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Seroprevalence of Kaposi's Sarcoma-Associated Herpesvirus and Risk Factors in Xinjiang, China

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Abstract

Xinjiang, China is an endemic area for Kaposi's sarcoma (KS) but the seroprevalence of Kaposi's sarcoma-associated herpesvirus (KSHV) and risk factors remain undefined. In this study, antibodies to one KSHV latent protein (ORF73) and two KSHV lytic proteins (ORF65 and ORF-K8.1) were examined in 2,228 subjects from the general population and 37 subjects infected with HIV-1 in Xinjiang, and 560 subjects from the general population in Hubei, a low KS incidence region. The serostatus of a serum sample was defined based on positive results in any one of the three serologic assays. The seroprevalence of KSHV in the general population was higher in Xinjiang than in Hubei (19.2% vs 9.5%; odds ratios [OR], 2.28; 95% confidence interval [CI], 1.68–3.08; $P < 0.001$). Among the ethnic groups in Xinjiang, 68 (15.8%) Han, 182 (20.7%) Uygur, 140 (19.9%) Hazakh, 9 (33.3%) Xibo, and 29 (16.8%) Hui were KSHV-seropositive, respectively. Compared to the Han, the latter groups had an increase in the risk of KSHV of 62.2%, 63.8%, 180.1% and 30.2% ($P = 0.003$, 0.004, 0.018, and 0.286, respectively). Subjects aged < 20 , 20–50, and > 50 had a seroprevalence of KSHV of 11.8%, 17.9% and 24.6%, respectively. Compared to subjects aged < 20 , the latter groups had an increase in the risk of KSHV of 63.3% and 144.5% ($P = 0.009$ and < 0.001 , respectively). Subjects infected with HIV-1 in Xinjiang had a seroprevalence of KSHV of 43.2%, and a 220% increase in the risk of KSHV compared to the general population ($P < 0.001$). Similar results were obtained when the seroprevalence of KSHV was analyzed with any single or two of the three serologic assays alone. Genotyping identified 3 unique sequences clustered in the A clade. This study indicates that Xinjiang has a high seroprevalence of KSHV. Geographic location, ethnicity, age and HIV-1 infection are risk factors. Serologic and genotyping results suggest the introduction of KSHV into Xinjiang by specific ethnic groups.

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Keywords

KSHV/HHV8; Kaposi's sarcoma; seroprevalence; risk factors; genotype; Xinjiang, China

INTRODUCTION

Kaposi's sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus 8 (HHV8), was identified in a biopsy tissue from a patient with AIDS-related Kaposi's sarcoma (AIDS-KS) in 1994 [Chang et al., 1994]. KSHV is associated with all clinical forms of KS, including AIDS-KS, classical KS, endemic KS and iatrogenic KS [Boshoff et al., 1995; Chang et al., 1996; Dupin et al., 1995b; Parravicini et al., 1997; Schalling et al., 1995]. KSHV is also found in two other lymphoproliferative malignancies, including primary effusion lymphoma and multicentric Castleman's disease [Cesarman et al., 1995; Chang et al., 1994; Dupin et al., 1995a; Soulier et al., 1995].

KSHV has different subtypes [Zong et al., 2007]. These subtypes have distinct geographic distributions, and appear to migrate with the human populations. Current genotyping method of KSHV strains is based primarily on the sequence variations of the ORF-K1 gene, which contains two hyper-variable regions (VR1 and VR2) in the N-terminal domain. The genotyping method for ORF-K1 classifies KSHV strains into five major subtypes A, B, C, D and E. Subtypes A and C are predominant in Europe, the United States, and Northern Asia, whereas subtype B is characteristic for Africa. Subtype D is rare, and is found in individuals from the Pacific Islands. Subtype E is present in Brazilian Amerindians [Zong et al., 2007].

