



## Detection and Characterization of *Kaposi's sarcoma Herpes Virus (KSHV)* from Archival Tissues fixed in Formalin and Paraffin Wax-Embedded at Kenyatta National Hospital, Kenya

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### Abstract

The DNA of Human Herpes virus (HHV-8) has been detected in patients with HIV/AIDS (Human Immunodeficiency Virus/Acquired Immunodeficiency Disease). Kaposi's sarcoma (KS) is a cancer seen in patients who have developed immunosuppression due to HIV. In order to characterize KS, archived tissues fixed in formalin and paraffin wax-embedded were obtained and three distinct gene region were determined by PCR (Polymerase chain reaction) and sequencing: K1, K15 and ORF75. The results showed that the three targeted gene were distinct and were present in the archived samples. The total number of the archived samples that were screened for the presence of HHV-8 DNA were 81. The study was able to detect K1, K15 and ORF75 gene in all the 81 archived samples. The extraction of DNA was by use of the Gene Read™ DNA FFPE Kit and detected by nested PCR using Qiagen® Taq PCR Core Kit where us the PCR products were isolated using 1% agarose gels. The KS gene products were cloned into pTOP V2-TA vector for the sticky-end and sequenced using M13 primers. The study cloned and sequenced 20 samples for each targeted K1, K15 (P) and ORF75 gene. Good sequences result was obtained 50% (10/20) ORF75, 40% (8/20) K15(P) and 75% (15/20) K1 gene. Double gene sequences were also detected. The study can authoritatively state that K1, K15 (P) and ORF75 genes sequences for the KS were detected in the archived samples.

**Keywords:** Kaposi's sarcoma, Human Herpes virus (HHV-8), Genotyping, Sequencing

### Introduction

The DNA sequences for Human herpesvirus-8 (HHV-8) was identified first in a tissue biopsy

from a patient who had Acquired immunodeficiency syndrome (AIDS) associated Kaposi's sarcoma<sup>(1)</sup>. An easy way to comply with

the Recent Science journal paper formatting requirements is to use this document as a template and simply type your text into it. In Uganda sexually transmitted diseases, people living in urban settings, people who are constantly on transit away from home are prone to develop AIDS associated KS<sup>(2)</sup>. Immunosuppression, low CD4 cell count, duration of time since HIV infection, adherence to antiretroviral treatment are among the risk factors for KS. The use of PCR (Polymerase Chain Reaction) technique to screen for HHV-8 gene can predict the likelihood of on ending up developing KS<sup>(3)</sup>. Most studies on KS 1 gene involve subjects from Africa and more specifically in East and Central Africa. The KSHV (Kaposi's sarcoma Herpes virus) ORF K1 has been amplified from PBMC (Peripheral blood mononuclear cells) or plasma DNA by use of a nested PCR technique. Among the circulating strains of KSHV, there exist a significant K1 gene that is diverse<sup>(4)</sup>. A study conducted in Uganda on evolution of HHV-8, K 15 M variant was reported. The sequence analysis of the K15 gene indicated that P allele was more prominent than M allele<sup>(5)</sup>. On a whole KS genome, the K15 gene is located on the right and it is involved in signal transduction. The P allele which is significantly more among people with HHV-8 gene is often associated with all the five K1 subtypes. The KS subtypes A, B and C are known to be associated with rare M allele and have been reported in parts of Africa not the East Africa. It is worth noting that K15 allele is unrelated to K1 subtype<sup>(6)</sup>. Kaposi's sarcoma is a persistent infection which is lifelong and is associated with lymphoproliferative disease and tumour. The ORF75 gene is known to play a vital role in viral replication and prodromal viral gene expression<sup>(7)</sup>.

The aim of this study was to detect and determine the circulating KS genes in the archived tissue samples.

