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Research Article

# Detection of SV40 and BKV in Patients with Non-Hodgkin's Lymphoma (NHL) in Khartoum State, Sudan

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## Abstract

**Background:** The history of SV40 and its role in the development of primary brain cancers began soon after its discovery in 1960 when it was demonstrated to induce central nervous system malignancies following injection into newborn hamsters.

**Objective:** Investigate the association of SV40 and BK with non-Hodgkin's lymphoma (NHL) patients in Khartoum State.

**Methods:** Paraffin embedded blocks of tumor specimen from 200 Sudanese patients with NHL and 50 controls were collected from isotope center-Khartoum, Sudan, during the period from, March to October 2013. Real-time PCR was used to investigate the presence of SV40 and BK viruses in these specimens.

**Results:** The results show that Twenty out of 200 patients were positive for SV40 virus (10%). Of these positive patients, 14 (10.4%) were males while 6 (9%) were females, one male out of the 50 control was positive for SV40 virus (2%). Ninety four out of 200 patients were positive for BK virus in patient with NHL (24.5%). Of these positive patients, 36 (26.8%) were males while 13 (19.6%) were females, while nine out of these 50 controls were positive for BK virus (18%) of these positive control, 6 (16.6%) were males while 3 (21.4%) were females. No significant differences were found between males and females or the control and the test groups as regards infection by these two viruses.

**Conclusion:** The incidence of SV40 and BKV in NHL patients in Khartoum State, was documented through the molecular detection of SV40 and BKV indicating high prevalence rates among NHL patients in Khartoum State. Detection of SV40 and BKV using real-time PCR was established. Generally, these findings are useful for future studies since there is little information available about of SV40 and BKV infection in Sudan.

***Keywords:*** *SV40 and BKV; Non-Hodgkin's Lymphoma; Khartoum State; Sudan*

## Introduction

Polyomaviruses are widely distributed in vertebrates. The era of polyomavirus began with the accidental discovery of murine polyomavirus (MPyV) in 1958 followed by the discovery of simian virus 40 (SV40) in 1960 in monkey kidney cells that were used to prepare the polio vaccine [1]. BK polyomavirus (BKPyV) was independently discovered in 1972 [2]. BKPyV was isolated from the urine of a kidney transplant (KT) patient with the initials BK suffering from ureteric stenosis. SV40 and BKV are icosahedral in shape that contains small, circular, double-stranded DNA genomes [3]. There are three members in the family that infect humans; Simian Virus 40 (SV40), BK virus (BKV) and JC virus (JCV). SV40, JCV and BKV DNAs contain 5,243, 5,130, and 4,963 base pairs respectively [4].

BKV, JCV and SV40 share a high degree of nucleotide sequence homology. The JCV genome shares 75% homology with the BKV genome and 69% with SV40 [5].

The history of SV40 and its role in the development of primary brain cancers began soon after its discovery in 1960 when it was demonstrated to induce central nervous system malignancies following injection into newborn hamsters [6,7]. Several studies have detected the DNA of Simian virus 40 (SV40) in tumor tissues obtained from non-Hodgkin's lymphoma (NHL) patients [8]. A link between SV40 and NHL is biologically plausible because SV40 causes leukemia and lymphoma in laboratory rodents [9]. Transformation of rodent and human cells by SV40 is induced by the two oncoproteins encoded in the early region of the viral genome, the large tumor antigen (Tag), and the small tumor antigen (tag) [10]. Laboratories that have used sensitive polymerase chain reaction (PCR) technologies to detect SV40 DNA in NHL tumor tissue have obtained variable results [11].

BKV was isolated from urine of renal transplantation in 1971 [12]. The BK virus is ubiquitous in human populations worldwide. BKV Infection typically occurs in childhood, with a seroprevalence up to 90% in adults.

