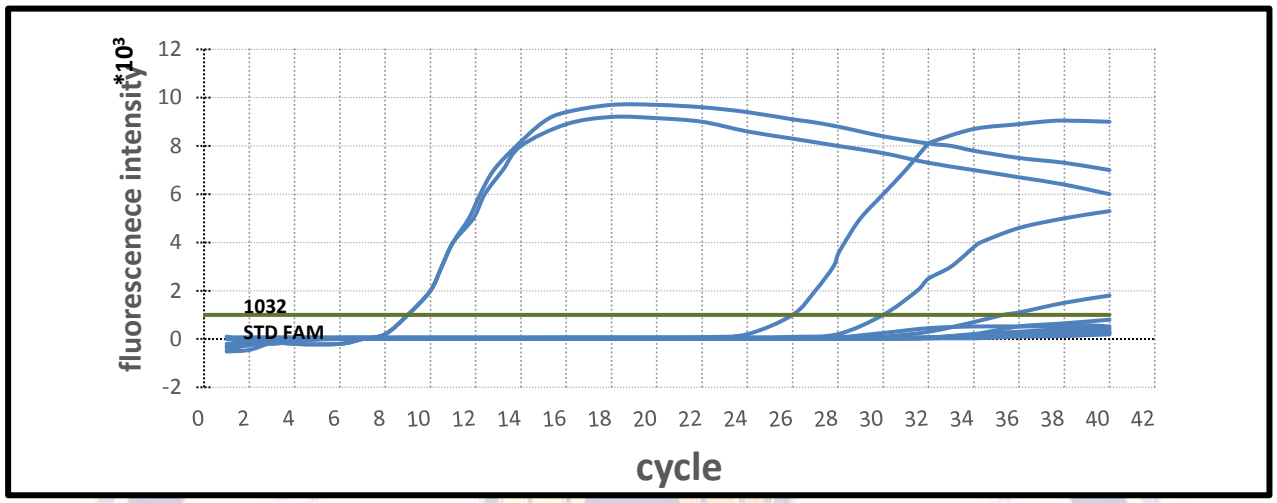

Figure (1): vp1 Bk virus amplification chart according to RT-PCR in positive and negative hematuria patient samples by using Taq Man probe (FAM dye) reaction.

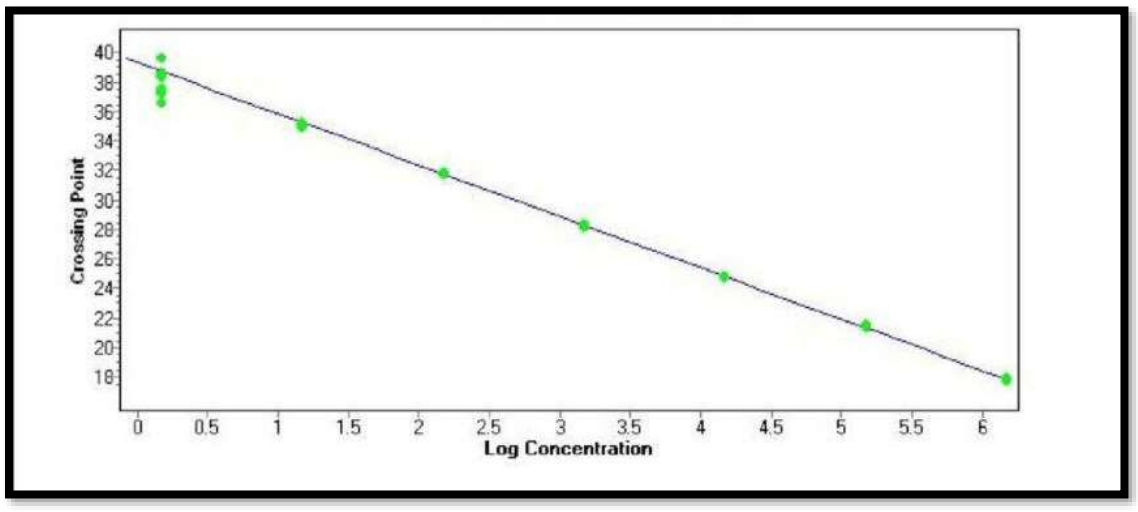

Figure (2):standard curve for BK virus amplification chart according to real time PCR

Journal of University of Babylon for Pure and Applied Sciences, Vol. (28), No. (2): 2020
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Table (5): Real-Time PCR threshold cycle (CT) of polyoma virus in positive and control sample. Where, positive samples CT: 9.05 -35.54 and NA: Non amplification as control samples.

