# Kaposi's Sarcoma-associated Herpesvirus Infection and Its Association with All-Cause and Cardiovascular Mortality in the General Adults: A Prospective Cohort Study

Xiaoping Huang1,2\*, Xueliang Huang1,\*, Yushao Liu1,\*, Lixia Li1,\*, Jiaman Liao1, Hao Huang1, Ying Zhao1, Yiqiang Zhan2

1 Longgang Central Hospital, Shenzhen, China

2 Department of Epidemiology, School of Public Health (Shenzhen), Sun Yat-Sen University, China

**Corresponding to:**

Ying Zhao, Longgang Central Hospital, Shenzhen, China; Yiqiang Zhan, Department of Epidemiology, School of Public Health (Shenzhen), Sun Yat-Sen University, China. Email: zhanyq8@mail.sysu.edu.cn

**Running title:** KSHV Infection and Mortality

## Abstract

**Object:** To investigate the association between Kaposi's sarcoma-associated herpesvirus (KSHV)/human herpesvirus 8 (HHV8) infection and both all-cause and cardiovascular mortality in a representative cohort of US adults.

**Methods:** Data from the National Health and Nutrition Examination Survey III (NHANES III; 1988-1994) were analyzed, including 13,993 participants aged 18-90 years who underwent KSHV serology evaluations. Mortality outcomes were ascertained through December 2019 using the National Death Index. Cox proportional hazards models were employed to examine the association between KSHV seropositivity and mortality, adjusting for potential confounders such as age, sex, ethnicity, body mass index, and smoking status.

**Results:** Over a median follow-up period of 26.5 years, 5,503 deaths were recorded. KSHV seropositivity was associated with an increased hazard of all-cause mortality (Hazard Ratio [HR]: 1.32, 95% Confidence Interval [CI]: 1.03-1.69) and cardiovascular mortality (HR: 1.58, 95% CI: 1.00-2.50) after adjusting for age, sex, ethnicity, and body mass index. However, further adjustment for smoking status attenuated these associations. Notably, the association between KSHV infection and all-cause mortality persisted among women (HR: 1.32, 95% CI: 1.02-1.72) after adjusting for all confounders, whereas the association with cardiovascular mortality was only statistically significant for men (HR: 1.90, 95% CI: 1.02, 3.53).

**Conclusions:** KSHV infection may represent an independent risk factor for all-cause and cardiovascular mortality among US adults. These findings highlight the need for further research to validate these associations in independent populations and to elucidate the biological mechanisms underlying the observed increased mortality associated with KSHV infection.

**Keywords:** Human herpesvirus 8; Kaposi's sarcoma-associated herpesvirus; mortality; NHANES; cohort study

**Introduction**

Double-stranded DNA virus Human Herpesvirus-8 (HHV8), also known as Kaposi sarcoma Herpesvirus (KSHV), establishing latency in their host cells for a lifetime1. However, earlier research has shown that KSHV infection produces major health implications, including B cell lymphoproliferative disorders and Kaposi sarcoma (KS), especially in persons with immunodeficiency, malnutrition, and solid organ transplantation. KSHV infection is not ubiquitous with seroprevalence, risk factors and transmission routes exhibiting considerable variations across populations in different geographic regions. The global prevalence of KSHV was about 5% to 20% and estimated to be 1% to 5% in the general U.S. population2.

The definition of cardiovascular mortality is death owing to disorders of the heart or blood vessels. Cardiovascular disease (CVD), remaining the first leading causes of death in the US for 100 years, costs around $555 billion in 2016, and the cost will skyrocket to $1.34 trillion by 20503. A variety of modifiable risk factors have been associated with increased risk of CVD and mortality, namely raised blood pressure, raised blood glucose, raised blood lipids, tobacco use and harmful use of alcohol4. One case reported cardiac involvement by HHV8-positive diffuse large B-cell lymphoma. Primary effusion lymphoma has been confirmed to classically occur in pericardial spaces and blood vessels5. Conclusive evidence is not yet established the association of HHV8 infection with CVD. Against this backdrop, the imperative for early identification of individuals at heightened mortality risk gains paramount significance, offering a pivotal avenue for mitigating the burden imposed by HHV8 infection.

However, no studies have shown a link between HHV8 seropositivity and mortality in general population, and it is still unknown whether there is a nonlinear relationship between HHV8 with CVD mortality and all-cause mortality. To address these important research gaps, we aimed to elucidate the association of HHV8 seropositivity with all-cause and CVD mortality using the most recent data from the National Health and Nutrition Examination Survey (NHANES) III and the National Death Index (NDI) as of December 2019. We also conducted a stratified analysis to examine if sex could modify these association.

