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# Kaposi's sarcoma-associated herpesvirus seropositivity is associated with type 2 diabetes mellitus: A case–control study in Xinjiang, China

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ABSTRACT

*Objective:* To assess the potential relationship between Kaposi's sarcoma-associated herpesvirus (KSHV) infection and type 2 diabetes mellitus (DM-2) in Xinjiang, China.
*Methods:* A case–control study of consecutively included DM-2 patients and normal controls was conducted among the Uygur and Han populations in Xinjiang Uygur Autonomous Region, China. Blood samples were collected and KSHV seroprevalence, antibody titers, and viral load were investigated. Logistic regression analysis and multiple linear regression analysis were applied to explore determinants of the main outcome measures.
*Results:* A total of 324 patients with DM-2 and 376 normal controls were included. The seroprevalence of KSHV was 49.1% (95% confidence interval (CI) 43.6–54.5%) for diabetic patients and 23.7% (95% CI 19.4–28.0%) for the control group. After adjusting for variables of ethnicity, sex, body mass index, occupation, educational level, marital status, age, and smoking and alcohol consumption habits, the association between DM-2 and KSHV infection still existed (odds ratio (OR) 2.94, 95% CI 2.05–4.22), and the risk of KSHV infection increased with glucose concentration (OR 1.35, 95% CI 1.21–1.51). KSHV was more likely to express both the latent and lytic antigens in diabetic patients (latent: OR 3.27, 95% CI 2.25–4.75; lytic: OR 3.99, 95% CI 2.68–5.93). Antibody titers and viral load increased in patients with higher blood glucose levels ($p < 0.001$).
*Conclusions:* Patients with DM-2 have an elevated risk of KSHV infection. Both antibody titers and viral load increased with blood glucose levels.


## Introduction

Kaposi's sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus 8 (HHV-8), has been shown to be the etiological agent of all clinical forms of Kaposi's sarcoma (KS), primary effusion lymphoma (PEL), and the plasmablastic variant of multicentric Castleman disease (MCD) (Cesarman et al., 1995; Soulier et al., 1995; Whitby et al., 1995). The virus has been studied worldwide since its discovery by Chang et al. in 1994 (Chang et al., 1994). According to previous epidemiological studies, the prevalence of KSHV in the general population varies geographically; it is high in Sub-Saharan Africa, moderate in the Mediterranean region, and low in northern Europe, Asia, and USA (Dukers and Rezza, 2003; Zhang and Wang, 2017). In China, the prevalence of KSHV also varies across different regions. Xinjiang Uygur Autonomous Region has been regarded as an epidemic area where KSHV prevalence is higher than in other regions of China (Zhang et al., 2012). However, the risk factors associated

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with viral infection that may explain the regional disparity have not yet been fully elucidated.

Previous studies have shown higher KSHV seroprevalence and increased KS risk in HIV-infected, transplant, and other immunocompromised patients (Anderson et al., 2008; Goedert et al., 2002; Uldrick and Whitby, 2011), indicating the significant influence of immunosuppression on the primary viral infection and subsequent disease development. Diabetes mellitus (DM) is a non-communicable disease diagnosed when there is a high glucose level in the blood. Due to multiple factors, such as suppression of immune defense, microangiopathy, and the hyperglycemic environment, patients with DM are more vulnerable to infection and tend to experience increased mortality from infectious diseases (Korbel et al., 2018; Zoppini et al., 2018). It has been suggested that hepatitis B/C virus and tuberculosis infections are more prevalent in diabetic patients (Ba-Essa et al., 2016; Ronacher et al., 2015; Zhu et al., 2016). Furthermore, increasing reports indicate that there is an association between type 2 diabetes mellitus (DM-2) and KSHV infection or the development of KS (Caselli et al., 2014; Ingianni et al., 2007; Sobngwi et al., 2008), while the evidence is still limited. High glucose-mediated signaling pathways are postulated to increase susceptibility to KSHV infection through involvement in the induction of KSHV lytic reactivation, although the detailed molecular mechanisms have not yet been fully described (Chang et al., 2017).

According to the International Diabetes Federation, nearly half a billion people are currently living with diabetes. Low- and middle-income countries assume almost 80% of the diabetes burden (International Diabetes Federation, 2017). DM-2, characterized by insulin resistance and hyperglycemia, is the most prevalent metabolic disorder in China (Zhu et al., 2016). There is an urgent need to improve the prognosis of diabetic patients and reduce the global burden of this chronic disease. To date, the association between diabetes and infection with KSHV has not been well documented. In this study, it was hypothesized that DM-2 might be associated with KSHV infection and this hypothesis was tested in Xinjiang Province, China where the prevalence of KSHV is high. The current study appears to be the first to explore the relationship between DM-2 and KSHV infection in this population, and the findings will contribute to enhanced awareness of KSHV infection among diabetic patients.