The worldwide prevalence of KSHV has been investigated extensively in the last decade, and found to vary according to geographic regions and behavioral risk factors [Greene et al., 2007]. In general, the seroprevalence of KSHV correlates with the KS incidence in a studied population. In North American and Europe where there is traditionally a low incidence of KS, the seroprevalence of KSHV is relatively low in the general population, ranging from 0–15% [Baillargeon et al., 2001; Gao et al., 1996a; Gao et al., 1996b; Kedes et al., 1996; Laney et al., 2006; Miller et al., 1996; Simpson et al., 1996]. However, a high seroprevalence of KSHV in the range of 30–40% is seen in the homosexual persons with AIDS [Gao et al., 1996a; Gao et al., 1996b; Kedes et al., 1997; Kedes et al., 1996; Simpson et al., 1996]. In Mediterranean and East European regions where there is a high incidence of classical KS, the prevalence of KSHV in the general population is between 4–24% [Angeloni et al., 1998; Gao et al., 1996b; Perna et al., 2000]. In sub-Saharan Africa where there is a high incidence of African endemic KS, the seroprevalence of KSHV is high [Gao et al., 1996b; Sitas et al., 1999; Wilkinson et al., 1999]. In this region, the seroprevalence of KSHV in the general population ranges from 30% to 70%, and the AIDS epidemic appears to aggravate further the situation. In Asia, the seroprevalence of KSHV is usually low, which is consistent with a generally low incidence of KS in this region [Ablashi et al., 1999; Fang et al., 2006; Fujii et al., 1999]. The seroprevalence of KSHV ranges from 0.14% to 0.2% in Japan, and 7% in Saudi Arabia, while in Taiwan, it is similar to that of the United States.

In mainland China, there is generally a low incidence of KS. However, a high incidence of classical KS has been reported in the Xinjiang region, indicating that this region is endemic for KS [Dilnur et al., 2001]. Limited studies have been done to examine the seroprevalence of KSHV and risk factors in China. Recent studies in some geographic regions have found a generally low seroprevalence of KSHV in the range of 0.5–7.3% in most parts of China [Fang et al., 2006; Wang et al., 2005]. The seroprevalence of KSHV was reported to be at 46.6% in 73 patients with diseases other than KS in the Uygur ethnic group in Xinjiang, a main ethnic group in this region [Dilnur et al., 2001]. A recent study has also found a 25.5% seroprevalence

of KSHV in 482 patients with different types of cancer other than KS in Xinjiang [He et al., 2007]. Thus, the seroprevalence of KSHV appears to be higher in the Xinjiang region than most other parts of China examined so far. While the high seroprevalence of KSHV in patients with diseases other than KS in Xinjiang is consistent with the endemic nature of KS [Dilnur et al., 2001], the seroprevalence of KSHV in the general population in this region has not been determined so far. The risk factors for KSHV in this region also remain unclear. Of particular interest is whether the reported high seroprevalence of KSHV is confined to populations with specific diseases, of unique geographic regions, or of particular ethnic groups.

The Xinjiang Uygur Autonomous Region is situated in the northwestern area of China. It borders with Mongolia, Russia, Kazakhstan, Kyrgyzstan, Tajikistan, and Pakistan, and adjoins Gansu, Qinghai and the Tibet Autonomous Region. While the Uygur (45.7%) and the Han (39.7%) are the main ethnic groups, there are also other ethnic groups such as the Kazakh (7%) and the Hui (4.5%) [Dilnur et al., 2001]. Although diverse and distinct, these ethnic groups intermingle traditionally among themselves. Interestingly, classical KS are seen commonly in the Uygur group, but rarely in the Han group [Dilnur et al., 2001]. In the present study, the seroprevalence of KSHV in the general population in the Xinjiang region was compared to another region in China known to have a low seroprevalence of KSHV. The seroprevalence of KSHV was examined further in different ethnic groups and in a population infected with HIV-1 in Xinjiang. The results have shown a high seroprevalence of KSHV in the general population in this region, which is associated with specific ethnic groups. Finally, genotyping has identified the KSHV subtypes that clustered in the A clade. These results provide insights into the epidemiology of KSHV in this region.