## Methods

### *Data source*

Data for this study were sourced from the National Health and Nutrition Examination Survey III (NHANES III), which was conducted from 1988 to 1994. NHANES III is a repeated cross-sectional survey designed to provide a comprehensive and nationally representative overview of the health and nutritional status of the U.S. population. This survey incorporates extensive sample coverage and a variety of health indicators through a combination of structured interviews and physical examinations conducted by trained professionals6. NHANES III employs a multi-stage probabilistic sampling methodology to ensure representativeness at the national level. The survey protocol was approved by the National Center for Health Statistics (NCHS) Research Ethics Review Committee, and all adult participants provided written informed consent. Detailed information on the NHANES methodology and ethical considerations is available on the websites of the Centers for Disease Control and Prevention (CDC) and the National Center for Health Statistics (NCHS) (https://www.cdc.gov/nchs/nhanes/index.htm).

### *Study participants*

For this analysis, we initially reviewed data from 33,994 participants collected during NHANES III. We excluded 14,398 participants who were either older than 90 years or younger than 18 years, as they were not eligible for the mortality follow-up. Additionally, participants with incomplete or missing follow-up data, sample weights, clustering, and stratification information were excluded. Non-responses or "I don't know" answers were treated as missing values. After applying these exclusion criteria, our final analysis comprised 13,993 participants. Among these, 308 individuals were seropositive for KSHV, while 13,685 individuals were seronegative. This final cohort was used to assess the association between KSHV seropositivity and the outcomes of all-cause and cardiovascular mortality.

### *Kaposi sarcoma Herpesvirus / Human Herpesvirus 8 seropositivity assessment*

A physical examination was conducted at a mobile examination center for all participants in NHANES III, where blood samples were obtained. To estimate the seroprevalence of HHV8 serostatus, HHV8 antibody testing of sera specimens was conducted by measuring K8.1 IgG through an enzyme-linked immunosorbent assay (ELISA)7. Earlier studies have shown that the K8.1 glycoprotein are unique to KSHV, with no counterparts in other herpesviruses8. NHANES III released the results of the HHV8 K8.1 assessment as *positive* or *negative*.

### *All-cause and cardiovascular mortality*

### Mortality data for this study were obtained from the National Death Index (NDI) up to December 2019. The NDI comprises death certificate information sourced from state vital statistics offices and is linked to NHANES III to facilitate tracking the health outcomes of its participants. NDI coverage is limited to individuals aged 18 years or older. The underlying cause of death among participants was classified according to the International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10). Specifically, cardiovascular disease (CVD) mortality was defined as death resulting from heart disease, encompassing ICD-10 codes I00–I09, I11, I13, and I20–I51.

### *Potential confounders*

We selected additional covariates to minimize bias due to confounding, including age9,10, sex11, body mass index (BMI)12 and serum triglycerides (TG)13. Information on these covariates was collected through an interview or physical examination. Ethnicity was determined by self-reported race and ethnicity and categorized as non-Hispanic White, non-Hispanic Black, Mexican American, or other. BMI can be found directly in the examination files and calculated as weight in kilograms divided by height in meters squared. Specifically, BMI level was stratified into four categories: Underweight (<18.5 kg/m2), normal weight (≥18.5 kg/m2 and <24.9kg/m2), overweight(≥25 kg/m2 and <29.9kg/m2) and obese(≥30 kg/m2), while serum TG level was stratified into three categories: Healthy (<1.69 mmol/L), borderline high (≥1.69 mmol/L and <2.25 mmol/L) and high(≥2.25 mmol/L).

**Statistical Analyses**

We used mean (standard deviation) and count (percentage) to describe the distributions of continuous and categorical variables, respectively. We calculated the mortality rate for participants with positive or negative KSHV and used a log-rank test to examine if the probability of survival curve differed by KSHV serostatus. The mortality rates presented as mortality per 1000 person-years of follow-up. The multivariable Cox proportional hazard model was used to estimate the association of KSHV seropositivity with risks of all-cause mortality and CVD mortality, generating a hazard ratio (HR) with a 95% confidence interval (CI) as an effect measurement. Attained age was used as the underlying time scale. Sex, ethnicity, BMI, family income and serum TG were additionally considered confounders. We also conducted stratified analysis by sex to examine whether the association of KSHV seropositivity with all-cause and CVD mortality would differ between men and women. Within each subgroup, we adjusted for all other covariates to isolate the specific effect of each stratification variable on the relationship between KSHV infection and mortality. A two-tailed P < 0.05 was set as the threshold for statistical significance. All analyses were conducted using the stratification and weighting scheme recommended by the National Center for Health Statistics for NHANES. All statistical computations were performed utilizing R 4.0 (R Core Team, Vienna, Austria).