Nephropathy BKV excretion in the urine is usually insignificant, although very rare cases of BKC associated nephropathy are on record BKV occurs in 1% to 10% of kidney transplant recipients, usually manifesting in the first year following transplantation [13]. BK virus is detectable in both blood and urine. After BK reactivation, the virus is first detectable in the urine, with viremia developing several weeks later [13].

To the best of our knowledge no data is available in the literature on the association of SV40 with non-Hodgkin's lymphoma (NHL) and human brain cancers in Sudan.

## Material and Method

### Demographic data

The collected data included name, age, sex, date of collection, date of infection of non-Hodgkin's lymphoma, and clinical symptoms and non-Hodgkin's lymphoma infection subtype .The obtained data were analyzed using the statistical package (SPSS) version 16 (SPSS Inc., Chicago, U.S.A.).

### Patient Criteria and Specimen Collection

Paraffin embedded blocks of tumor specimens from 200 Sudanese patients with non-Hodgkin's lymphoma and 50 controls were collected from isotope center-Khartoum, Sudan, during the period from, March to October 2013.Written consents were obtained from recruited patients and controls before being enrolled in the study. Collected specimens were stored at 4˚C till test was performed.

### DNA extraction

### Specimen deparaffinization

The Specimens were deparaffinized according to the protocol of the manufacturer (Aid lab Biotecim, China) in brief; two 20-µ sections were cut from each tissue specimen block by the same person. To avoid cross-contamination, the microtome block was cleaned and the blade replaced between specimens. All Specimen were deparaffinized by adding xylene for one hour and then washed by ethanol 100%, 80% ,60% and 40% respectively then deionized water for 10 seconds for rehydration.

### DNA extraction procedure

DNA was extracted from re-hydrated tissue by using DNA extraction Kit according to the protocol of the manufacturer (Aid lab Biotecim, China). DNA was stored at -20°C till used.

### Real-time PCR for detection of SV40 virus

The qualitative detection system was carried out by using q PCR (Anlitka jene company, Germany), real-time PCR master mix for one reaction was prepared as follows: 4 µl of Real mod$^{R}$ green 5X (Intron biotechnology, South Korea), 2 µl of each 10 P mol/ml forward and reverse primer, 5 µl of DNA, the volume was completed to 20 µl by nuclease free water. The reaction mix was cycled 94°C for 1 minute, 52°C for 1 minute, and 72°C for 1 minute (35 cycles).

### Real-time PCR for detection of BK virus

The real-time PCR qualitative detection system was carried out by using q PCR (Anlitka jene company, Germany), The real-time PCR master mix for one reaction was prepared as follows: 4 µl of Real modR green 5X (Intron biotechnology, South Korea), 2 µl of each 10 P mol/ml forward and reverse primer, 5 µl of DNA, The volume was completed to 20 µl by nuclease free water. The reaction mix was cycled 95°C for 12 minute, 95°C for 15 sec, 60°C for 30 minutes, and 72°C for 30 minute (35 cycles).

| Primer Name | Primer Sequence; 5 – 3 |
|---|---|
| RA3 | 5-GCGTGACAGCCG GCGCAGCACCA-3 |
| RA4 | 5-GTCCATTAGCTGCAAAGATTCCTC-3 |

***Table 1:*** *The primer Sequences used for detection SV40 using real-time PCR.*

| Primer Name | Primer Sequence; 5 – 3 |
|---|---|
| PEP-1 | 5-AGTCTTTAGGGTCTTCTACC-3′ |
| PEP-2 | 5′-GGTGCCAACCTATGGAACAG-3 |

***Table 2:*** *The primer Sequences used for detection BK virus using real-time PCR.*

## Results

### Detection of SV40 using Real-time PCR in non- Hodgkin's lymphoma patients

Twenty out of 200 patients were positive for SV40 virus (10%). Of these positive patients, 14 (10.4%) were males while 6 (9%) were females (Table 3) and one male control out of 50 was positive for SV40 virus (2%) (Table 4) but with no significant differences.

