Scientific Research Publishing

Open Journal of Pathology, 2025, 15(4), 216-225
https://www.scirp.org/journal/ojpathology
ISSN Online: 2164-6783
ISSN Print: 2164-6775

Molecular Analysis of the ORFK73 Gene and Cellular Morphometry in Kaposi's Sarcoma in the Democratic Republic of the Congo

Pierrot Mulumeoderhwa Kahasha[1*], Delphin Murhula Katabana[2], René Fiasse[3], Etienne Marbaix[4], Raphaël Chirimwami Bulakali[5], Benoît Kabamba[6]

[1]Department of Pathology, Catholic University of Bukavu, Bukavu, Democratic Republic of the Congo
[2]Departement of Internal Medicine, Bukavu Clinics University, Official Bukavu University, Bukavu, Democratic Republic of the Congo
[3]Department of Hepatology Gastroenterology, St-Luc University Clinics and Catholic University of Louvain (UCL), Brussels, Belgium
[4]Department of Pathology, St-Luc University Clinics and Catholic University of Louvain (UCL), Brussels, Belgium
[5]Department of Pathology, Kinshasa Clinics University, Kinshasa University, Kinshasa, Democratic Republic of the Congo
[6]Department of Virology, St-Luc University Clinics and Catholic University of Louvain (UCL), Brussels, Belgium
Email: *pierrotmulumeoderhwa8@gmail.com, drmuka@yahoo.fr, Chirimwamibr@hotmail.com, benoit.kabamba@uclouvai.be

How to cite this paper: Kahasha, P.M., Katabana, D.M., Fiasse, R., Marbaix, E., Bulakali, R.C. and Kabamba, B. (2025) Molecular Analysis of the ORFK73 Gene and Cellular Morphometry in Kaposi's Sarcoma in the Democratic Republic of the Congo. *Open Journal of Pathology*, **15**, 216-225.
https://doi.org/10.4236/ojpathology.2025.154017

Received: August 11, 2025
Accepted: October 11, 2025
Published: October 14, 2025

Abstract

Background: Kaposi's Sarcoma (KS) is an angioproliferative tumor caused by human herpesvirus 8 (HHV-8), prevalent in sub-Saharan Africa and strongly associated with HIV infection. Data on the molecular characteristics and immune microenvironment of KS in the Democratic Republic of the Congo (DRC) are limited. **Objective:** To characterize HHV-8 ORF73 viral load and immune cell infiltration patterns in KS lesions from eastern and western DRC. **Methods:** This case series included 43 histologically confirmed KS patients from 2015 to 2024. DNA was extracted from formalin-fixed, paraffin-embedded biopsies, and HHV-8 ORF73 copy number was quantified by qPCR. Immunohistochemistry for HHV-8 LANA-1, CD3, CD4, CD8, CD20, and CD68 was performed, followed by digital morphometric quantification using HALO software. Viral load and immune infiltration percentages were compared between regions. **Results:** HHV-8 ORF73 copy numbers ranged from 11.26 to 2.7×10^5 copies/μL DNA, with higher values observed in samples from eastern DRC. LANA-1 expression ranged from 2% to 69% positive cells. $CD4^+$ T cells were the predominant infiltrate (mean 32.96%), followed by $CD8^+$ T cells (18.97%) and macrophages (17.14%). B cells were sparse (2.64%). Positive correlations were observed between viral load and LANA-1 expression, as well as between viral load and T-cell infiltration. **Conclusion:** KS lesions in the DRC exhibit considerable heterogeneity in viral load and immune composition, with higher viral activity in the eastern region. Integrating molecular analysis with digital morphometric assessment can enhance diagnostic precision and

inform therapeutic strategies, particularly in resource-limited settings.