## Results

### *Characteristics of the study participants*

**Table 1** describes the baseline characteristics of the 13,993 participants. The weighted analysis showed that the mean age was 43.54±17.14 years and the mean BMI was 26.41±5.66 kg/m2 at baseline. Men accounted for 46.65% whereas non-Hispanic white people accounted for 75.78%, of the participants. The prevalence of KSHV seropositivity was 1.56%, with 52.16% in men and 47.84% in women. Further details can be found in **Table 1**.

### *Mortality rate*

During a median follow-up of 26.5 years, we observed 5,510 deaths, including 1,653 CVD deaths. The all-cause mortality rate was 30.12 and 17.29 per 100,000 person-years for participants with KSHV seropositivity and participants without KSHV, respectively, leading to a mortality rate ratio of 1.76 (95% CI: 1.52, 2.05) (**Table 2**). **Figure 1** depicts the survival probability by KSHV serostatus, showing a statistically significant difference between the two groups (log-rank test: *P*=0.0402). The CVD mortality rate was 10.15 and 5.17 per 100,000 person-years for participants with KSHV seropositivity and participants without KSHV, respectively, leading to a mortality rate ratio of 1.96 (95% CI: 1.51, 2.53) (**Table 4**). **Figure 2** depicts the CVD survival probability by KSHV serostatus, showing marginal statistical difference between the two groups (log-rank test: P=0.0593).

### *Association between KSHV infection and all-cause and CVD mortality*

We fitted three different Cox regression models to explore the relationship of KSHV infection with all-cause and CVD mortality. Survival curves were plotted with the Kaplan–Meier method. Significant associations between KSHV seropositivity and all-cause mortality were found in three models as shown in **Table 3**. KSHV seropositivity was associated with a higher hazard of all-cause mortality (HR=1.32, 95% CI 1.03, 1.29, *P*=0.03) when adjusting for attained age. Comparable results were obtained when additionally adjusting for sex and ethnicity (HR=1.31, 95% CI: 1.03, 1.67, *P*=0.03), BMI (HR=1.32, 95% CI: 1.03, 1.69, *P*=0.03) and serum TG (HR=1.31, 95% CI: 1.02, 1.68, *P*=0.03). While significant associations between KSHV seropositivity and CVD mortality were found in the third model adjusting for sex and ethnicity and BMI (HR=1.58, 95% CI: 1.00, 2.50, *P*=0.04) as shown in **Table 5**.

### *Stratified analysis*

The association of KSHV infection with all-cause mortality was statistically significant only in women (HR: 1.32, 95% CI: 1.01, 1.72, *P*=0.04) but not in men (*P*=0.24), while a statistically association of HHV8 infection with CVD mortality was observed in men (HR: 1.90, 95% CI: 1.02, 3.53, *P*=0.04) but not in women (*P*=0.43).

**Discussion**

In the present study involving a nationally representative population of 13,993 non-institutionalized U.S. adults, we found that Kaposi's sarcoma-associated herpesvirus (KSHV) infection was associated with a 32% increased risk of all-cause mortality and a 58% increased risk of cardiovascular disease (CVD) mortality when considering several potential confounders. This significant association underscores the potential impact of KSHV infection on public health, particularly in relation to all-cause mortality and CVD mortality.

There exists a scarcity of studies investigating the association between Human Herpesvirus 8 (HHV-8) seropositivity and mortality. Notably, a study with 159 participants reported that elevated KSHV viral loads were associated with increased mortality (OR: 6.5, 95% CI, 1.3-32.4) in patients without tuberculosis or other microbiologically confirmed coinfections14. However, the findings across studies have not always been consistent. For instance, a recent study involving 5022 HIV-1-infected, antiretroviral-naïve U.S. persons did not report an association between a history of KSHV seropositivity and mortality (P=0.34)4. The discrepancy between studies may be partly attributed to differences in KSHV-specific antibody titres, which correlate with viral load. Individuals with a low viral load consequently have lower antibody titres that might be missed by current serological assays, leading to possible underestimation of the overall prevalence of KSHV15.