## Materials and methods

### *Study design and participants*

The study was conducted from January 2014 to July 2015 in Xinjiang Uygur Autonomous Region, China. All consecutive patients of Han or Uygur ethnicity diagnosed with DM-2 according to the 1999 World Health Organization diagnostic criteria (World Health Organization, 2006) were included. Patients without diabetes in the same region were recruited as controls. Exclusion criteria were (1) not Han or Uygur, and (2) diagnosed with type 1 diabetes. Demographic and clinical data were obtained from the medical records. All study participants provided written informed consent.

### *Blood collection*

Blood samples were collected from participants using disposable sterile needles and ethylenediaminetetraacetic acid (EDTA) tubes by professional nurses. Sera were separated and stored at −80 °C for serological testing. All specimens were anonymized and coded with unique identification numbers.

### *Serological testing*

KSHV seropositivity was determined by the presence of anti-KSHV antibodies detected by immunofluorescence assay (IFA) in the serum samples of the study participants. All samples were confirmed using two IFAs, as described previously (Zheng et al., 2017). First, a monoclonal-enhanced IFA (mIFA) (Minhas et al., 2008) was applied to test the 1:40 phosphate buffered saline (PBS) diluted serum samples. In brief, sera were incubated with BC3 cells fixed on glass microscopy slides. The specimens of KS patients and healthy individuals were used as positive and negative controls. Mouse monoclonal anti-human IgG (CRL-1786; American Type Culture Collection, Manassas, VA, USA) was used as secondary antibody and DyLight488-conjugated donkey anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA, USA) was employed as the fluorescent marker. The slides were examined by epifluorescence microscopy (Nikon, Japan). A negative fluorescence reaction is indicated by the appearance of only red cells, while bright apple-green fluorescence indicates the presence of anti-KSHV antibodies in the sample. Second, *Spodoptera frugiperda* clone 9 cells infected with baculovirus expressing ORF65, ORF-K8.1 (lytic antigens) and ORF73 (latent nucleic antigen, LANA) were coated on the slides and used for further testing (Fu et al., 2009). A sample was considered seropositive only if it was positive with both assays. The levels of antibodies were analyzed by performing dilutions of the serum samples and were reported as titers.

### *Determination of viral load*

A TaqMan Real-Time PCR assay was conducted to quantitate the number of KSHV genomic copies in DNA samples of IFA-positive participants after nested PCR amplification of ORF26 and gB. Human β-globin and KSHV ORF26 were amplified using primers and probes described previously (Olp et al., 2013). Each 25 μl reaction mixture contained the following: 5 μl sample and 20 μl PCR mixture consisting of 12.5 μl Universal PCR Master Mix (PE Applied Biosystems) and primers and probe at concentration of 200 nM, respectively. The Master Mix contained uracil-*N*-glycosylase, which eliminated previously amplified PCR products to protect against carryover contamination. Amplification conditions were 50 °C for 2 min, 95 °C for 10 min, and 40 cycles of 95 °C for 15 s and 60 °C for 1 min. All samples were run in triplicate and the mean KSHV copy number per cellular equivalent was calculated.

### *Statistical analysis*

Both laboratory test results and medical records were double-entered and managed in EpiData 3.1. All data were subsequently transferred to SAS 9.4 (SAS Institute, Inc., Cary, NC, USA) and GraphPad Prism 7.0 (GraphPad, La Jolla, CA, USA) for further statistical analysis. Continuous variables were expressed as the mean ± standard deviation or median and interquartile range (IQR) according to the statistical distribution. Categorical variables were described as numbers or proportions. A non-parametric test (Mann–Whitney *U*-test) was used to assess the difference in geometric mean titers (GMT) of KSHV antibodies and medians of viral load between different groups. Logistic regression analysis was applied to examine the risk factors for KSHV seropositivity. Multiple linear regression analysis was conducted to determine whether a variable was associated with the KSHV antibody titer or viral DNA load. The correlation with antibody titers and viral DNA load was further assessed by Spearman's rank correlation coefficient and simple linear regression. Ninety-five percent confidence intervals (95% CI) were calculated for all of the coefficients of regression. A two-sided $p$-value of $<0.05$ was considered statistically significant.

## Results

### Participant characteristics

A total of 324 patients with DM-2 and 376 normal controls were included in this study. Sociodemographic and clinical characteristics of the participants, including ethnicity, sex, body mass index (BMI), occupation, educational level, marital status, smoking and alcohol consumption habits, age, and blood glucose concentration, are described in Table 1. Specifically, the diabetes group had a slightly higher percentage of Uygur (51.2%, 166/324) and male (59.6%, 193/324) participants than the control group. The median age was 53 (IQR 41–64) years in the diabetes group and 49 (IQR 37–62) years in the control group. Among the patients with diabetes, the median blood glucose concentration was 7.2 (IQR 6.5–8.0) mmol/l; the value was 5.3 (4.4–5.8) mmol/l among normal controls.