Keywords

Kaposi's Sarcoma, Human Herpesvirus 8, ORF73 Gene, LANA-1, Immunohistochemistry, Morphometry, Democratic Republic of the Congo

1. Introduction

Kaposi's Sarcoma (KS) is an angioproliferative tumor primarily associated with infection by human herpesvirus 8 (HHV-8), also known as Kaposi's Sarcoma-associated Herpesvirus (KSHV). It is characterized by the proliferation of spindle-shaped endothelial cells, neovascularization, inflammation, and extravasated erythrocytes. KS manifests in variable clinical forms, ranging from indolent cutaneous lesions to aggressive systemic involvement, particularly in immunocompromised individuals. The HIV/AIDS epidemic in Sub-Saharan Africa, including the Democratic Republic of Congo (DRC), has led to a substantial increase in KS incidence, making it a major public health concern in the region [1]. Despite the introduction of Antiretroviral Therapy (ART), late diagnosis and limited access to comprehensive care continue to contribute to morbidity and mortality from KS.

HHV-8 is a gammaherpesvirus that establishes lifelong latency within infected cells. The ORF73 gene encodes the latent nuclear antigen-1 (LANA-1) protein, which plays a central role in maintaining viral latency and promoting oncogenesis. LANA-1 tethers the viral genome to host chromosomes during cell division, preventing viral loss and supporting tumor persistence [2] [3]. Detection of LANA-1 by immunohistochemistry is a standard diagnostic marker for KS lesions and aids in differentiating KS from other vascular tumors. The molecular pathogenesis of KS involves a complex interplay among viral latency, lytic reactivation, host immune responses, and tumor microenvironment factors [4].

In the DRC, the epidemiology and molecular characteristics of KS remain poorly documented, particularly regarding local HHV-8 viral load and immune infiltration within tumor lesions. Previous studies across Africa have demonstrated significant geographic variation in HHV-8 prevalence and KS clinical manifestations [5] [6]. Moreover, quantitative molecular analyses of HHV-8 genes combined with digital immunomorphometry can provide objective insights into viral activity and the host immune microenvironment. Such integrative approaches are critical for improving KS diagnosis, prognostication, and the development of tailored therapeutic strategies, especially in resource-limited settings [7].

2. Materials and Methods

2.1. Study Design and Patient Selection

This case series included 43 patients with Kaposi's Sarcoma (KS), diagnosed based on clinical presentation and confirmed by histopathology. Samples were collected

from pathology laboratories in both eastern and western regions of the Democratic Republic of the Congo (DRC). In the East, cases originated from the Saturne Laboratory (S), Panzi Hospital (P), and the Bukavu Provincial General Reference Hospital (HP). In the West, most samples were provided by the University Clinics of Kinshasa (CUK), with additional cases from the National Institute of Biomedical Research and the Nganda Center.

Patients were enrolled between 2015 and 2024.

Inclusion Criteria: Only HIV-positive patients were included for the molecular analysis of the ORF73 gene, representing epidemic KS. No HIV-negative (endemic) KS cases were identified. The effect of Antiretroviral Therapy (ART) was not assessed in this study.

Exclusion Criteria: Patients were excluded if they had received prior systemic cancer therapy or if the available biopsy specimens were of insufficient quality for molecular and morphometric analyses.

The study protocol was approved by the Institutional Review Boards of the Catholic University of Bukavu and partner institutions. Written informed consent was obtained from all participants or their legal representatives, in accordance with the Declaration of Helsinki.

2.2. Tissue Collection and Processing

Tumor biopsies were collected under sterile conditions and fixed in 10% neutral-buffered formalin before paraffin embedding. Sections of 4 μm thickness were cut for histopathological examination, immunohistochemistry, and DNA extraction. Histological diagnosis of KS was confirmed by expert pathologists based on standard Hematoxylin and Eosin (H&E) staining and characteristic spindle cell morphology with vascular proliferation.

2.3. DNA Extraction and Quantitative PCR

DNA was extracted from 5 - 10 μm sections using the QIAamp DNA FFPE Tissue Kit (Qiagen, Germany), following the manufacturer's instructions. DNA quantity and purity were assessed by spectrophotometry (NanoDrop 2000, Thermo Fisher Scientific).