## Material and Methods

**Study population.** This was a retrospective descriptive study where archived tissue samples

were retrieved from the year 2016 to 2013. The tissue samples were formalin fixed paraffin embedded (FFPE) and were sectioned (10 µm) using a rotary microtome. Different blades were used per tissue to prevent carryover of DNA and after each tissue section, the surfaces of the microtome were sterilized with DNAZap™ PCR DNA degradation solutions. MacroGen kit Gene Read DNA FFPE was used for DNA extraction. The Gene Read DNA FFPE removes paraffin and reverses formalin cross-links from the DNA sample before it is bound to the QIAamp Min Elute column. The DNA eluted was ready to be used for nested PCR. Taq PCR Core Kit- Qiagen was used to detect KS genome. Three (K1, K15 and ORF75) gene locus of KSHV were detected using two sets of primers in table 1. The PCR cycling condition of all the three regions were similar and it consisted of 30 number of cycles which entailed; initial denaturation at 94°C for 3 minutes, denaturation at 94°C for 1 minute, annealing at 63°C for 1 minute, extension at 72°C for 1 minute and final extension at 72°C for 10 minutes. The amplified PCR products were analysed by electrophoresis on a 1% agarose gel containing ethidiumbromide (1µl/ml of agarose solution) and were visualized under ultraviolet light alongside 1Kb DNA ladder. A known case of KS was used as positive control and RNase free water used as a negative control.

**Table 1:** KSHV sets of Primers used for thenested PCR

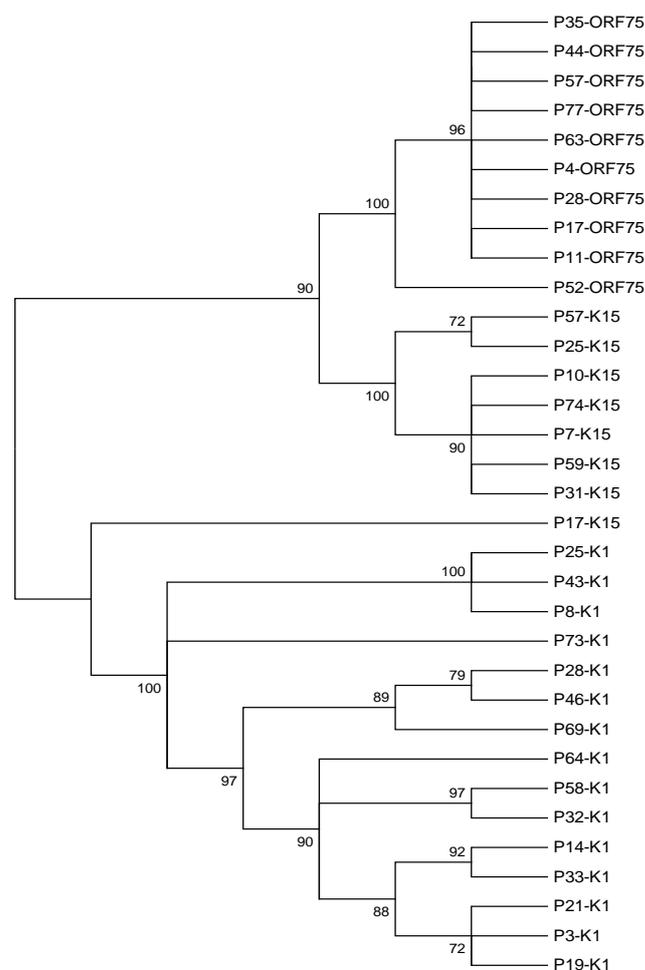
Gene Locus	Product	
	Size	Primer sequence
K1	868	K1a-f ATGTTCTGTATGTTGTCTGC K1a-r AGTACCAATCCACTGGTTGCG
	840	K1b-f GTCTGCAGTCTGGCGTTTGC K1b-r CTGGTTGCGTATAGTCTTCCG
K15 (P)	365	K15P-OF TGCAGGCTTGGTCATGGGTTAC K15P-OR GGGACCACGCTGCAATTAATG
	285	K15-3C ACGCATAACATGTACTGCCAC K15-4C CTTTGATATTGCCAGTGGTG
ORF75	895	KS 1000 CGGTTTCGGTGGCATAACAGGC KS 1034 CTGACTACAGAGGGTGTCCCCG
	804	LGH 2000 GGAAACAGGGTGTCTGTG LGH 2034 CATGGCCTACGACGTCAC

**Cloning and sequencing:** PCR products were isolated using 1% agarose gels. The KS gene products were cloned into pTOP V2-TA vector for the sticky-end and sequenced using M13 primers. The study cloned and sequenced 20 samples for each targeted K1, K15 (P) and ORF75 gene. Three clones were sequenced for each of the sample and the purpose of this was to verify the sequence. Both the forward and reverse PCR products were sequenced. The products of the DNA sequence were aligned using MAFFT version 7. Neighbour joining analysis of the aligned sequences were carried out in MEGA version6 software packages.