### The distribution of KSHV antibody titers and viral load in the participants

According to the IFA test results, the seroprevalence of KSHV was 49.1% (95% CI 43.6–54.5%) in the DM-2 group and 23.7% (95% CI 19.4–28.0%) in the control group. The GMT of antibodies against KSHV was 246.4 (95% CI 208.2–291.5) for diabetic patients and 105.9 (95% CI 85.3–131.5) for controls. A significant difference in the GMT of KSHV antibody titers was detected between the two groups (Mann–Whitney $U$-test = 4008, $p < 0.001$) (Figure 1a). The average viral load was 0.540 (IQR 0.129–0.832) copies/$10^3$ cells in the DM-2 group and 0.063 (IQR 0.043–0.085) copies/$10^3$ cells in the control group. This difference was also statistically significant (Mann–Whitney $U$-test = 1490, $p < 0.001$) (Figure 1b).

**Table 1**
Characteristics of study subjects with and without type 2 diabetes mellitus.

| | DM-2 | Control | Total |
|---|---|---|---|
| Total number (%) | 324 (46.3) | 376 (53.7) | 700 (100.0) |
| Ethnicity, *n* (%) | | | |
| Han | 158 (48.8) | 215 (57.2) | 373 (53.3) |
| Uygur | 166 (51.2) | 161 (42.8) | 327 (46.7) |
| Sex, *n* (%) | | | |
| Male | 193 (59.6) | 171 (45.5) | 364 (52.0) |
| Female | 131 (40.4) | 205 (54.5) | 336 (48.0) |
| BMI (kg/m$^2$), *n* (%) | | | |
| <18 | 21 (6.5) | 31 (8.2) | 52 (7.4) |
| 18–24 | 185 (57.1) | 305 (81.1) | 490 (70.0) |
| >24 | 118 (36.4) | 40 (10.6) | 158 (22.6) |
| Occupation, *n* (%) | | | |
| Student | 12 (3.7) | 8 (2.1) | 20 (2.9) |
| Cadre | 60 (18.5) | 98 (26.1) | 158 (22.6) |
| Worker | 48 (14.8) | 82 (21.8) | 130 (18.6) |
| Farmer | 77 (23.8) | 59 (15.7) | 136 (19.4) |
| Retired | 97 (29.9) | 87 (23.1) | 184 (26.3) |
| None | 30 (9.3) | 42 (11.2) | 72 (10.3) |
| Educational level, *n* (%) | | | |
| Elementary school or lower | 56 (17.3) | 37 (9.8) | 93 (13.3) |
| Middle school | 79 (24.4) | 72 (19.2) | 151 (21.6) |
| High school | 104 (32.1) | 127 (33.8) | 231 (33.0) |
| College or higher | 85 (26.2) | 140 (37.2) | 225 (32.1) |
| Marital status, *n* (%) | | | |
| Single | 22 (6.8) | 24 (6.4) | 46 (6.6) |
| Married | 265 (81.8) | 285 (75.8) | 550 (78.6) |
| Widowed/divorced | 37 (11.4) | 67 (17.8) | 104 (14.9) |
| Ever smoker, *n* (%) | | | |
| Yes | 174 (53.7) | 133 (35.4) | 307 (43.9) |
| No | 150 (46.3) | 243 (64.6) | 393 (56.1) |
| Regular alcohol consumption, *n* (%) | | | |
| Yes | 168 (51.9) | 154 (41.0) | 322 (46.0) |
| No | 156 (48.2) | 222 (59.0) | 378 (54.0) |
| Age (years), median (IQR) | 53 (41–64) | 49 (37–62) | 51 (39–63) |
| Glucose (mmol/l), median (IQR) | 7.2 (6.5–8.0) | 5.3 (4.4–5.8) | 6.0 (5.1–7.1) |

DM-2, type 2 diabetes mellitus; BMI, body mass index; IQR, interquartile range.