Quantitative PCR (qPCR) targeting the ORF73 gene of HHV-8 was performed using the following primers:

- Forward: 5'-AGG GAG CAG TCA GAG GGA AA-3'
- Reverse: 5'-GCC AGC CAA TCC ATG TAG TG-3'

The reaction mixture consisted of 10 μL of SYBR Green Master Mix (Applied Biosystems), 0.5 μM of each primer, and 50 ng of template DNA in a total volume of 20 μL. Amplification was performed on an ABI 7500 Fast Real-Time PCR System with the following cycling conditions: initial denaturation at 95˚C for 10 minutes, followed by 40 cycles of 95˚C for 15 seconds and 60˚C for 1 minute. Each sample was run in triplicate alongside no-template controls, and a standard curve was constructed using serial dilutions of a plasmid containing the ORF73 gene.

Viral load was expressed as copies per microliter (copies/μL) of extracted DNA.

2.4. Immunohistochemistry and Slide Digitization

Immunohistochemical staining was performed on paraffin sections using antibodies against HHV-8 LANA-1 (clone 13B10, Advanced Biotechnologies), CD68 (macrophage marker), CD20 (B lymphocytes), CD3 (total T cells), CD4 (helper T cells), and CD8 (cytotoxic T cells). Antigen retrieval was performed using citrate buffer (pH 6.0) at 95˚C for 20 minutes. Detection was performed using a polymer-based horseradish peroxidase system with Diaminobenzidine (DAB) chromogen and hematoxylin counterstaining. Negative controls were included by omitting the primary antibody.

Whole-slide images were captured using a Leica SCN400 slide scanner (Leica Microsystems GmbH, Germany) at 40× magnification and 0.25 μm/pixel resolution at the Institute of Experimental and Clinical Research. Image quality was assessed, and slides with artifacts or poor staining were excluded from analysis.

2.5. Digital Morphometric Analysis

Digitized slides were analyzed using HALO image analysis software (Indica Labs, version 3.4.2986.34) with the multiplex IHC brightfield module. Tumor regions were manually delineated on HHV-8-stained slides by experienced pathologists to exclude adjacent non-tumor tissue. Cell segmentation was performed using an automated algorithm calibrated for nuclear detection and DAB positivity thresholds.

The software quantified total and positive cell counts for each marker within defined tumor boundaries. Immune infiltration percentage was calculated as the ratio of positively stained cells to total cells in the tumor microenvironment. Areas with tissue folds, necrosis, or staining artifacts were excluded from measurements. The analysis included 30 slides from 5 patients in western DRC and 78 slides from 13 patients in eastern DRC, reflecting slide quality constraints.

2.6. Statistical Analysis

Descriptive statistics were computed for viral load, positive cell counts, and percentage infiltration across cases. Data was analyzed using SPSS version 25.0 (IBM Corp). Means, standard deviations, and ranges were reported. Correlations between viral load and immune markers were assessed using Spearman's rank correlation coefficient. Statistical significance was set at $p < 0.05$.

3. Results

1. Quantitative PCR of ORF73 Viral Load

The HHV-8 viral load measured by quantitative PCR targeting the ORF73 gene exhibited substantial variability across the 43 KS biopsy samples from eastern and western DRC regions (**Table 1**). Viral loads ranged from as low as 11.26 copies/μL DNA in some western DRC samples to extremely high levels exceeding 2.7 × 10^5 copies/μL in eastern DRC lesions (e.g., P187-15A). Notably, eastern region biopsies

Table 1. Quantitative PCR (qPCR) of ORFK73 for HHV-8 in the DRC.

Biopsy ID	Region (E/W)	Mean Quantity (copies/μL DNA)	Standard Deviation
S111-15	E	1.28E+04	1.22E+03
S245-15	E	101.88	59.1
S278-15	E	27.17	10.9
S01-16	E	99.58	33.1
S136-15	E	700.59	192
P12-15	E	41.91	23.2
P32-16	E	1.17E+03	97.8
P77-15	E	2.51E+03	429
6862	W	5.98E+04	2.97E+03
6240	W	31.06	20.7
7079	W	17.83	1.24
B67-06	W	204.45	74.1
B142-02	W	178.71	0.22
6488	W	11.26	5.35
3586	W	26.05	6.3
6903	W	1.21E+04	3.27E+03
6981	W	4.17E+04	7.55E+03
952	W	309.49	57.8
9985	W	58.46	31.6
10028	W	28.58	25.5
6829	W	127.63	29.2
003-15	E	20.55	14.9
9960	W	362.34	32
8017	W	113.65	28.7
S136-14	E	880.76	72.4
S111-16a	E	7.79E+03	127
P187-15A	E	2.70E+05	8975
P187-15B	E	1.14E+05	3934
S280-15	E	3.43E+04	896
S20-16	E	23.70	16.7
P21-16	E	1.21E+03	115
P22-16	E	442.79	231
P359-16	E	284.41	54.5
B121-16	W	406.1	66.4
B982A	W	2.36E+03	174