Well	Fluor	**Target**	Content	Sample	C q	C q Mean	C q std. Dev
A1	FAM	VP1	Unkn	Human polyoma virus	25.95	25.95	0
A5	FAM	Agno	Unkn	Human polyoma virus	9.08	9.08	0
A6	FAM	Agno	Unkn	Human polyoma virus	9.05	9.05	0
A8	FAM	VP1	Unkn	Human polyoma virus	30.11	30.11	0
B2	FAM	VP1	Unkn	Human polyoma virus	35.54	35.54	0
C3	FAM	VP1	NTC	Human polyoma virus	0.00	0.00	0
D3	FAM	VP1	Neg Ctrl	Human polyoma virus	0.00	0.00	0

Estimation of Immunological Parameter in Serum Patients and Control CD4, CD8 level by ELISA

The levels of CD4, CD8, TNF-α, IFN-α, IFN-ɤ were estimated by ELISA procedure according to Elabscience Company (USA).All these parameters have the same principle of Sandwich-ELISA method as well as the same analytical procedure.

ELISA kits

The ELISA kits are used in this study listed in table 6

KITs	**Origin**	**Company**
Human CD-4 ELISA Kit	**USA**	**Elabscience**
Human CD-8 ELISA Kit	**USA**	**Elabscience**

Journal of University of Babylon for Pure and Applied Sciences, Vol. (28), No. (2): 2020
Online ISSN: 2312-8135, Print ISSN: 1992-0652

Result

Two hundred and thirty bloody urine specimens were collected from patients and subjected for Special media have been used to differentiate between viral and bacterial causes of bloody urine in two hundred and thirty patients. We eliminate 154 specimen of urine (67%) due to growth of bacteria in this media and focus on 76 (33%) specimen without growth which might relate to different diseases. These samples were recorded as a viral cause of hematuria for detection of BK infection in figure (3).

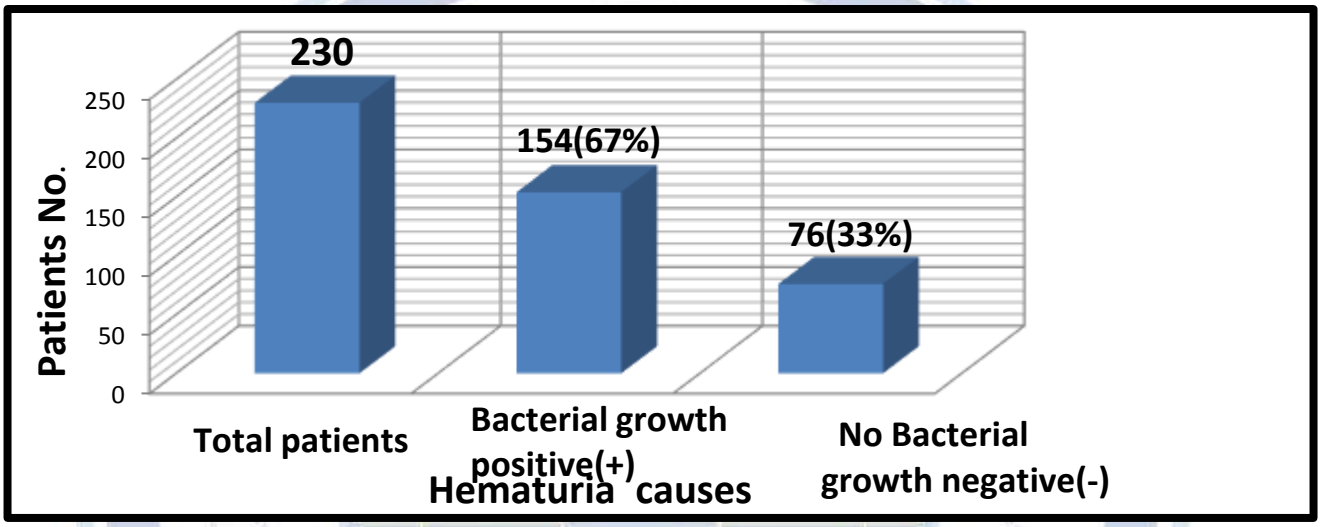

Figure (3): Patients group according to causative factor of hematuria

These samples were recorded in the study as a viral cause of hematuria for detection of BK infection in figure (3). All negative culture cases were diagnosed by urologist physician and classified as: 4(5.26%) patients underwent glomerulonephritis, 15(19.73%) chronic renal failure, 14(18.42%) urinary tract infection, 27(35.52%) kidney transplantation and 16 (21.5%) cystitis. The distribution of these study groups are listed in table 7.

Journal of University of Babylon for Pure and Applied Sciences, Vol. (28), No. (2): 2020
Online ISSN: 2312-8135, Print ISSN: 1992-0652

Table (7): patients of hematuria according to the study groups

Study group	N0 of patients	Total Percentage %	NO &Percentage of positive BK infection
Glomerulonephritis	**4**	**5.26**	**1 (1.3)**
Chronic renal failure	**15**	**19.73**	**1(1.3)**
Urinary tract infection	**14**	**18.42**	**3(3.9)**
Kidney transplantation	**27**	**35.52**	**5(6.5)**
Cystitis	**16**	**21.5**	**3(3.9)**

About age distribution 2.6% of cases between 1-10 years ,13.1% from 11-20 years ,22.36% from 21-30, 18.4% from 31-40 , 15.7 % from 41-50, and 27.6% more than 50 years appear table (8). The highest frequency was in the age group over 50 years, while the lowest frequency 2.6% was in the age range (1-10)

Table (8) Distribution of human polyoma virus (BKVS) according to gender and age groups.