### Risk factors of KSHV infection markers

Infection with KSHV was found to be significantly associated with the following variables on univariate logistic regression analysis (Table 2): (1) ethnicity (Uyghur vs. Han: odds ratio (OR) 1.50, 95% CI 1.10–2.05), (2) BMI (18–24 vs. <18 kg/m$^2$: OR 0.55, 95% CI 0.31–0.97), (3) occupation (student vs. none: OR 6.66, 95% CI 2.02–22.01), (4) educational level (college or higher vs. elementary school or lower: OR 0.54, 95% CI 0.33–0.89), (5) marital status (married vs. single: OR 0.41, 95% CI 0.22–0.75; widowed/divorced vs. single: OR 0.33, 95% CI 0.16–0.67), (6) DM-2 (OR 3.11, 95% CI 2.25–4.29), and (7) glucose (mmol/l) (OR 1.35, 95% CI 1.22–1.50). After adjusting for variables of ethnicity, sex, BMI, occupation, educational level, marital status, smoking and alcohol consumption habits, age, and diabetic status in model 1, only the association between DM-2 and KSHV infection remained significant (OR 2.94, 95% CI 2.05–4.22). To avoid multicollinearity, diabetic status was replaced with blood glucose level in model 2 and the risk of infection was found to increase with glucose concentration (OR 1.35, 95% CI 1.21–1.51). Being a student was also shown to be a risk factor (OR 7.53, 95% CI 1.43–39.58), although the confidence interval was wide.

The seropositivity rate of antibodies to ORF73, ORF65, and ORF-K8.1 was 92.7% (230/248), 56.0% (139/248), and 45.6% (113/248), respectively, in the IFA-positive participants. A total of 175 serum samples tested positive for antibodies to both latent (ORF73) and lytic (ORF65 or ORF-K8.1) antigens. Thus, 76.1% (175/230) of sera that were positive for latent antibodies were also positive for lytic antibodies. In contrast, 90.7% (175/193) of sera positive for lytic antibodies were also positive for latent antibodies. It was found that the virus was more likely to express both the latent and lytic antigens in diabetic patients (latent: OR 3.27, 95% CI 2.25–4.75; lytic: OR 3.99, 95% CI 2.68–5.93). In addition, Uygur ethnicity appeared to be a risk factor for ORF73 antibody seroconversion (OR 1.48, 95% CI 1.01–2.16) (Table 3).

### Correlation between KSHV viral load and antibody titers, and correlates

Antibody titers and viral DNA load were tested for the 248 IFA-positive participants. By multiple linear regression analysis (Table 4), it was found that KSHV antibody titers were increased in Uygur participants ($p < 0.001$), while the Han population had a higher viral load level ($p < 0.001$). Furthermore, both antibody titers and viral load increased in patients with higher blood glucose levels ($p < 0.001$).

A further pairwise comparison between the logarithmic transformation of KSHV antibody titers and viral load (copies/$10^3$ cells) revealed a linear relationship (slope = 0.08, 95% CI 0.05–0.11), with a significant Spearman's rank correlation ($r = 0.34$, $p < 0.001$) (Figure 2).

## Discussion

The prevalence of diabetes is increasing remarkably in low- and middle-income countries, especially in the Southeast Asia and Western Pacific regions. China alone has a diabetic population of approximately 121 million. Patients from these areas develop disease earlier and suffer a worse prognosis than their counterparts in wealthier countries (International Diabetes Federation, 2017). It is therefore imperative that we learn more about the potential complications of diabetes and improve medical care for these patients. As the etiological agent of several tumors including

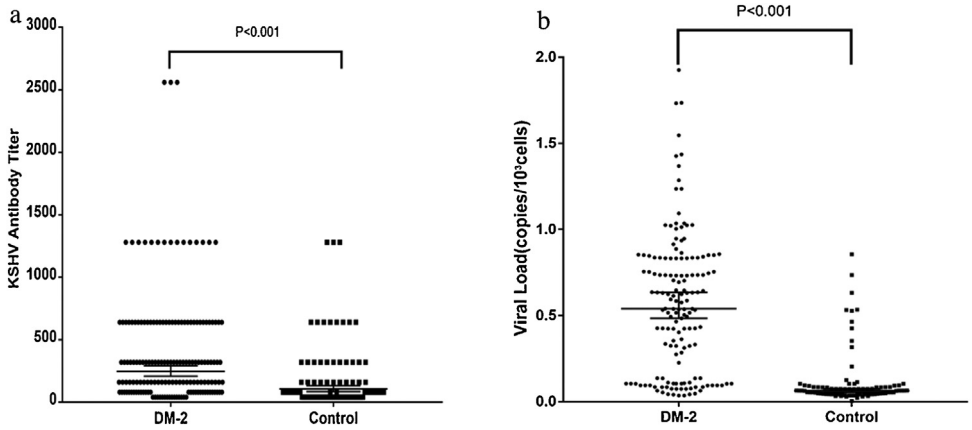


**Figure 1.** (a) Titer distribution of Kaposi's sarcoma-associated herpesvirus (KSHV) among patients with type 2 diabetes mellitus (DM-2) and normal controls. (b) Distribution of viral load across the DM-2 and control groups.