### Detection of BK virus using Real-time PCR in non- Hodgkin's lymphoma patients

Ninety four out of 200 patients were positive for BK virus (24,5%). Of these positive patients, 36 (26.8%) were males while 13 (19.6%) were females (Table 3) but with no significant differences between male and females. Nine out of 50 controls were positive for BK virus (18%). Of these positive controls, 6 (16.6%) were males while 2 (21.4%) were females (Table 4) but with no significant differences There was also no significant differences between the control and the test groups in the prevalence of BK infection (Table 5).

| Patients | Total tested | No.+ve SV40 | No. +ve BK |
|---|---|---|---|
| Male | 134 | 14 (10.4%) | 36 (26.8%) |
| Female | 66 | 6 (9%) | 13(19.6%) |
| Total | 200 | 20 (10%) | 49(24.5%) |

***Table 3:*** *Detection of SV40 and BKV virus using Real-time PCR in non- Hodgkin's lymphoma Patients.*

| Patients | Total tested | No.+veSV40 | No. +ve BK |
|---|---|---|---|
| Male | 36 | 1(2.7%) | 6 (16.6%) |
| Female | 14 | 0 | 3 (21.4%) |
| Total | 50 | 1(2%) | 9 (18 %) |

***Table 4****: Detection of SV40 and BKV virus using Real-time PCR in the control group.*

| | | Gender | | | Sig. |
|---|---|---|---|---|---|
| | | Male | Female | Total | |
| BK in cases | Positive | 36 | 12 | 48 | 0.176 |
| | Negative | 98 | 54 | 152 | |
| | Total | 134 | 66 | 200 | |
| BK in controls | Positive | 6 | 3 | 9 | 0.776 |
| | Negative | 30 | 12 | 42 | |
| | Total | 36 | 15 | 51 | |
| SV40 in cases | Positive | 14 | 6 | 20 | 0.764 |
| | Negative | 120 | 60 | 180 | |
| | Total | 134 | 66 | 200 | |
| SV40 in controls | Positive | 1 | 0 | 1 | 0.529 |
| | Negative | 35 | 14 | 49 | |
| | Total | 36 | 14 | 50 | |

***Table 5:*** *Shows association of gender with BK virus and SV40 in cases and controls.*

## Discussion

SV40 infection in the human host was seen as a rare event and only restricted to people living in contacts with the natural hosts-monkeys such as inhabitants of Indian villages located close to the jungles and workers attending to monkeys in zoos and animal facilities [4]. Its association with the human host dates back to the 1950s and 1960s when SV40 contaminated vaccines occurred due to the ability of the virus to survive in the formalin treatment used to inactivate the poliovirus [4].

The BK virus rarely causes disease, many people who are infected with this virus are asymptomatic.

The present study indicates that the prevalence of SV40 in NHL patients in Khartoum State was 10% (20/200). This is in line with previous study reported by Regis., *et al*. in 2003 [14].

Our study indicates that males 14(10.4%) are more susceptible to SV40 infection than females 6(9%). Similar result was reported in Central Europe by Butel., *et al*. (1999) [15]. Moreover, SV40 infection was detected among control group with prevalence rate of 1(2%).

In this study, the prevalence of BK detected in NHL patients in Khartoum State (24%, 48/200) was lower than that reported in asymptomatic kidney transplant recipients (32%) in Sudan by Ibrahim., *et al*. in 2016 [16].

The present study showed that males 16 (26.8%) had higher prevalence of BK infection than females 9 (19.6%) This agrees with a previous a study conducted by Haysom., *et al.* in 2003 [17]. In addition BKV infection was detected among control group (16%). This is in agreement with international prevalence of BKV in healthy individuals which ranged from 1% to 25% [18].

This study showed that the prevalence of BK virus infection in NHL patients is higher than SV40 virus infection and that the prevalence of BK virus infection was as well higher than SV40 virus infection among the control group in Khartoum State.