Year	Number. of cases %	Gender	
		Men	Women
1 – 10	2 (2.6)	1	1
11 – 20	10 (13.1)	6	3
21 – 30	17 (22.36)	3	2
31 – 40	14 (18.4)	8	6
41 – 50	12 (15.7)	9	3
≥ 50	21 (27.6)	24	5
Total	76	56 (73.68%)	20 (26.32%)

Seventy six patient samples and (25) control were extracted to get the eluate DNA which were examined to detect the VP1 and Agno gene of human polyoma BKVS through conventional gradient polymerase chain reaction protocols, as shown in Figure (4) and (5).

Journal of University of Babylon for Pure and Applied Sciences, Vol. (28), No. (2): 2020
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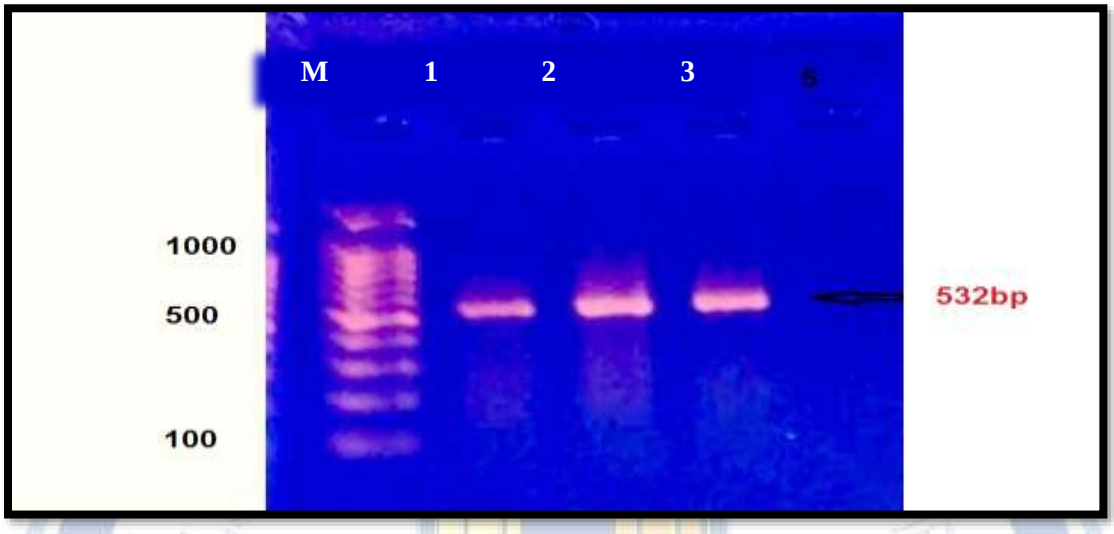

Figure (4) : vp1 gene of 532bp PCR producte in Human polyomavirus(BKV)in electrophoresis image of Agrose Where lane (1-3) positive, DNA ladder M: Marker (100-1100 bp) from hematuria patient sample.

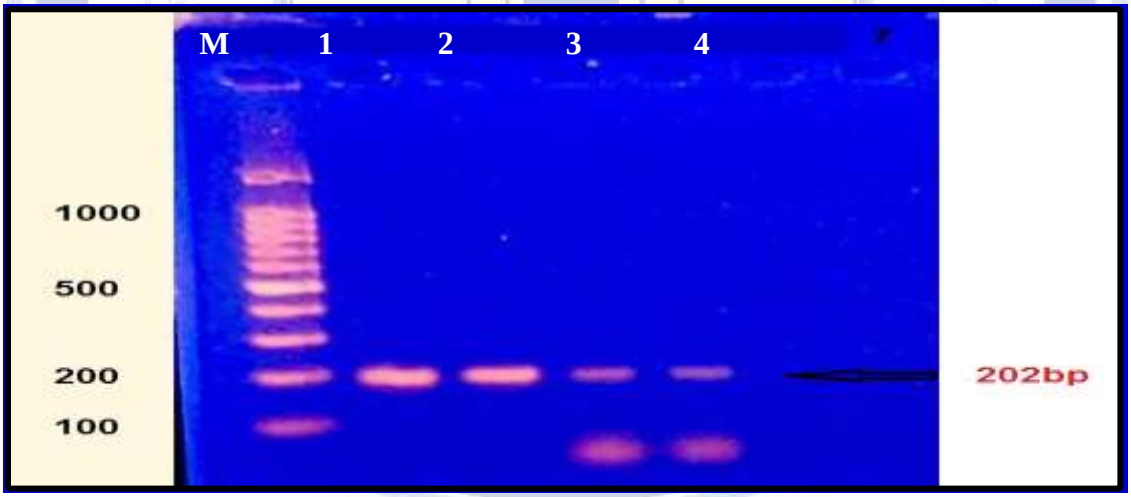

Figure (5): Agno gene of 202bp PCR producte in Human polyomavirus(BKV)in electrophoresis image of Agrose Where lane (1-4) positive, DNA ladder M: marker (100-1100 bp) from hematuria patient sample

Journal of University of Babylon for Pure and Applied Sciences, Vol. (28), No. (2): 2020
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DNA sequencing was performed for detection BKVS humanpolyomavirus in positive urine samples. After that the sequencing of the 532 bp PCR product of VP1 gene, was sent to Macrogen Company in Korea for performed the DNA sequencing by AB DNA sequencing system .Phylogenetic analysis was performed based on NCBI-Blast Alignment identification. Table (9).