**Table 2**
Unadjusted and adjusted analysis of risk factors associated with Kaposi's sarcoma-associated herpesvirus infection according to immunofluorescence assay results ($n = 700$).

| | Unadjusted | | Adjusted (model 1) | | Adjusted (model 2) | |
|---|---|---|---|---|---|---|
| | OR (95% CI) | *p*-Value | OR (95% CI) | *p*-Value | OR (95% CI) | *p*-Value |
| Ethnicity | | | | | | |
| Han | 1.00 | | 1.00 | | 1.00 | |
| Uygur | 1.50 (1.10, 2.05) | 0.011 | 1.42 (0.98, 2.06) | 0.062 | 1.42 (0.98, 2.05) | 0.064 |
| Sex | | | | | | |
| Male | 1.00 | | 1.00 | | 1.00 | |
| Female | 0.78 (0.57, 1.06) | 0.113 | 0.81 (0.50, 1.31) | 0.386 | 0.78 (0.49, 1.27) | 0.321 |
| BMI (kg/m$^2$) | | | | | | |
| <18 | 1.00 | | 1.00 | | 1.00 | |
| 18–24 | 0.55 (0.31, 0.97) | 0.039 | 0.99 (0.44, 2.20) | 0.971 | 0.97 (0.43, 2.16) | 0.932 |
| >24 | 0.88 (0.47, 1.65) | 0.695 | 0.98 (0.41, 2.34) | 0.956 | 0.94 (0.39, 2.26) | 0.893 |
| Occupation | | | | | | |
| None | 1.00 | | 1.00 | | 1.00 | |
| Student | 6.66 (2.02, 22.01) | 0.002 | 5.21 (0.98, 27.66) | 0.053 | 7.53 (1.43, 39.58) | 0.017 |
| Cadre | 0.69 (0.38, 1.23) | 0.206 | 1.05 (0.42, 2.66) | 0.911 | 1.35 (0.54, 3.38) | 0.525 |
| Worker | 0.66 (0.36, 1.22) | 0.187 | 0.53 (0.24, 1.20) | 0.129 | 0.65 (0.29, 1.45) | 0.294 |
| Farmer | 1.03 (0.57, 1.86) | 0.917 | 0.78 (0.40, 1.52) | 0.458 | 0.91 (0.47, 1.79) | 0.792 |
| Retired | 1.02 (0.58, 1.80) | 0.936 | 0.96 (0.45, 2.05) | 0.919 | 1.09 (0.51, 2.31) | 0.829 |
| Educational level | | | | | | |
| Elementary school or lower | 1.00 | | 1.00 | | 1.00 | |
| Middle school | 0.79 (0.47, 1.35) | 0.391 | 1.00 (0.53, 1.89) | 0.994 | 0.97 (0.51, 1.85) | 0.929 |
| High school | 0.90 (0.55, 1.47) | 0.673 | 1.44 (0.70, 2.94) | 0.319 | 1.45 (0.71, 2.97) | 0.311 |
| College or higher | 0.54 (0.33, 0.89) | 0.016 | 0.63 (0.26, 1.50) | 0.293 | 0.57 (0.24, 1.37) | 0.210 |
| Marital status | | | | | | |
| Single | 1.00 | | 1.00 | | 1.00 | |
| Married | 0.41 (0.22, 0.75) | 0.004 | 0.75 (0.30, 1.88) | 0.535 | 0.73 (0.29, 1.84) | 0.510 |
| Widowed/divorced | 0.33 (0.16, 0.67) | 0.002 | 0.60 (0.21, 1.67) | 0.326 | 0.54 (0.19, 1.49) | 0.232 |
| Ever smoker | | | | | | |
| No | 1.00 | | 1.00 | | 1.00 | |
| Yes | 1.14 (0.84, 1.56) | 0.405 | 0.88 (0.57, 1.33) | 0.534 | 0.90 (0.59, 1.37) | 0.617 |
| Regular alcohol consumption | | | | | | |
| No | 1.00 | | 1.00 | | 1.00 | |
| Yes | 1.10 (0.81, 1.51) | 0.534 | 0.94 (0.63, 1.39) | 0.741 | 0.92 (0.62, 1.37) | 0.686 |
| DM-2 | | | | | | |
| No | 1.00 | | 1.00 | | – | – |
| Yes | 3.11 (2.25, 4.29) | <0.001 | 2.94 (2.05, 4.22) | <0.001 | – | – |
| Age (years) | 1.00 (0.99, 1.01) | 0.947 | 1.00 (0.98, 1.02) | 0.848 | 1.00 (0.98, 1.02) | 0.987 |
| Glucose (mmol/l) | 1.35 (1.22, 1.50) | <0.001 | – | – | 1.35 (1.21, 1.51) | <0.001 |

OR, odds ratio; CI, confidence interval; BMI, body mass index; DM-2, type 2 diabetes mellitus.