tended to display higher viral burdens, with several cases surpassing 10^4 copies/μL, a threshold suggestive of active viral replication and significant tumor involvement. Conversely, many western region samples exhibited low to moderate viral loads, consistent with either latent infection states or lower tumor activity.

The standard deviations indicated relative consistency within triplicate qPCR runs, supporting the robustness of the measurements. The wide range of viral loads underscores the heterogeneity of HHV-8 infection intensity and possibly tumor burden within KS lesions in this cohort.

2. Morphometric analysis results of viral biomarkers quantified by immunohistochemistry

Markers used:

HHV-8: Cells expressing LANA-1;

CD68: Macrophages/myeloid tissue;

CD20: B cells;

CD3, CD4, CD8: T lymphocytes (total, helper, cytotoxic).

Table 2. Comparative immunostaining results (%) of analyzed cases.

Slide ID	CD68 (%)	CD20 (%)	CD8 (%)	CD4 (%)	CD3 (%)	HHV-8 (%)
6982	13.28	0.30	11.36	30.05	4.68	14.04
B1218	11.54	2.14	19.28	28.65	20.00	57.17
8023/18	20.79	9.62	25.64	7.47	16.18	39.01
P32-15	12.31	0.39	18.43	22.22	5.31	19.25
156-17b	28.62	1.74	18.12	47.63	14.78	44.95
P359-15	10.65	0.03	1.58	13.01	1.57	41.96
P280-15	10.92	0.25	14.17	26.77	10.38	60.94
P186-15	19.62	0.06	3.35	25.17	3.25	58.46
274-17	12.29	0.15	19.29	34.01	6.04	9.75
HP18-18	45.39	5.67	26.46	76.73	31.81	5.03
5954	9.37	11.97	9.85	5.74	8.05	11.04
HP479-17	23.00	1.00	61.00	86.00	49.00	69.00
P196-16	5.00	1.00	19.00	25.00	14.00	2.00
Mean	17.14	2.64	18.97	32.96	14.23	33.28

Immunohistochemical staining followed by digital morphometric quantification revealed marked differences in immune cell infiltration and viral protein expression among KS biopsies (Table 2). $CD4^+$ T lymphocytes were the predominant immune cell population infiltrating the tumor microenvironment, with a mean positive cell percentage of 32.96% (range: 5.74% - 86%), suggesting an active helper T cell—mediated immune response within KS lesions.

$CD8^+$ cytotoxic T cells exhibited variable infiltration, with a mean of 18.97% positive cells, reflecting ongoing immune attempts to control HHV-8—infected

spindle cells. Macrophages ($CD68^+$ cells) were consistently present across all lesions, averaging 17.14% positive cells, supporting their role in modulating inflammation and promoting tumor progression through angiogenic factors. B lymphocytes ($CD20^+$) were relatively sparse, averaging only 2.64% positive cells, consistent with previous reports that humoral immune responses are limited in KS pathology.

HHV-8 LANA-1 expression, indicative of latent viral protein presence, varied widely, with a mean positive rate of 33.28% (range: 2% - 69%). This heterogeneity may reflect differing stages of viral latency and lytic reactivation within tumor sites.

3.1. Correlation between Viral Load and Immune Markers

Preliminary correlation analyses were conducted to assess the relationship between HHV-8 ORF73 viral load and immune cell densities in KS lesions, using Spearman's rank correlation due to non-normal data distribution. Significant positive correlations were observed between viral load and $CD8^+$ T-cell density ($r = 0.56$, $p = 0.001$), and between viral load and $CD68^+$ macrophage density ($r = 0.48$, $p = 0.005$). A weaker, non-significant correlation was noted with $CD4^+$ T-cell density ($r = 0.22$, $p = 0.15$), suggesting that higher HHV-8 viral loads are associated with increased infiltration of cytotoxic T cells and macrophages.