Table (9) Homology sequence identity for human polyoma virus local isolate by using NCBI-Blast Gene bank Database

Sequencing analysis of VP1 Gene					
No.	Accession Number	Isolates	New strain	Local sequence identity	
				Identities	Gaps
1	KY488564	VP1	FK0001	99%	
2	KY488565	VP1	FK0002	99%	
3	KY488567	VP1	FK0003	99%	
4	KY488566	VP1	FK0004	99%	

Journal of University of Babylon for Pure and Applied Sciences, Vol. (28), No. (2): 2020
Online ISSN: 2312-8135, Print ISSN: 1992-0652

A- BLAST Results: VP1 Gene. VP1 sequencing of human polyoma virus

```
SCORE        EXPECT     Identities     Gaps          Strand
 128 bits(64)      5e-26        84/91          1/91          plus/plus
Query  71
GGGTTCTTTTGGAGCTCTTGAAGCACTCCTTGACCTGTTGAAGATCCCGTGA-
GGTTACC  129
|||||  ||||||||||  |||  ||||||||||||  |||||||||||||||||||    |||
sbjct1106
GGGTTATTTTGGAGCTGTTGGAGCACTCCTTGAGCTGTTGAAGATCCCGTGATCATTAC
C1165

Query  130   TGGGGAGGGGTTCCTGAAACCTTCCACAATG  160
||||||||||||||||||||||||||||||
Sbjct  1166  TGGGGAGGGGTTCCTGAAACCTTCCACAATG  119

Query  71  GGGTTCTTTTGGAGCTCTTGAAGCACTCCTTGACCTGTTGAAGATCCCGTGA-
GGTTACC  129
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1062
GGGTTATTTTGGAGCTGTTGGAGCACTCCTTGAGCTGTTGAAGATCCCGTGATCATTACC 1121

Query  130   TGGGGAGGGGTTCCTGAAACCTTCCACAATG  160
||||||||||||||||||||||||||||||
Sbjct  1122  TGGGGAGGGGTTCCTGAAACCTTCCACAATG  1152

Query  71  GGGTTCTTTTGGAGCTCTTGAAGCACTCCTTGACCTGTTGAAGATCCCGTGA-
GGTTACC  129
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1546
GGGTTATTTTGGAGCTGTTGGAGCACTCCTTGAGCTGTTGAAGATCCCGTGATCATTACC 1605

Query  130   TGGGGAGGGGTTCCTGAAACCTTCCACAATG  160
||||||||||||||||||||||||||||||
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Journal of University of Babylon for Pure and Applied Sciences, Vol. (28), No. (2): 2020
Online ISSN: 2312-8135, Print ISSN: 1992-0652

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Sbjct  1606  TGGGGAGGGGTTCCTGAAACCTTCCACAATG  1636

Query  71  GGGTTCTTTTGGAGCTCTTGAAGCACTCCTTGACCTGTTGAAGATCCCGTGA-
GGTTACC  129
|||||  ||||||||||  |||  ||||||||||||  ||||||||||||||||||    |||||
Sbjct  1393
GGGTTATTTTGGAGCTGTTGGAGCACTCCTTGAGCTGTTGAAGATCCCGTGATCATTACC
1452


Query  130  TGGGGAGGGGTTCCTGAAACCTTCCACAATG  160
||||||||||||||||||||||||||||||
Sbjct  1453  TGGGGAGGGGTTCCTGAAACCTTCCACAATG  1483


Query  71  GGGTTCTTTTGGAGCTCTTGAAGCACTCCTTGACCTGTTGAAGATCCCGTGA-
GGTTACC  129
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct1738
GGGTTATTTTGGAGCTGTTGGAGCACTCCTTGAGCTGTTGAAGATCCCGTGATCATTACC 1797

Query  130  TGGGGAGGGGTTCCTGAAACCTTCCACAATG  160
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Sbjct  1798  TGGGGAGGGGTTCCTGAAACCTTCCACAATG  1828
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B – BLAST Results: vp1 gene . Vp1 sequencing of human polyoma virus

```
122bits(66)     2e-24       83/91       1/91       plus/plus
Query 71   GGGTTCTTTTGGAGCTCTTGAAGCACTCCTTGACCTGTTGAAGATCCCGTGA-
GGTTACC  129
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1117
GGGTTATTTTGGAGCTGTTGGAGCACTCCTTGAGCTGTTGAAGATCCCCTGATCGTTACC 1176
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Journal of University of Babylon for Pure and Applied Sciences, Vol. (28), No. (2): 2020
Online ISSN: 2312-8135, Print ISSN: 1992-0652