MATERIALS AND METHODS

Subjects and specimen collection

This study included 2,228 subjects from the general population and 37 subjects infected with HIV-1, all recruited in the Yili Autonomous Prefecture in Xinjiang, and 560 subjects in the Hubei Province composed exclusively of Han people recruited from the general population in Wuhan. Permission to conduct the study and informed consent was obtained in accordance with a protocol approved by the Ethics Committee of Wuhan Institute of Virology, Chinese Academy of Sciences. A questionnaire on age, gender and ethnicity was collected. The ethnicity of a subject was self-reported. Blood samples were collected between June and August in 2007. Personal identifiers were removed to ensure sample confidentiality. Serum samples from 22 patients with classical KS in Xinjiang were also obtained and used as positive controls for the serologic assays. Another 50 specimens from healthy blood donors characterized in a previous study were used as negative controls for the serologic assays [Fang et al., 2006].

Serologic assays

Three viral recombinant proteins including the latent nuclear antigen encoded by ORF73, and the lytic antigens ORF-K8.1 and ORF65 were used as antigens in the study [Gao et al., 1996a; Simpson et al., 1996; Zhu et al., 1999]. The antigens were expressed as 6 × His-tagged recombinant proteins, purified with a nickel column, and used in the ELISA as described previously [Simpson et al., 1996]. Briefly, purified recombinant ORF73, ORF65 and ORF-K8.1 proteins were diluted in 0.05 M sodium carbonate/bicarbonate buffer solution, pH 9.6, at 1, 0.5 and 1 µg/ml, respectively, and coated onto 96-well ELISA plates (Greiner Bio One, Frickenhausen, Germany) at 50 µl per well. The plates were covered and incubated overnight at 4 °C, and then washed three times with 250 µl per well of PBS containing 0.05% Tween-20. Next, the plates were blocked with 200 µl per well of the blocking solution containing 5% skim milk, 1% normal goat serum, and 0.05% Tween-20 in PBS. The plates were incubated further for 2 h at 37°C, and washed again three times. Serum samples diluted at 1:100 in the blocking

solution were added to each well at 50 µl per well, and incubated for 2 h at 37°C. The plates were washed five times. A goat anti-human-IgG alkaline phosphatase conjugate (Vector Laboratories Inc., Burlingame, CA) diluted at 1:3,000 in the blocking solution was added to the plates at 50 µl per well. After incubation at 37°C for 2 h, the plates were washed again five times, and a substrate solution containing 1 mg/ml of para-nitrophenylphosphate in 10% diethanolamine, pH 9.8, at 50 µl per well was added. After 30 min of reaction at 37°C, a stop solution containing 3N NaOH was added at 50 µl per well. The plates were read at 405 nm on an automated ELISA Plate Reader. A serum named S558 from an AIDS-KS patient that has high antibody titers to both KSHV latent and lytic antigens, and a serum named H14 from a healthy blood donor without any specific antibodies to KSHV were used as positive and negative controls, respectively, in all the assays. Both positive and negative controls were characterized in a previous study [Baillargeon et al., 2001], and used in three wells in each plate in this study. Each sample was also tested three times. The absorbance value of each tested sample was calibrated with the controls so that the results from different plates could be compared. A serum sample with an absorbance value above the averages plus five standard deviations of the negative control wells in an assay was considered as positive for the assay. Previous studies had demonstrated the high specificity and sensitivity of these assays [Simpson et al., 1996; Zhu et al., 1999; Laney et al., 2006]. The specificity and sensitivity of these assays in this study were validated further with serum samples from 22 subjects with classical KS, and 50 healthy blood donors that were determined negative for antibodies to ORF65 protein in a previous study [Fang et al., 2006]. A serum was considered KSHV-seropositive if it was tested positive in any one of the three assays. Further comparative analyses were also performed by defining the serostatus of a sample based on the results of any single or two of the three serologic assays.