KS, PEL, and MCD, KSHV is also prevalent in these less developed regions with a heavier diabetes burden. The present research appears to be one of the largest studies in China to investigate the relationship between KSHV infection and DM-2.

According to a previous meta-analysis (Zhang et al., 2012), the pooled prevalence of KSHV was 11.3% (95% CI 7.2–15.5%) for the general population and 22.2% (95% CI 12.7–31.8%) for immunocompromised patients in China. The KSHV prevalence varied in different areas and was higher in the Xinjiang Uygur Autonomous Region (i.e., 21.2% among the general population and 25.6% among immunocompromised persons). In the present study of 324 patients and 376 normal controls from this region, the

**Table 3**
Risk factors for Kaposi's sarcoma-associated herpesvirus infection markers (latent antigen: ORF73; lytic antigens: ORF65, ORF-K8.1) ($n = 700$).

| | Latent antigen | | Lytic antigens | |
|---|---|---|---|---|
| | OR (95% CI) | *p*-Value | OR (95% CI) | *p*-Value |
| Ethnicity | | | | |
| Han | 1.00 | | 1.00 | |
| Uygur | 1.48 (1.01, 2.16) | 0.043 | 1.27 (0.85, 1.90) | 0.243 |
| Sex | | | | |
| Male | 1.00 | | 1.00 | |
| Female | 0.74 (0.45, 1.22) | 0.241 | 0.65 (0.38, 1.11) | 0.115 |
| BMI (kg/m$^2$) | | | | |
| <18 | 1.00 | | 1.00 | |
| 18–24 | 0.89 (0.39, 2.01) | 0.777 | 0.69 (0.29, 1.61) | 0.388 |
| >24 | 0.88 (0.36, 2.14) | 0.783 | 0.79 (0.31, 1.97) | 0.606 |
| Occupation | | | | |
| None | 1.00 | | 1.00 | |
| Student | 4.03 (0.75, 21.80) | 0.106 | 2.52 (0.49, 13.14) | 0.271 |
| Cadre | 0.94 (0.36, 2.42) | 0.894 | 0.69 (0.26, 1.87) | 0.469 |
| Worker | 0.46 (0.20, 1.06) | 0.067 | 0.48 (0.20, 1.14) | 0.097 |
| Farmer | 0.71 (0.36, 1.40) | 0.328 | 0.56 (0.27, 1.15) | 0.111 |
| Retired | 0.80 (0.37, 1.74) | 0.575 | 0.95 (0.43, 2.12) | 0.902 |
| Educational level | | | | |
| Elementary school or lower | 1.00 | | 1.00 | |
| Middle school | 0.92 (0.48, 1.76) | 0.802 | 1.21 (0.62, 2.39) | 0.578 |
| High school | 1.29 (0.62, 2.67) | 0.490 | 1.35 (0.64, 2.88) | 0.433 |
| College or higher | 0.57 (0.23, 1.41) | 0.223 | 0.74 (0.30, 1.83) | 0.509 |
| Marital status | | | | |
| Single | 1.00 | | 1.00 | |
| Married | 0.71 (0.28, 1.81) | 0.473 | 0.94 (0.34, 2.65) | 0.911 |
| Widowed/divorced | 0.60 (0.21, 1.70) | 0.335 | 0.84 (0.27, 2.65) | 0.768 |
| Ever smoker | | | | |
| No | 1.00 | | 1.00 | |
| Yes | 0.85 (0.55, 1.30) | 0.446 | 0.94 (0.59, 1.48) | 0.782 |
| Regular alcohol consumption | | | | |
| No | 1.00 | | 1.00 | |
| Yes | 0.89 (0.59, 1.34) | 0.577 | 0.87 (0.57, 1.34) | 0.534 |
| DM-2 | | | | |
| No | 1.00 | | 1.00 | |
| Yes | 3.27 (2.25, 4.75) | <0.001 | 3.99 (2.68, 5.93) | <0.001 |
| Age (years) | 1.00 (0.98, 1.01) | 0.567 | 0.99 (0.98, 1.01) | 0.571 |

OR, odds ratio; CI, confidence interval; BMI, body mass index; DM-2, type 2 diabetes mellitus.