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Query  130    TGGGGAGGGGTTCCTGAAACCTTCCACAATG  160
||||||||||||||||||||||||||||||||
Sbjct  1177   TGGGGAGGGGTTCCTGAAACCTTCCACCATG  1207

Query  71  GGGTTCTTTTGGAGCTCTTGAAGCACTCCTTGACCTGTTGAAGATCCCGTGA-
GGTTACC  129
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Sbjct 1200
GGGTTATTTTGGAGCTGTTGGAGCACTCCTTGAGCTGTTGAAGATCCCCTGATCGTTACC 1259

Query  130   TGGGGAGGGGTTCCTGAAACCTTCCACAATG  160
||||||||||||||||||||||||||||||||
Sbjct  1260  TGGGGAGGGGTTCCTGAAACCTTCCACCATG  1290

Query  71  GGGTTCTTTTGGAGCTCTTGAAGCACTCCTTGACCTGTTGAAGATCCCGTGA-
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Sbjct 1166
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Query  130   TGGGGAGGGGTTCCTGAAACCTTCCACAATG  160
||||||||||||||||||||||||||||||||
Sbjct  1226  TGGGGAGGGGTTCCTGAAACCTTCCACCATG  1256


Query  71  GGGTTCTTTTGGAGCTCTTGAAGCACTCCTTGACCTGTTGAAGATCCCGTGA-
GGTTACC  129
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1232
GGGTTATTTTGGAGCTGTTGGAGCACTCCTTGAGCTGTTGAAGATCCCCTGATCGTTACC 1291



Query  130   TGGGGAGGGGTTCCTGAAACCTTCCACAATG  160
||||||||||||||||||||||||||||||||
Sbjct  1292  TGGGGAGGGGTTCCTGAAACCTTCCACCATG  1322
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Journal of University of Babylon for Pure and Applied Sciences, Vol. (28), No. (2): 2020
Online ISSN: 2312-8135, Print ISSN: 1992-0652

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Query  71  GGGTTCTTTTGGAGCTCTTGAAGCACTCCTTGACCTGTTGAAGATCCCGTGA-
GGTTACC  129
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1236
GGGTTATTTTGGAGCTGTTGGAGCACTCCTTGAGCTGTTGAAGATCCCCTGATCGTTACC 1295
```

. C - BLAST Results: vp1 Gene. Vp1 sequencing of human polyoma virus

Score	Expect	Identities	Gaps	Strand
167 bits(90)	1e-37	111/121 (92%)	2/121(2%)	Plus/Plus

```
Query  42  TTGCACTGATTTTTGGTGTATTTTCTTTT-
GGGTTCTTTTGGAGCTCTTGAAGCACTCCT  100
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 3937
TTGCACTTATTTTTGGTGTATTTTCTTTCAGGGTTATTTTGGAGCTGTTGGAGCACTCCT39434

Query  101 TGACCTGTTGAAGATCCCGTGAGG-
TTACCTGGGGAGGGGTTCCTGAAACCTTCCACAAT  159
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct39435
TGAGCTGTTGAAGATCCCGTGATCATTACCTGGGGAGGGGTTCCTGAAACCTTCCACAAT39494

Query  160    G  160
|
Sbjct  39495  G  39495


Query  42  TTGCACTGATTTTTGGTGTATTTTCTTTT-
GGGTTCTTTTGGAGCTCTTGAAGCACTCCT  100
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct35952
TTGCACTTATTTTTGGTGTATTTTCTTTCAGGGTTATTTTGGAGCTGTTGGAGCACTCCT36011

Query  101 TGACCTGTTGAAGATCCCGTGAGG-
TTACCTGGGGAGGGGTTCCTGAAACCTTCCACAAT  159
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Journal of University of Babylon for Pure and Applied Sciences, Vol. (28), No. (2): 2020
Online ISSN: 2312-8135, Print ISSN: 1992-0652

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Sbjct36012
TGAGCTGTTGAAGATCCCGTGATCATTACCTGGGGAGGGGTTCCTGAAACCTTCCACAAT36071

Query  160    G  160
|
Sbjct  36072  G  36072

Query  42    TTGCACTGATTTTTGGTGTATTTTCTTTT-
GGGTTCTTTTGGAGCTCTTGAAGCACTCCT  100
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 124655
TTGCACTTATTTTTGGTGTATTTTCTTTCAGGGTTATTTTGGAGCTGTTGGAGCACTCCT
124596

Query  101   TGACCTGTTGAAGATCCCGTGAGG-
TTACCTGGGGAGGGGTTCCTGAAACCTTCCACAAT  159
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 124595
TGAGCTGTTGAAGATCCCGTGATCATTACCTGGGGAGGGGTTCCTGAAACCTTCCACAAT12453
6