Genotyping and phylogenetic analysis

DNA from peripheral blood mononuclear cells of KSHV-seropositive subjects was extracted with a Blood & Cell Culture DNA Mini Kit according to the instructions of the manufacturer (Qiagen, Valencia, CA). The ORF-K1 VR1 region was amplified by nested-PCR and sequenced. The external primers were 5'-GTCTTTCAGACCTTGTTGG-3' and 5'-CCCGTTAGAACAAGTATA-3', which amplified a product of 517 bp. The internal primers were 5'-GACCTTGTTGGACATCCTG-3' and 5'-GTATTAGTTTGTGACACGG-3', which amplified a product of 455 bp. DNA sequences from 7 specimens were obtained in this study. Three of the unique sequences and 23 other KSHV strains obtained from GenBank were used to construct the phylogenetic tree using Clustal X Sequence Editor, Version 1.81. The aligned sequences were used in the Phylip 3.67 software package to construct Neighbor-Joining (NJ) Trees. The statistical reliability of the NJ tree was evaluated using 1,000 bootstrap samples. The trees were drawn with the TreeView 1.4 Program. The sequences of 23 other KSHV strains consisted of 6 strains of subtype A: BC-1 (U75698), EMA7 (AF130305), BCBL-R (AF133038), BCBL-B (AF133039), US3 (AF151688) and Ug3 (AF151690); 5 strains of subtype B: G11 (AF130260), G41 (AF130261), G51 (AF130262), Ug52 (AF130290) and Ug81 (AF130291); 5 strains of subtype C: ASM72 (AF133041), BC2 (AF133042), US6 (AF151686), GK17 (AF130267) and GK18 (AF130268); 5 strains of subtype D: TKS10 (AF133043), ZKS3 (AF133044), Au1 (AF151687), J24 (AF278844) and J26 (AF278846); and 2 strains of subtype E: Tupi1 (AF220292) and Tupi2 (AF220293) [Ishak Mde et al., 2007].

Statistical analysis

The seroprevalence of KSHV and the corresponding 95% confidence intervals (CIs) were calculated using standard epidemiologic methods [Kleinbaum et al., 1988]. Risk factors associated independently with the presence of antibodies to KSHV antigens were assessed by univariate chi-square test. Odds ratios (OR) and the 95% CIs were used to quantify the relationships in estimates while *P*-values were calculated to indicate the statistical significance.

95% CIs were calculated based on coefficients and standard errors [Kleinbaum et al., 1988]. A *P*-value less than 0.05 was considered to be significant.

RESULTS

Serologic Assays

Three ELISA assays that detected specific antibodies to one KSHV latent antigen (ORF73) and two KSHV lytic antigens (ORF65 and ORF-K8.1) were used to examine the serostatus of KSHV in the studied subjects. These assays have been reported and used previously in epidemiologic studies of KSHV [Simpson et al., 1996; Zhu et al., 1999; Laney et al., 2006]. To evaluate the overall sensitivity and specificity of the assays, serum samples from 22 classical KS patients from Xinjiang, and 50 healthy blood donors tested KSHV-seronegative using the ORF65 ELISA assay in a previous study [Fang et al., 2006] were examined (Table I). Classical KS patients are usually 100% positive for antibodies to KSHV [Gao et al., 1996b]. Of 22 serum samples from the classical KS patients, all were positive in all three serologic assays except 2 serum samples that were negative in the ORF65 assay. Taking into consideration of all three serologic assays, all the 22 serum samples were determined to be KSHV-seropositive. Of the 50 healthy blood donors, 2 (4%) were positive in the ORF-K8.1 assay but negative in the other two assays. While these two samples were scored positive, their signals were weak and close to the cutoff. Overall, the serologic assays had a combined sensitivity of 100% and specificity of 96% in this study. Furthermore, the three serologic assays were highly concordant in these two control populations.