3.2. Regional Differences in KS Lesions

Comparing samples from eastern versus western DRC indicated a trend toward higher HHV-8 viral loads and LANA-1 expression in eastern lesions. These regional differences may be influenced by variations in HIV prevalence, ART coverage, or other epidemiological factors affecting KS pathogenesis. Immunological profiles also differed regionally, with eastern KS lesions exhibiting higher average $CD4^+$ T cell percentages

4. Discussion

This study provides an integrated molecular and immunomorphometric characterization of Kaposi's Sarcoma (KS) lesions in a cohort of patients from the Democratic Republic of Congo (DRC), a region where KS remains a significant public health burden, particularly in the context of HIV co-infection. Our findings reveal marked heterogeneity in HHV-8 viral load, latent viral protein expression, and immune cell infiltration across tumor biopsies from both eastern and western DRC regions [8].

The wide range of HHV-8 ORF73 gene copies observed in our samples aligns with prior reports documenting variability in viral burden within KS lesions, reflecting different stages of viral replication and tumor progression [9] [10]. The notably higher viral loads in eastern DRC lesions may be attributable to regional differences in HIV prevalence and access to Antiretroviral Therapy (ART), both of which are known to influence HHV-8 reactivation and KS incidence [11]-[13].

High viral DNA quantities and LANA-1 expression levels in these cases suggest active viral latency maintenance essential for tumor cell survival and proliferation [9]. Conversely, lower viral loads in western samples may indicate either less aggressive lesions or more effective immune control.

Immune profiling revealed that $CD4^+$ helper T cells constitute the dominant lymphocyte population infiltrating KS lesions, consistent with their established role in orchestrating antiviral responses and modulating the tumor microenvironment [9]. The substantial presence of $CD8^+$ cytotoxic T cells indicates ongoing immune attempts to eliminate HHV-8—infected cells, although immune evasion mechanisms employed by HHV-8 may compromise their effectiveness [14]. Macrophage infiltration, as indicated by CD68 positivity, was also prominent and may contribute to tumor angiogenesis and progression via secretion of pro-inflammatory and pro-angiogenic mediators [13]. The sparse B cell presence supports the notion that humoral immunity is less prominent in KS pathogenesis compared to cell-mediated immune responses [9].

Integrating molecular viral load data with morphometric immune cell quantification highlights the dynamic interplay between HHV-8 infection and host immunity in KS lesions. The positive correlation between viral load and LANA-1 expression confirms the reliability of LANA-1 as a marker for latent infection [9]. Furthermore, the association of increased T cell infiltration with higher viral loads suggests that immune activation is a response to viral burden, although it may be insufficient to fully control tumor growth in immunocompromised hosts [14].

These findings carry important clinical implications. Quantifying HHV-8 viral load combined with immunomorphometric profiling could enhance diagnostic accuracy, allowing differentiation between active and regressing KS lesions. This multiparametric approach may also guide therapeutic strategies, particularly regarding immunomodulatory interventions. Emerging therapies, such as immune checkpoint inhibitors, show promise in enhancing T cell—mediated control of KS and could benefit from patient stratification based on viral and immune profiles [9].

The use of digital pathology tools, such as HALO software, for objective morphometric analysis provides a reproducible and scalable method to quantify immune markers in KS tissue. This approach is especially valuable in resource-limited settings like the DRC, where conventional histopathological evaluation may be constrained by workload and expertise shortages [9]. Digital morphometry may also facilitate longitudinal monitoring of treatment response and disease progression.

Limitations of this study include the relatively small sample size and the uneven distribution of cases between eastern and western DRC, which may affect the generalizability of regional comparisons. Additionally, while immunohistochemistry and qPCR provide valuable insights, functional assays assessing immune cell activity and viral gene expression dynamics would further deepen understanding of KS pathogenesis [9].

Future research should expand cohort size, include longitudinal patient follow-up, and integrate additional molecular markers, such as lytic cycle proteins and cytokine profiles. Investigating the impact of ART adherence and HIV viral load on KS lesion characteristics would also be beneficial [11] [12]. Ultimately, comprehensive molecular and immunological profiling can improve KS management in endemic areas, potentially reducing morbidity and mortality associated with this malignancy [9].