Query  160     G  160
|
Sbjct  124535  G  124535
```

D - BLAST Results: vp1 Gene..

Score	Expect	Identities	Gaps	Strand
121bits(65)	8e-24	80/87(92%)	1/87(1%)	Plus/Plus

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Query  71  GGGTTCTTTTGGAGCTCTTGAAGCACTCCTTGACCTGTTGAAGATCCCGTGA-
GGTTACC 129
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Journal of University of Babylon for Pure and Applied Sciences, Vol. (28), No. (2): 2020
Online ISSN: 2312-8135, Print ISSN: 1992-0652

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Sbjct 2073
GGGTTATTTTGGAGCTGTTGGAGCACTCCTTGAGCTGTTGAAGATCCCCTGATCGTTACC2132

Query  130   TGGGGAGGGGTTCCTGAAACCTTCCAC  156
             ||||||||||||||||||||||||||||
Sbjct  2133  TGGGGAGGGGTTCCTGAAACCTTCCAC  2159

Query  71   GGGTTCTTTTGGAGCTCTTGAAGCACTCCTTGACCTGTTGAAGA-
TCCCGTGA-GGTTAC 128
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1838 GGGTTATTTTGGAGCTGTTGGAGCACTCCTTGAGCTGTTGAAGATTCCC-
TGATCGTTAC1896

Query  129   CTGGGGAGGGGTTCCTGAAACCTTCCACAATG  160
             |||||||||||||||||||||||||||| |||
Sbjct  1897  CTGGGGAGGGGTTCCTGAAACCTTCCACCATG  1928
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There are several immunological parameters that evaluate certain parameters of immune system including IFN ά, IFN-ɤ, ,TNF-α,CD4 and CD8 to maintain the disease status. The result of IFN-α, IFN-ɤ, CD4 and CD8 showed a higher significant level of what compared with control samples at P value (< 0.05) of Ttest, while the result of TNF-α showed no Significant difference in comparison with control group as shown in Table (10).

Journal of University of Babylon for Pure and Applied Sciences, Vol. (28), No. (2): 2020
Online ISSN: 2312-8135, Print ISSN: 1992-0652

Table(10): Immunological parameters hematuria patients in comparison with control

Parameter	Variable group	N	Mean± SD	P Value T-test
INF	Test	76	27.72 ± 8.12	H.S 0.010
	Control	25	18.52 ± 0.96	
INF α	Test	76	29.70 ± 8.01	H.S 0.005
	Control	25	17.37 ± 0.90	
TN Fα	Test	76	23.69 ± 2.31	N.S 0.512
	Control	25	23.52 ± 1.02	
CD4	Test	76	7.89 ± 2.04	H.S 0.007
	Control	25	3.27 ± 0.36	
CD8	Test	76	4.71 ± 1.30	H.S 0.007
	Control	25	1.74 ± 0.21	

The result of CD4 in relation to age group showed that patients at age > 50 Years give M±SD (10.22 ±0.72) have a higher level than others at a significant difference in comparison with control 3.27± 0.36 at LSD value (2.4).The same results were shown of each groups in comparison between each other,but the result of CD8 revealed that the age group(> 50 years) M±SD(6.028±0.94) has higher level than other age groups at a significant difference in comparison with control 1.74 ±0.22 at LSD value (1.8) .While the results showed no significant difference in each group in comparison to each other except with respect to the age group (21-30) and (31-40) years have a significant difference in table (11).

Journal of University of Babylon for Pure and Applied Sciences, Vol. (28), No. (2): 2020
Online ISSN: 2312-8135, Print ISSN: 1992-0652

Table (11) : Age relationship of CD4 and CD8 among hematuria patients and control.

Age group	N	CD4	CD8
		Mean±SD	Mean±SD
1- 10	2	8.21 ± 0.59	4.67 ± 0.43
11-20	10	6.614 ± 0.73	3.48 ± 0.58
21-30	18	5.03 ± 0.42	3.27 ± 0.56
31–40	14.00	8.28 ± 0.64	4.91 ± 0.58
41-50	12	8.84 ± 0.74	5.45 ± 0.52
> 50	20	10.22± 0.72	6.028 ± 0.94
Control	25	3.27 ± 0.36	1.74 ± 0.22
LSD		2.4	1.8

Due to the serious morbidity of viral infections, the study was planned to conduct and detect BKV infection in hemorrhagic cystitis and as a result limiting the rate of frequency of the infectious agent in the HC virus which are present in some patients' case in the hospitals in Iraq. Determining the clinical features relate to BKVS HC which may support us in distinguish it from other causes of HC virus.

The normal age distribution for hemorrhagic cystitis infection among children more than the adult since Childhood is minimally symptomatic or asymptomatic Primary infection in addition BK virus is acquired in early childhood and remain lifelong latent in genitourinary. More predominant in immunocompetent patients as a result of increase depression of immune system post tissue transplant and using the mycophenolic acid & tacrolimus are considered immune suppress medication effected immune response in spite of the multi drug increase the suppresser effect in case of BKVs infection. but all age groups is being susceptible to infection with such viruses at variable percentages the age more than 50 years with immunocompromised are most affected group might be due to reactivation of latent infection. The sex classification in the present survey of Human polyoma virus (BKV) infection in hemorrhagic cystitis cases observed that the men 56 (73.68%) had a higher