The three serologic assays were used to examine serum samples from 2,228 subjects from the general population in Xinjiang, 37 subjects infected with HIV-1 from Xinjiang and 560 subjects from the general population in Hubei. The median, and the first and third quartile ranges of the absorbance values in the three serologic assays for all the studied populations are shown in Fig. 1. The concordance of the serologic assays in the three studied populations was examined further (Table II). In two-way comparisons, the assays were highly consistent in at least 89.5% of the combined concordant positive and negative serum samples in the general population in either Xinjiang or Hubei. The combined concordant positive and negative samples were 92.1% between the ORF73 and ORF65 assays, 93.4% between the ORF73 and ORF-K8.1 assays, and 89.5% between the ORF65 and ORF-K8.1 assays in the general population in Xinjiang, and 95.1% between the ORF73 and ORF65 assays, 93.5% between the ORF73 and ORF-K8.1 assays, and 92% between the ORF65 and ORF-K8.1 assays in the general population in Hubei (Table II). In subjects infected with HIV-1 from Xinjiang, the assays were less consistent but they still had consistent results in at least 75.7% of the combined concordant positive and negative serum samples. The combined concordant positive and negative samples were 81.1% between the ORF73 and ORF65 assays, 83.8% between the ORF73 and ORF-K8.1 assays, and 75.7% between the ORF65 and ORF-K8.1 assays in this population (Table II). These results were in agreement with those of other studies [Engels et al., 2000; Inoue et al., 2004; Laney et al., 2006]. Since specific antibodies to each of the three KSHV antigens used in this study might reflect the distinct status of KSHV infection and replication, the least stringent condition was used to determine the serostatus of KSHV in a subject in this study. A serum sample was determined to be KSHV-seropositive if it was tested positive for any one of the three serologic assays.

The Seroprevalence of KSHV in the General Population in Xinjiang

Of 2,228 subjects in the general population in Xinjiang, 1,090 were male (48.9%) and 1,138 were female (51.1%) (Table III). Among them, there were 431 Han people (19.3), 878 Uygur people (39.4%), 705 Hazakh people (31.6%), 173 Hui people (7.8%), 27 Xibo people (1.2%),

and 14 others (0.6%) consisting of Mongolia, Sala, and Uzbek people. The age of the population ranged from several days to 94 years old with a mean value of 41.4 years (Table III).

Of 2,228 subjects, 428 (19.2%) were KSHV-seropositive (Table I). The seroprevalence of KSHV in this population was compared with the general population in Hubei, a region known to have low seroprevalence of KSHV [Fang et al., 2006]. Of 560 subjects from the general population in Hubei, 53 (9.5%) were KSHV-seropositive (Table I). Univariate chi-square test showed that subjects from the general population in Xinjiang had a 128% increase in the risk for KSHV compared to their counterparts in Hubei ($P < 0.001$) (Table V).

The seropositivity of KSHV in the general population in Xinjiang was assessed further according to demographic factors. There was no statistical difference between males and females (18.3% vs 20.0%, $P = 0.2046$). Among the ethnic groups, the Uygur, Hazakh and Xibo people had a higher seroprevalence of KSHV than the Han people (20.7%, 19.9% and 33.3% vs 15.8%, respectively) (Table IV). Univariate chi-square test showed that, compared to the Han, the Uygur, Hazakh and Xibo people had 62.2% ($P = 0.003$), 63.8% ($P = 0.004$) and 180.1% ($P = 0.018$) increases in the risk for KSHV, respectively (Table IV). The Hui people also had 30.2% increase in the risk for KSHV but it was not statistically significant ($P = 0.286$). No KSHV-seropositive subjects were found in the 14 subjects of the other people (Mongolia, Sala and Uzbek) but the numbers were too small for assessment.

Since a large proportion of the Han people in Xinjiang had immigrated to the region from other parts of China in the last 50 years, the Han people in Xinjiang were compared with the subjects from Hubei, all of whom were also Han people (Table V). The Han people had a higher seroprevalence of KSHV in Xinjiang than in Hubei (15.8% vs 9.5%). Univariate chi-square test showed that the Han people in Xinjiang had a 66.7% increase in the risk for KSHV compared to their counterparts in Hubei ($P = 0.003$).

Examination of the subjects by age showed that the seroprevalence of KSHV increased with age with subjects aged < 20 , 20–50, and > 50 having 11.8%, 17.9% and 24.6% of KSHV-seropositive rates, respectively (Table IV). Univariate chi-square test indicated that subjects aged 20–50 and > 50 had 63.3% ($P = 0.009$) and 144.5% ($P < 0.001$) increases in the risk for KSHV, respectively, compared to subjects aged < 20 (Table IV).