To the best of our knowledge, this is the first study to identify SV40 and BK in NHL patients in Khartoum state-Sudan using molecular techniques and can serve as baseline for future plans aiming to study SV40 and BK in Sudan.

## Conclusion

In conclusion presence and Incidence of SV40 and BKV in non-Hodgkin's lymphoma (NHL) patients in Khartoum State, was documented

through the molecular detection of SV40 and BKV - indicating high prevalence rates among non-Hodgkin's lymphoma (NHL). Detection of SV40 and BKV using real-time PCR was established.

Further surveillance and molecular detection at the country level is important in order to fully understand the true status of SV40 and BK infection in Sudan.

## Bibliography

1. Sweet BH and Hilleman MR. "The vacuolating virus, S.V. 40". *Proceedings of the Society for Experimental Biology and Medicine* 105 (1960): 420-427.

2. Gardner SD., *et al*. "New human papovavirus (B.K.) isolated from urine after renal transplantation". *Lancet* 1.7712 (1971): 1253-1257.

3. Carbone M., *et al*. "The pathogenesis of mesothelioma". *Seminars in Oncology* 29.1 (2002): 2-17.

4. Walker DL and Frisque RJ. "The biology and molecular biology of JC virus". In: Salzman NP ed. New York: Plenum Press (1986): 327-377.

5. Gardner SD., *et al*. "New human papovavirus (B.K.) isolated from urine after renal transplantation". Lancet 1 (1971): 1253-1257.

6. Eddy BE Borman, GS Grubbs, GE and Young, RD. "Identification of the oncogenic substance in rhesus monkey kidney cell culture as simian virus 40". *Virology* 17.1 (1962) 65-75.

7. Girardi AJ., *et al*. "Development of tumors in hamsters inoculated in the neonatal period with vacuolating virus, SV-40". *Proceedings of the Society for Experimental Biology and Medicine* 109.3 (1962): 649-660.

8. Tabuchi K., *et al*. "Screening of human brain tumors for SV40- related T antigen". *International Journal of Cancer* 21.1 (1978): 12-17.

9. Nakatsuka S., *et al*. "Simian Virus 40 sequences in malignant lymphomas in Japan". *Cancer Research* 63.22 (2003): 7606-7608.

10. Engels EA. "Does Simian Virus 40 Cause Non-Hodgkin's Lymphoma? A Review of the Laboratory and Epidemiological Evidence". *Cancer Investigation* 23.6 (2005): 529-536.

11. Shah KV. "Polyomaviruses". In: Fields BN, Knipe DM, editors. Virology. 2nd edition. New York: Raven Press (1990): 1609-1623.

12. Bechert CJ., *et al*. "Monitoring of BK viral load in renal allograft recipients by realtime PCR assays". *American Journal of Clinical Pathology* 133.2 (2010): 242-250.

13. Capello D., *et al*. "Simian Virus 40 infection in lymphoproliferative disorders". *Lancet* 361.9351 (2003): 88-89.

14. Regis A Vilchez and Janet S Butel. "SV40 in human brain cancers and non-Hodgkin's lymphoma". *Oncogene* 22.33 (2003): 5164-5172.

15. MacKenzie J., *et al*. "Association between Simian Virus 40 DNA and lymphoma in the United Kingdom". *Journal of the National Cancer Institute* 95.13 (2003): 1001-1003.

16. Ibrahim A Helibi., *et al*. "Molecular Characterization of Polyomaviruses (BKV, JCV) in a Symptomatic Kidney Transplant Recipients in Sudan". *American Journal of Infectious Diseases and Microbiology* 4.2 (2016): 44-51.

17. Haysom AR., *et al*. "BK Viral infection in an Australian pediatric renal transplant population". *Pediatric Transplantation* 8.5 (2004): 480-484.

18. Butel JS., *et al*. "Molecular evidence of simian virus 40 infections in children". *Journal of Infectious Diseases* 180.3 (1999): 884-887.

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