seroprevalence of KSHV was found to be 23.7% (95% CI 19.4–28.0%) for the control group and 49.1% (95% CI 43.6–54.5%) for DM-2 patients, which is generally consistent with the previous study results. In addition, a strong association was confirmed between DM-2 and KSHV infection (OR 2.94, 95% CI 2.05–4.22) in the Han and Uygur populations in Xinjiang Province, China. The results were also strengthened by the observation of increased antibody titers and viral DNA load in participants with higher blood glucose levels. These findings are generally in agreement with those of recent studies on KSHV infection among diabetic patients from European and African countries (Caselli et al., 2014; Ingianni et al., 2007; Piras et al., 2017; Sobngwi et al., 2008). In addition, it was found that ethnicity might be inversely associated with the level of antibody titers and viral load: the Uygur population had increased antibody titer levels, while the viral DNA load was higher in the Han population. The underlying mechanism is worth further study. It is speculated that the discrepancy in immunological responses to the virus among the two populations might be due in part to different genetic composition related to the immune system, such as the human leukocyte antigen (HLA) gene complex, as reported previously (Alkharsah et al., 2007; Guech-Ongey et al., 2010).

Moreover, it was found that the expression of both latent (OR 3.27, 95% CI 2.25–4.75) and lytic (OR 3.99, 95% CI 2.68–5.93) antigens was significantly higher among patients with DM-2. Combined with the observation of a positive correlation between KSHV antibody titers and viral load ($r = 0.34$, $p < 0.001$), it could be inferred that the virus is more likely to be reactivated and replicate in higher glucose conditions. Therefore, diabetic patients are at higher risk of KS and other KSHV-associated tumors (Anderson et al., 2008; Chang et al., 2017). Although the study results conflict with those of a study by Piras et al. (Piras et al., 2017), this observation is supported by other studies that have shown that high glucose levels can significantly increase lytic reactivation of KSHV. Multiple signaling pathways involved in the lifecycle of KSHV have been proven to be modulated by high glucose in various cell types (Chang et al., 2017; Ye et al., 2016).

Nevertheless, as a result of the case–control design of this study, it was not possible to determine whether KSHV infection preceded the onset of diabetes or not. Therefore, the causal relationship between KSHV infection and DM-2 remains unresolved. It was not possible to determine whether diabetes increases susceptibility to KSHV infection by reducing the efficiency of the immune system, or, in contrast, whether the metabolism of the infecting virus modifies the microenvironment thus leading to the onset of DM-2 (Piras et al., 2017). These two hypotheses have both been supported by previous studies. Using in vitro models, Chang et al. (Chang et al., 2017) found that high glucose significantly enhanced the susceptibility of various target cells to KSHV infection by elevating the levels of specific cellular receptors for KSHV entry, including integrin $\alpha 3\beta 1$ and xCT/CD98. In contrast, other studies have suggested that the onset of diabetes may be partly due to the activity of KSHV, including cytokine secretion, altering the metabolic pathways related to insulin resistance, as with other chronic viral infections (Aytug et al., 2003; Guillen et al., 2015; Mehta et al., 2003). An alternative theory is that direct infection could induce endoplasmic

**Table 4**
Multiple linear regression analysis of the Kaposi's sarcoma-associated herpesvirus antibody titers and viral load among the immunofluorescence assay-positive population ($n$ = 248).

| | KSHV antibody titers[a] | | Viral load[b] | |
|---|---|---|---|---|
| | β (95% CI) | *p*-Value | β (95% CI) | *p*-Value |
| Ethnicity | | | | |
| Uygur vs. Han | 1.03 (0.64, 1.42) | <0.001 | −0.15 (−0.23, −0.07) | <0.001 |
| Gender | | | | |
| Female vs. male | −0.43 (−0.95, 0.09) | 0.104 | −0.02 (−0.13, 0.09) | 0.753 |
| BMI (kg/m$^2$) | | | | |
| 18–24 vs. <18 | 0.18 (−0.63, 1.00) | 0.654 | −0.15 (−0.32, 0.01) | 0.073 |
| >24 vs. <18 | 0.14 (−0.74, 1.02) | 0.749 | −0.07 (−0.25, 0.11) | 0.457 |
| Occupation | | | | |
| Students vs. none | −0.32 (−1.76, 1.12) | 0.661 | −0.04 (−0.34, 0.26) | 0.809 |
| Cadre vs. none | 0.25 (−0.68, 1.19) | 0.593 | 0.01 (−0.18, 0.21) | 0.891 |
| Workers vs. none | 0.30 (−0.53, 1.14) | 0.475 | 0.04 (−0.14, 0.21) | 0.667 |
| Farmers vs. none | −0.19 (−0.88, 0.51) | 0.598 | 0.01 (−0.13, 0.15) | 0.895 |
| Retired vs. none | 0.23 (−0.52, 0.97) | 0.549 | 0.02 (−0.14, 0.17) | 0.847 |
| Educational level | | | | |
| Middle school vs. elementary school or lower | −0.41 (−1.06, 0.24) | 0.212 | 0.00 (−0.13, 0.14) | 0.947 |
| High school vs. elementary school or lower | −0.57 (−1.27, 0.12) | 0.104 | 0.04 (−0.11, 0.18) | 0.599 |
| College or higher vs. elementary school or lower | −0.86 (−1.70, −0.03) | 0.044 | −0.03 (−0.20, 0.14) | 0.724 |
| Marital status | | | | |
| Married vs. single | −0.57 (−1.52, 0.38) | 0.241 | −0.09 (−0.28, 0.11) | 0.382 |
| Widowed/divorced vs. single | −0.76 (−1.85, 0.33) | 0.169 | −0.03 (−0.25, 0.20) | 0.826 |
| Ever smoker | | | | |
| Yes vs. no | −0.38 (−0.84, 0.08) | 0.107 | −0.07 (−0.16, 0.03) | 0.177 |
| Regular alcohol consumption | | | | |
| Yes vs. no | 0.18 (−0.23, 0.60) | 0.377 | −0.01 (−0.09, 0.08) | 0.836 |
| Age (years) | 0.00 (−0.01, 0.02) | 0.634 | 0.00 (0, 00, 0.00) | 0.701 |
| Glucose (mmol/l) | 0.24 (0.17, 0.31) | <0.001 | 0.10 (0.08, 0.11) | <0.001 |