Several mechanisms may explain the observed association between KSHV seropositivity and increased mortality. One primary mechanism involves the inflammatory response induced by the virus, particularly KSHV inflammatory cytokine syndrome (KICS), which is characterized by a systemic elevation of pro-inflammatory cytokines such as interleukin-6 (IL-6) and interleukin-10 (IL-10)16,17. Elevated cytokine levels can contribute to immune system dysregulation and chronic inflammation, which are known to be associated with various adverse health outcomes, including cardiovascular disease and increased mortality18. Additionally, KSHV can induce angiogenesis and lymphangiogenesis, which are processes that could promote tumorigenesis and enhance the invasiveness of existing cancers, thereby worsening the prognosis and survival of affected individuals19. Furthermore, KSHV infection is known to lead to the depletion and dysfunction of immune cells, making individuals more susceptible to opportunistic infections and other comorbid conditions that can accelerate disease progression and mortality20. The virus also has mechanisms to evade the host immune response, which can lead to persistent infection and further complications21. The interplay between these factors likely contributes to the higher mortality observed in KSHV-seropositive individuals.

This study has several noteworthy strengths. Firstly, it utilized data from NHANES III, a well-conducted survey with long-term follow-up and a large sample size, thereby enhancing the reliability of the results. Secondly, the use of nationally representative sample weights, strata, and primary sampling units ensures that the estimates are generalizable to the U.S. population. Thirdly, to our knowledge, this is the first study to report the association between KSHV infection and both all-cause and CVD mortality in a representative sample of non-institutionalized adults. However, the study also has some limitations. One significant limitation is the inability to assess the relationship between the concentration of HHV-8 IgG and all-cause mortality due to the lack of data on the absolute values of HHV-8 IgG. This limitation restricts our understanding of how the immune response to KSHV influences mortality risk. Additionally, we cannot rule out the potential impact of the age at HHV-8 seroconversion. Previous studies have shown that early seroconversion is strongly associated with subsequent antibody values to both K8.1 and ORF7322. This variability may affect the observed associations, and future research should consider the timing of seroconversion in their analyses. Another limitation concerns the generalizability of our findings. The study focused exclusively on U.S. adults, which restricts the applicability of the results to other populations or geographical regions. It is imperative to consider demographic, cultural23, genetic24, and environmental differences25 when extrapolating these findings beyond the studied population. Replicating these results in diverse populations would enhance their external validity. Furthermore, causality cannot be inferred from our study due to its observational nature. While we identified significant associations between KSHV seropositivity and increased mortality risks, future studies should aim to establish causal relationships. Experimental studies or longitudinal cohort studies with detailed time-course data could shed more light on the causal pathways linking KSHV infection and mortality.

In conclusion, we found that HHV-8 seropositivity was associated with a higher risk of all-cause mortality and CVD mortality in a nationally representative sample of U.S. adults. These findings have significant implications for the study of viral infections, immune senescence, and longevity. If these results are replicated in other populations with larger sample sizes and diverse ethnic backgrounds, they could inform preventive strategies targeting HHV-8 infection. Given the increasing recognition of the role of viral infections in chronic disease progression and mortality, our study highlights the importance of integrating viral serology into public health surveillance and risk assessment frameworks. Overall, our findings contribute to the growing body of evidence on the public health significance of KSHV and highlight the need for continued research to uncover the complex interplay between viral infections and long-term health outcomes. By advancing our understanding of these relationships, we can move towards more effective interventions to enhance population health and longevity.

**Data availability statement:** The data are publicly available on https://wwwn.cdc.gov/Nchs/Nhanes/

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**Author contributions:** This manuscript was conceived and conceptualized by Yiqiang Zhan, while Yiqiang Zhan and Ying Zhao supervised the investigation, methodology, and analysis of the project. Xiaoping Huang generated the coding and analyzed online accessible data. Xiaoping Huang, Xueliang Huang, Yushao Liu, and Lixia Li wrote the original draft of the manuscript, and the review and editing were done by Jiaman Liao, and Hao Huang. All authors contributed to the final approval of the manuscript.

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**Tables and Figures**

**Table 1.** **Characteristics of the participants at baseline according to HHV8 K8.1 antibody status in NHANES III, the US**

**Table 2. Mortality rate in relation to HHV8 K8.1 antibody status in NHANES III, the US**

**Table 3. Multivariable adjusted hazard ratio (HR) and 95% confidence interval (CI) of all-cause death in relation to serum** **HHV8 K8.1 antibody status in NHANES III, the US**

**Table 4. CVD mortality rate in relation to HHV8 K8.1 antibody status in NHANES III, the US**

**Table 5.** **Multivariable adjusted hazard ratio (HR) and 95% confidence interval (CI) of CVD death in relation to serum** **HHV8 K8.1 antibody status in NHANES III, the US**

**Figure 1.** **Survival probability according to HHV8 K8.1 antibody in NHANES III, the US**

**Figure 2. CVD Survival probability according to HHV8 K8.1 antibody in NHANES III, the US**