prevalence compared to women 20(26.32%) table 8 This result might be due to irregular hard working with smoking behavior of male more than female and bad social stress of Iraqi population that will be lead to impaired in immune status and susceptible to infection with such viruses. All negative bacterial culture growth 76 specimen bloody urine and 25 control were prepared for analyzed by PCR for detection BKVS. The results of present study showed that, a well characterized done by (14). Who compare the sensitivity of PCR for purified DNA of urine sample which is increased markedly while urine sample without extraction have lower sensitivity due to presence of inhibitory substances which lead to false negative so new

Journal of University of Babylon for Pure and Applied Sciences, Vol. (28), No. (2): 2020
Online ISSN: 2312-8135, Print ISSN: 1992-0652

method of PCR developed to control the large concentration of these inhibitors (23, 1). the natural Human polyoma viruse infection induce different immunological parameters that may help in protection from such infection. Certain parameters such as IFN-ɤ, IFN-α, TNF-α, CD4 and CD8 were studied and give proper results reflected the immunological status of infected patients. In related studies done by (26)Who mentioned that BKinduction ofproinflammatory, Cytokines,phagocytosis, complement activation, and interferon's are usefull in most condition since they inactivate Humanpolyomaviruse (BKVs) rapidly.The adaptive and innate immune response began to react early after the virus infection , the innate immune response recognize the viral infection by intracellular and extracellular receptor including Toll-like receptor and release of inflammatory mediators (IL1, IL2, IFN-ɤ and TNF-α) occur as a consequence of viral recognition .These mediators prevents the viral aggregation and maturation by activation of other innate immune cells and direct anti viral response. The T- cells is response of directed to the viral capsid protein (27). Both Major histocompatibility complex class I and II molecule have been reported presenting polyomviral antigen , MHC class I as well as MHC–II restricted epitopes residing within the viral protein have been describe (24). However, the viral prptein 1 (VP1) of major capsid protein of late coding region is the main stimulus for the cell mediated immune response . Both specific CD4 and CD8, T-cell can recognize different vp1 protein epitopes, they can also recognize different BK subspecies CD4+ human polyomvirus specific cytotoxic T-cells are the main immune response against BK viral infection as they have the ability to lyse BK viral infected cells by a caveolar- dependent endocytosis mechanism (25).

Discussion

PCR technique developed to diagnose all genotype & subgroup of BKVs in short period. Due to its' high sensitivity by comparing to other method (Culture) require active virus and long duration to cultivation that may reach to 6weeks mention by (16). In the present study ,all negative bacterial culture growth 76 specimen bloody urine and 25 control were prepared for analyzed by PCR for detection BKVS. The results of present study showed that, a well characterized done by (14,28). Who compare the sensitivity of PCR for purified DNA of urine sample which is increased markedly while urine sample without extraction have lower sensitivity due to presence of inhibitory substances which lead to false negative so new method of PCR developed to control the large concentration of these inhibitors (23, 1). All patients with positive PCR For human polyoma virus had mainly hematuria symptoms with hemorrhagic cystitis ranging from mild to severe hematuria.The results Shows CT value were different.The viral load in urine specimen indicated by qPCR in which the CT value was less than 35 and represent the low titer of human polyom virus (Cq) value quantification cycle indicates the number of amplification round before the fluorescence signal exceeds the threshold value of back ground fluorescence. A low C q value is an indicator of low concentration of DNA in the sample, whereas high Cq value is an indicator of high concentration of DNA (31, 32). In this research, using a developed new method of accurate

