

RESEARCH ARTICLE

## Molecular detection and Characterization of Merkle Cell Polyoma Virus in Lymphomas among Sudanese Patients

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### ABSTRACT

**Objectives:** Lymphoma (Hodgkin and Non-Hodgkin) is one of the most common malignancies in Sudan. Contemporaneous studies from several countries including the United States, Japan and Germany suggested a possible etiological link between Merkle cell polyoma virus (MCPyV) and these lymphomas; however, there are no previous studies that have been reported on this topic in Sudan. This study was applied to investigate the role of this virus in lymphoma patients in Sudan. Objective is to detect and characterize Merkle Cell Polyomavirus in lymphoma patients by using molecular techniques

**Method:** Adult Sudanese patients (both females and males) diagnosed with lymphoma were included in this study. Blood samples were used for DNA extraction which was then subjected to Real-time PCR to detect MCPyV DNA. Samples, proved positive by Real-time PCR amplification, were sequenced and phylogenetically analyzed.

**Results:** MCPyV was detected in 16/225 from Sudanese (7.1%) of lymphoma patients of which five were Hodgkin and eleven were Non-Hodgkin. MCPyV sequences in Sudanese patients showed 100% similarity to each other and 100% identity with reference sequences from gene bank.

**Conclusion:** This study is the first evidence that MCPyV exists in a subset of Sudanese lymphoma patients. MCPyV is unlikely to have direct contribution in the pathogenesis of lymphoma in the majority of Sudanese cases. MCPyV identified from the Sudanese lymphoma subjects may constitute an African lineage and its pathogenicity needs to be investigated in future studies. *J Microbiol Infect Dis 2020; 10(1):47-52.*

**Keywords:** Merkle Cell Polyomavirus, Hodgkin Lymphoma, Non-Hodgkin, Real-time PCR, Sudan

### INTRODUCTION

Polyomaviruses are double stranded DNA, icosahedral, non-enveloped viruses. Their circular genome encodes certain oncogenic proteins; thus, they are considered as putative oncogenic viruses [1,2]. Currently, there are nine recognized human polyomaviruses but their roles in carcinogenesis are not well understood [3]. The fifth polyomavirus; Merkle cell polyomavirus (MCPyV); was reported in 2008 [4]. This virus was detected in approximately

80% of Merkle cell carcinoma (MCCs) which is an aggressive skin carcinoma. More recently Human polyomavirus 9 (HPyV9) was identified in the serum of kidney transplant patients who were undergoing immunosuppressive treatment [5]. MCPyV is phylogenetically closely related to the African green monkey-derived lymphotropic polyomavirus which is capable of infecting lymphoid cells [6]. The search for hematologic neoplasia's where MCPyV may have a role in its pathogenesis is at present an active area of

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research [7]. The possible association of MCPyV with lymphoid malignancies was first recorded by researchers from USA, but they did not find a significant association between CLL and MCPyV [8,9]. However, researchers from Canada and Germany were able to detect MCPyV-positive CLL cases and advocated the possible involvement of MCPyV in a subset of CLLs [10,11]. More recently, another group of researchers from USA also reported MCPyV-positive CLLs [12].

## METHODS

### *Clinical specimens*

Whole blood samples (225) were obtained from lymphoma patients (140 were HL and 85 were NHL) during the period April 2015 to September 2015. About 7 ml of EDTA venous blood was collected, of which 2 ml were used for full blood count. The rest of the blood was used for DNA extraction.

### *DNA preparation*

Commercial DNA extraction kits (Analytikjena, Germany) were used to extract DNA of MCPyV from 200 microliter whole blood samples according to procedure described by the manufacturer.

### *Real-time PCR for MCPyV*

Real-time one-step RT-PCR for the LT regions of MCPyV was carried out to detect viral DNA by using a commercial kit according to the manufacturer's directions (Real-Time RT-PCR kit, Analytikjena, Germany) Real-time-PCR was carried out by using primers/probe as shown in Table 1 for MCPyV LT regions [13].

The master mix for single real time PCR reaction was prepared as follows: 10 µl of 2X PCR reaction mix, 1 µl of each primer forward and reverse / 0.5 probe, 2.5 µl of molecular grade water, and 5 µl of total DNA. The final volume was 25 µl for a single reaction (Analytikjena, Germany). The reaction was performed in Roter gene Q (Qiagen, Germany). The thermal cycling conditions were 2 minutes at 95 °C for initial denaturation and 45 cycles of 15 seconds at 95 °C for denaturation and 60 seconds at 60 °C for annealing and extension.

### *Sequencing and phylogenetic analysis for MCPyV*

The product of RT-PCR was sequenced by the company BGI (CHINA) and the sequences were then used to draw the phylogenetic tree.

## RESULTS

Sixteen (7.1%) out of two hundred and twenty five specimens were proved positive to MCPyV. Out of these 3(4.3%) samples were from Hodgkin and 13(8.4%) samples were from Non-Hodgkin lymphoma patients with no significant difference between the two groups (Table 1).

According to the gender, MCPyV was detected in 11 (8.8%) male and 5 (5%) female, with no significant difference between the two sexes (Table 3). The frequency of MCPyV positivity was highest in the age group 10-30 years old (16%) and lowest in the age group 31-50 (4.3 %) but with no significant differences between the three age groups tested (Table 4).