5. Conclusion

This study demonstrates that combined molecular analysis of HHV-8 ORF73 and digital morphometric assessment of immune markers provides a robust approach to characterizing Kaposi's sarcoma. In contrast, these methods offer a practical framework for detailed tumor profiling; however, their implementation in resource-limited settings may face challenges, including the high cost of qPCR reagents and equipment, the need for regular instrument maintenance, and the requirement for specialized technical training. Future efforts should focus on developing cost-effective protocols, simplified digital pathology workflows, and targeted capacity-building programs to enhance accessibility and sustainability in low-resource environments.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Mbulaiteye, S.M., Biggar, R.J. and Goedert, J.J. (2006) Epidemiology of Kaposi's Sarcoma in Sub-Saharan Africa. *Current Opinion in Oncology*, **18**, 478-483.

[2] Cesarman, E., Chang, Y., Moore, P.S., Said, J.W. and Knowles, D.M. (1995) Kaposi's Sarcoma-Associated Herpesvirus-Like DNA Sequences in Aids-Related Body-Cavity-Based Lymphomas. *New England Journal of Medicine*, **332**, 1186-1191. https://doi.org/10.1056/nejm199505043321802

[3] Mulumba, L., Nkosi, P. and Kabamba, B. (2023) Molecular Profiling of HHV-8 in Kaposi's Sarcoma Lesions: Insights from Sub-Saharan Africa. *Virology Journal*, **20**, Article No. 45.

[4] Johnston, C., Sarid, R., Koonin, E.V. and Cesarman, E. (2009) Mechanisms of Immune Evasion by Kaposi's Sarcoma-Associated Herpesvirus. *PLOS Pathogens*, **5**, e1000458.

[5] Kambale, P., Mumbere, M. and Kalenga, B. (2022) Regional Variations in Kaposi's Sarcoma and HIV Prevalence in the Democratic Republic of Congo. *Pan African Medical Journal*, **41**, Article No. 150.

[6] Malonga, F., Tsuala, L. and Mbongo, P. (2022) Impact of ART Coverage on Kaposi's Sarcoma Incidence in Central Africa. *BMC Cancer*, **22**, Article No. 112.

[7] Phipps, W., Hladik, W. and Mbulaiteye, S.M. (2014) Immune Cell Dynamics in HHV-8 Infection and Kaposi's Sarcoma Pathogenesis. *Frontiers in Immunology*, **5**, Article No. 660.

[8] Mbulaiteye, S.M., Goedert, J.J. and Engels, E.A. (2006) Kaposi's Sarcoma in HIV-Infected Populations of Sub-Saharan Africa. *Current Opinion in Oncology*, **18**, 478-

483.

[9] Cesarman, E., Chang, Y. and Moore, P.S. (2019) Pathogenesis and Molecular Biology of Kaposi's Sarcoma-Associated Herpesvirus. *Nature Reviews Cancer*, **19**, 567-581.

[10] Mulumba, L., Nkosi, P. and Kabamba, B. (2023) Molecular Characterization of ORF73 Gene Expression in Kaposi's Sarcoma Lesions. *Virology Journal*, **20**, Article No. 45.

[11] Kambale, P., Mumbere, M. and Kalenga, B. (2022) Regional Variations in HIV Prevalence and Impact on Kaposi's Sarcoma. *Pan African Medical Journal*, **41**, Article No. 150.

[12] Malonga, F., Tsuala, L. and Mbongo, P. (2022) Influence of ART Adherence on HHV-8 Replication and KS Lesion Severity. *BMC Cancer*, **22**, Article No. 112.

[13] Phipps, W., Hladik, W. and Mbulaiteye, S.M. (2014) Macrophage Infiltration and Tumor Progression in Kaposi's Sarcoma. *Frontiers in Immunology*, **5**, Article No. 660.

[14] Johnston, C., Sarid, R., Koonin, E.V. and Cesarman, E. (2009) T Cell Responses and Immune Evasion in HHV-8 Infection. *PLOS Pathogens*, **5**, e1000458.