and rapidly recognized of Human polyoma virus called PCR-DNA sequencing technique was performed for conformational identification of genotype of human polyoma virus in positive urine samples by PCR method. The 5 human polyoma virus DNA positive amplicons analyzed in present study after that the sequencing of the 532 bp PCR by product for *vp1* protein sent to Company in South Korea for performed the DNA sequencing by AB-DNA sequencing system to determine the subgroup of BKVs distribution in Iraq that causes for HC (29, 30). Approving the sequences of the nucleotide by firstly examine the results of DNA-sequencing through using NCBI-BLAST nucleotide and it's relationship with other strains. All these sequences have been recorded of Iraqi sequences in NCBI Gene-Bank and DDBJ of INSDC Human polyoma virus of sequences were isolated from clinical specimens a in Najaf and Baghdad city each sequence have Accession number Human polyoma viruse infection induce different immunological parameters that may help in protection from such infection. Certain parameters such as IFN-ɤ, IFN-α, TNF-α, CD4 and CD8 were studied and give proper results reflected the immunological status of infected patients. In related studies done by (26)Who mentioned that BK induction of proinflammatory, Cytokines,phagocytosis, complement activation, and interferon's are usefull in most condition since they inactivate Humanpolyomaviruse (BKVs) rapidly.The adaptive and innate immune response began to react early after the virus infection the innate immune response recognize the viral infection by intracellular and extracellular receptor including Toll-like receptor and release of inflammatory mediators (IL1, IL2, IFN-ɤ and TNF-α) occur as a consequence of viral recognition .These mediators prevents the viral aggregation and maturation by activation of other innate immune cells and direct anti viral response. The T- cells is response of directed to the viral capsid protein (27). The present study relatively consistence with result of other researcher such as Loeches *et al.*,(2009)(34). Also it agreement with the study done by Knowles *et al.*,*(* 2006)(33). Who stated that primary hemorrhagic cystitis infection account with the incidence of BKV positivity male children and it is associated in older patients attributed to increase viral activity as consequence of weak T cell immunity.also agree the studies in other countries such as Iran (35).

Conflict of Interests.
There are non-conflicts of interest .

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الخلاصة

يعتبر ألتهاب المثانة الدموي من المضاعفات المهمة لأشكال التهابات الجهاز البولي السفلي والتي تظهر بشكل عسر بول او بول دموي من جراء نزف من المثانة. ان ألتهاب المثانة الدموي يمكن ان يشاهد بول مصحوبا بالدم لفتره كنتيجة للإصابة الفيروسية . التهاب المثانة الفيروسي يمثل شكل اخر من التهابات الجهاز البولي غير البكتيرية والتي تصيب البالغين و الأطفال على حد سواء.بالإضافة للدراسة الحالية BK توجد فايروسات أخرى مثل الفايروس CMV, HAdVS هدف الدراسة إيجاد علاقة الأنماط المصلية للفايروس BK بالمتلازمة الحادة للبول الدموي و دراسة هذه الأنماط المصلية من الناحية الجزئية و تتابع السلسلة الجزيئي لمثل هذا الفيروس والتأكيد على العوامل المناعية المرتبطة به لدى الأشخاص المصابين. أجريت الدراسة الحالية على مئتين وثلاثون نموذج بول حاوي على دم ومئتين وثلاثون دم جمعت من ٢٣٠ مريض تم اختيارهم للتخلص من الأسباب الأخرى لوجود الدم للادرار، مجموعة المرضى شملت ١٧٠ذكر و ٦٠ انثى تراوحت أعمارهم بين ٦الى ٦٥ سنوات هؤلاء المرضى يراجعون بعض من المستشفيات . عينات المرضى (الادرار و الدم) و التي جمعت للتحري السريع والمتقدم عن فايروس BK بأستخدام الفحص السايتولوجي السريع للأدرار وتفاعل أنزيم البلمرة . جميع نماذج الادرار المصحوبة بالدم تمت زراعتها على المزارع الاعتيادية لغرض التفريق بين الإصابة البكتيرية و فايروس BK . الدراسة الحالية وجدت ١٥٤ عينة (٦٧%) موجبة للزرع البكتيري و قد تم استبعادها و ٧٦ عينة (٣٣%) كانت سالبة للزرع البكتيري والتي من المتوقع ان تكون كمسبب فايروسي للادرار الدموي .كل الحالات السالبة للزرع البكتيري صنفت اعتمادا على راي أخصائي الجراحة البولية كالآتي (٥,٢٦%)٤مرضى التهاب الكبيبات الكلوي و (١٩,٧٦%)١٥فشل كلوي مزمن و (١٨,٤٢%)١٤التهاب الجهاز البولي ، (٣٥,٥٢%)٢٧زرع الكليه، (٢١,٥%)١٦التهاب المثانه. الفئه العمرية أكثر من خمسين سنه ظهرت كأعلى نسبه مئوية (٢٧,٦%) ٢١بالمقارنة بالمجاميع العمرية الأخرى بالإضافة إلى النسبة المئوية للذكور اعلى من الاناث. كل بول المرضى ٧٦ عينة مع ٢٥ عينة بول مجموعة سيطرة تم تحليلها بواسطة تقنية تفاعل انزيم البلمرة بالإضافة للتقنيات الأخرى المستخدمة لتشخيص الفايروسات في عينات أدرار الدراسة الحالية تناولت بعض العوامل المناعية لتقييم الجهاز المناعي مثل CD8, CD4,IFN−α,IFN−γ,TNF−α لتقييم الحالة المناعية. نتيجة CD4,CD8,IFN−α,IFN−γ أظهرت مستويات معنوية عالية بالمقارنة مع عينات السيطرة في قيمة (اقل من P(٠,٥ بينما نتيجة TNF−α لم تظهر اختلاف معنوي بالمقارنة مع مجموعة السيطرة مع ملاحظة CD4 و CD8وعلاقتها بالفئات العمرية وجدت بأن المرضى في العمر اكبر من 50سنة ايضا تناولت الدراسة جانب من استخدام برامج البايوانفورماتك لتصميم بعض البرايمرات للجينات كما اثبتت النتائج بوجود سلالات جديدة من خلال تقنيات sequence DNA technology وتم تسجيل بعض الجينات لفاريوس BK اعتمادا على جينات ال VP1,Agno وسجلت العزلات الجديدة في Nucleotide/Blast في بنك الجين العالمي الامريكي باعتبارها عزلات جديدة.

الكلمات الدالة: فايروس ال بي كي ,التهاب المثانة الدموي, تفاعلات البلمرة المتسلسل والفحوات المناعية