**Ethical consideration:** Ethical approval (P682/11/2014) was obtained from Kenyatta National Hospital/University of Nairobi

### Results

All the 81 archived tissue samples had the K1, K15 (P) and ORF75 genes detected. Out of the 81 samples that the HHV-8 gene was detected, only 20 samples each for the three (K1, K15 (P) and ORF75) genes were subjected to cloning. The proceeded with cloning and sequencing for the samples that had been well amplified and gave good gel band using the nested primers in table 1. Good sequences result was obtained 50% (10/20) ORF75, 40% (8/20) K15 (P) and 75% (15/20) K1gene (Figure 1).It was noted that four samples had double genes detected; sample P57 (Patient 57) had ORF75 gene and K15 (P) gene, sample P17 (Patient 17) had ORF75 gene and K15 (P) gene, sample P28 (Patient 28) had ORF75 gene and K1 gene and sample P25 (Patient 25) had K15 (P) gene and K1 gene.



**Fig. 1** Thirty-three samples were subjected to bootstrap analysis to create the consensus tree. Neighbour-joining analysis was carried out using MEGA Version 6 software package. Bootstrap above 70 represent significant branching.

### Discussion

In this study we can report that the three (K1, K15 (P) and ORF75) KS genes were successfully detected and can be said to be circulating among patients with AIDS-KS and are living in Nairobi. Earlier studies on KS that have been conducted in Africa have detected HHV-8 genome and noted that their sequences were heterogeneous<sup>(8,9,10)</sup>. A study conducted in Zambia<sup>(11)</sup> which detected ORF26, ORF75 and K1 gene among its subjects reported that K1 glycoprotein gene was the most prominent. The findings of the Zambian study are in agreement with this study that also found K1 gene to be the most frequent at 75% (15/20) which was later followed by 50% (10/20) ORF75 gene, 40% (8/20) K15 (P) gene. The K1 subtype A and

B have also been detected in Zimbabwe<sup>(13)</sup>, in this study the intertype K1 (B) differed with the one conducted in Uganda at a P value of 0.0004 which was considered significant. The K1 gene has also been detected in Botswana and the sequences for this study were obtained from the Bantu and San subjects<sup>(3)</sup>.

This study reported that four samples had double genes detected. These double genes were between ORF75 gene and K15 (P) gene, ORF75 gene and K1 gene and also K15 (P) gene and K1 gene. A similar finding of double gene detection was also noted in a study outside Africa in Russia<sup>(12)</sup>, where there was diversity on the HHV-8 gene. In the Russian study, there was no correlation between K1 and K14/1/K15 molecular subtypes and that it was also noted that K5 (M) and K15 (P) genotype can also be observed in the K1 subtype.

Only 40% ((8/20) samples had K15 (P) gene detected in this study. Similar results of the existence of K15 (P) have been reported in Uganda where the K15 (P) was noted to be the most predominant compared to the K15 (M) allele<sup>(5)</sup>. The K15 gene exist in two different alleles and the sequence in their amino acids has 70% divergence. The K15 (P) allele has been reported as the most prevalent and its known to have association with K1 genes of the subtype A, B, C and D. The K15 (M) allele is less predominant and has not been reported to have any form of association with the rare subtype D<sup>(14)</sup>. Reports from previous studies that looked at K15 gene used PCR technique to the two (P and M) alleles<sup>(15)</sup>. The K15 (M) allele has been reported to be rare in Southern, Central and Westparts of Africa<sup>(9)</sup>, however there has been a single case where it was reported in a 70-year-old patient from Uganda<sup>(16)</sup>.

In Botswana ORF75 sequence were amplified from their subjects, clustered together later they showed that they were aligned to subtype B and subtype C. In South Africa, type N and Q of the ORF75 sequences have been reported in KS strains<sup>(3)</sup>. In our results we were only detecting the existence of the ORF75 gene and its sequences

and were able to report that 50% (10/20) ORF75 genes. The only consistent results in our study with those done in Botswana and South Africa is that we were both able to detect the ORF75 gene sequences in our study participant, however in their studies they went a notch higher and did divergence in the ORF75 gene.

Before the advent of HIV and AIDS African endemic KS was the most prevalent in East and Central Africa, however the AIDS pandemic altered the distribution of KS and tumour associated with it become the most prevalent in sub-Saharan Africa<sup>(17,18)</sup>.

### Conclusion and Recommendation

K1, K15 (P) and ORF75 genes for the KS were detected in the archived samples. The three genes are in circulation among the population in Nairobi Kenya. There is need to conduct a study on genetic diversity on the AIDS-KS subtypes.

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