CI, confidence interval; BMI, body mass index.
[a] After $\log_2$ transformation.
[b] Copies/$10^3$ cells.

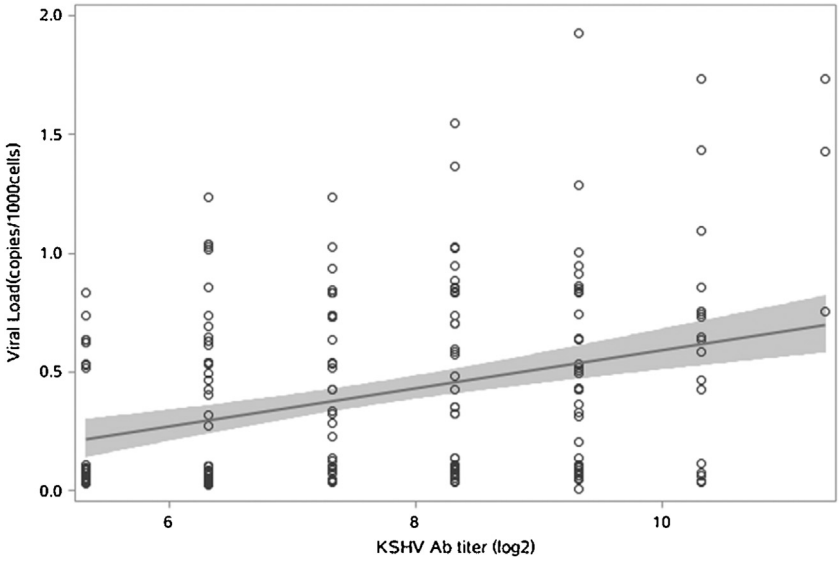


**Figure 2.** Correlation between Kaposi's sarcoma-associated herpesvirus (KSHV) antibody titers (after logarithmic transformation) and viral load (copies/$10^3$ cells) among the immunofluorescence assay-positive population ($n$ = 248).

reticulum stress in pancreatic β-cells, affecting their functions (Sobngwi et al., 2008). In the study by Lontchi-Yimagou et al., a significantly lower HOMA-β level was found in KSHV-DNA-positive diabetic patients compared with the KSHV-DNA-negative group, while no difference was observed between patients with and without KSHV antibodies. Thus, they proposed that impaired insulin secretion might be associated with the replication of the virus (Lontchi-Yimagou et al., 2018). However, most of the studies with a cross-sectional design have not provided the time at which patients acquired diabetes and the viral infection. In addition, the level of insulin secretion and insulin resistance as specific biomarkers for diabetic patients should also be taken into account in future studies. Therefore, there is a need for additional well-designed prospective epidemiological cohort studies with a large sample size to elucidate whether KSHV predisposes to DM-2 or vice versa.

In conclusion, this study proposes an association between DM-2 and KSHV infection in the Chinese Han and Uygur populations, adding to the previously reported findings that have mainly been observed in European and African countries. Given the fact that diabetes poses a heavy disease burden in China, the prevention of viral infection and development of related diseases is imperative. More well-designed longitudinal prospective cohort studies and biomedical research are warranted to better our understanding of the detailed pathophysiology of these two related conditions.

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## Ethical approval

The study protocol and collection of samples were approved and supervised by the Medical Ethics Committee of the First Affiliated Hospital, Shihezi University School of Medicine, China.

## Conflict of interest

The authors declare no conflicts of interest.

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