Nucleotide Sequencing and Phylotyping analysis of Merkle cell polyoma virus:

Sequencing of MCPyV, LT gene was done in eight RT-PCR positive samples of which three samples (20, 58 and 77) were successfully sequenced and were compared with other reference sequences published in GeneBank (MK561422.1, LC148301.1, MH136801.1 and KX781279.1). The sequences of the three amplified fragments showed 100% similarity to each other and 100% identity with the reference sequences (data not shown).

## DISCUSSION

Several epidemiological studies indicated the high frequency of exposure to MCPyV in the human population [14,15], similar to frequencies reported for the other Polyomaviridae members [16,17]. It is reported that in most of the cases, the primary infection occurs during childhood but reaching up to 85% in adulthood according to some researchers [18,19], who reported the occurrence of antibodies against MCPyV antigens in serum samples of healthy adults.

Table 1. Primers and probes used for the LT regions of MCPyV Real-time PCR.

No.	Primer sets	Nucleotide sequence position	sequence 5'-3'	PCR amplicons (bp)
1	LT.1F	1,034–1,053	CCACAGCCAGAGCTCTTCCT	146
2	LT.1R	1,179–1,157	TGGTGGTCTCCTCTCTGCTACTG	
3	LT. probe	1,065–1,088	FAM-TCCTTCTCAGCGTCCCAGGCTTCA-TAMRA	

Table 2. Frequency of Merkle cell polyoma virus among Hodgkin and Non-Hodgkin lymphoma.

Lymphoma types	Merkle cell polyoma virus		Total	P value
	Positive	Negative		
Hodgkin	3 (4.3%)	67 (95.7%)	70 (31.1%)	0.268
Non-Hodgkin	13 (8.4%)	142 (91.6%)	155 (68.9%)	
Total	16 (7.1%)	209 (92.9%)	225 (100%)	

Table 3. Total number of positive MCPyV patients according to gender.

Sex	Merkle cell polyoma virus		<i>P. value</i>
	Positive	Negative	
Male 125 (55.6%)	11 (8.8%)	114 (91.2%)	0.270
Female 100 (44.4%)	5 (5%)	95 (95%)	
Total 225 (100%)	16 (7.1%)	209 (92.9%)	

Table 4. Total number of positive MCPyV patients according to age group.

Age group in years (no, tested, %)	Merkle cell polyoma virus		<i>P. value</i>
	Positive	Negative	
10-30 (25, 11.1%)	4 (16%)	21(84%)	0.106
31-50(115, 51.1%)	5 (4.3 %)	110(95.7)	
≥ 51 (85, 37.5%)	7 (8.2%)	78(91.8%)	
Total (225,100%)	16 (7.1%)	209(92.9%)	

In the present study, the sequences of the amplified fragments showed 100% similarity to each other and 100% identity with the reference sequences from gene bank confirming our RT-PCR results of MCPyV detection in some of our lymphoma patients.

In the current study 16 out of 225 (7.1%) specimens were positive for MCPyV, similar results with low prevalence rates were detected using PCR in Non-Hodgkin (6.6%) and Hodgkin (3.3%) lymphoma specimens from Canada by Toracchio et al. [10].

However, Pantulu et al., [11] using PCR in Japan reported a prevalence rate of 27% (32/120) of MCPyV in CLL cases. Other studies using RT-PCR by Haugg et al [15] Goh et al., [19] and by Lam et al. [20] detected 9/27 (33.3%), 27.1% (19/70), and 33.3% (6/18) of MCPyV in CLL cases respectively. The high rates of MCPyV among these CLL cases compared to our results may be due to the limitation of these studies to CLL (one of the lymphoma types) population compared to the wide range of our population which contained many types of lymphoma. In addition, higher MCPyV DNA detection rates have been shown to consistently occur in CLL subjects, although prevalence and viral load are low [5,10].

Our results also indicated no significant differences in the rate of MCPyV detection according to type of disease (Hodgkin vs Non-Hodgkin); gender (male vs female); or age (Tables 2-4). This is similar to previous studies which found no significant association between gender and age and the prevalence of MCPyV in patients with acute and chronic respiratory diseases including lung cancer. MCPyV is also known to be a common infection in the general population [21,22]. Thus there appears to be an equal opportunity for the virus to be the cause of cancer in different groups (as a part of the general population) of our study population.

In this respect, some limitations of our study comprise the absence of data on insertion sites pattern of the virus in cells of patients. In addition our study also lacks information on presence or absence of T-antigen oncoproteins and viral T-antigen expression in cells of our

patients and mutations that might be present in the viral T antigen. These limitations are largely due to limited funds and resources available.

## Conclusion

In conclusion the presence and incidence of MCPyV in lymphoma patients in Sudan, was documented through the molecular detection using RT-PCR and sequencing indicating low prevalence rates among lymphoma patients. Further surveys and molecular investigations at the country level are important in order to fully unravel the true status of MCPyV infection in Sudan.

Finally, this study provides the first evidence that MCPyV exists in a subset of Sudanese lymphoma patients.

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