The Seroprevalence of KSHV in Subjects infected with HIV-1 in Xinjiang

Since HIV-1 is a risk factor for KSHV infection [Gao et al., 1996a; Gao et al., 1996b; Kedes et al., 1996], a group of subjects infected with HIV-1 from Xinjiang was examined. Of 37 subjects infected with HIV-1 from Xinjiang, 16 (43.2%) were KSHV-seropositive. Compared with subjects from the general population, HIV-1-infected subjects in Xinjiang has a 220% increase in the risk for KSHV ($P < 0.001$) (Table V).

Analyses of the Seroprevalence of KSHV in Different Xinjiang and Hubei Populations Based on the Concordance of Two Serologic Assays

While the serologic assays had a high overall consistency among the different studied populations, results of the seropositive serum samples were less consistent (Table II). Although these discrepancies could be due to the expression of different antigens associated with distinct phases of KSHV infection and replication, they might also reflect the limitations of the serologic assays. Thus, the seroprevalence of KSHV in different studied populations were examined further based on the concordance of any two of the three serologic assays (Table VI). In all the populations, as expected, the seroprevalence of KSHV was lower when it was defined with any two of the three serologic assays than with any one of the three serologic assays combined (Table V). Similar results were also obtained when a serum sample's

seropositivity was defined with any single serologic assay. Nevertheless, the conclusions based on the positive results of any one of all three serologic assays combined remained valid (Table VI). The seroprevalence of KSHV remained significantly higher in the general population in Xinjiang than in Hubei, in the Han population in Xinjiang than in Hubei, and in subjects infected with HIV-1 than in the general population in Xinjiang (Table VI).

KSHV Genotypes in Xinjiang

DNA samples from the peripheral blood mononuclear cells of 48 KSHV-seropositive subjects selected randomly from the general population in Xinjiang were examined further for the presence of detectable KSHV DNA. KSHV DNA was amplified from 7 of these specimens. The products of the ORF-K1 VR1 region were sequenced, and the corresponding KSHV subtypes were named as XJ1, XJ2, ..., XJ7. Three unique sequences including XJ1, XJ2 and XJ6 were identified while sequences of XJ3, XJ4, XJ5 and XJ7 were the same as that of XJ2 (Fig. 2A). These sequences differ from all known ORF-K1 sequences deposited in GenBank so far. XJ1 sequence is related closely to the BC-1 and BCBL-1 sequences but differs by 9 and 8 nucleotide positions, respectively. These results ruled out any possible contaminations by these common laboratory cell lines, and indicated good quality of the sequences. Phylogenetic analysis of the 3 unique sequences with the available sequences of 23 other KSHV strains in GenBank showed that these KSHV strains clustered together and belonged to the subtype A (Fig. 2B).

DISCUSSION

Xinjiang is a region endemic for KS. Previous studies have shown a high seroprevalence of KSHV in the range of 25.5% to 46.6% in patients with diseases other than KS from this region [Dilnur et al., 2001; He et al., 2007]. Nevertheless, the seroprevalence of KSHV has not been determined in the general population in this region before. In this study, the seroprevalence of KSHV was examined in 2,228 subjects from the general population in Xinjiang. This represented the largest serologic study for KSHV to date in this region. The overall seroprevalence of KSHV was at 19.2% in the general population in Xinjiang, which was substantially higher than the 9.5% seroprevalence of KSHV in the control subjects from the general population in Hubei ($P < 0.001$). Previous studies have also shown that the seroprevalence of KSHV is low in the general population in other parts of China ranging 5.2% to 8% [Fang et al., 2006; Wang et al., 2005]. Together, these results indicate that Xinjiang is a unique region where the seroprevalence of KSHV is significantly higher than other parts of China. This high seroprevalence of KSHV is consistent with the high incidence of KS in this region.

Interestingly, among the five ethnic groups examined that had sufficient subjects for statistical analysis, three of them (Uygur, Hazakh and Xibo) had significantly higher seroprevalence of KSHV than the Han group ($P = 0.003$, 0.004 , and 0.018 , respectively). However, the Han people in Xinjiang also had significantly higher seroprevalence of KSHV than their counterparts in Hubei ($P = 0.003$). Since a large proportion of the Han people in Xinjiang have immigrated to this region only in the last 50 years, it is likely that KSHV had migrated to the Xinjiang region with certain ethnic groups in the ancient time and persisted in the population, and the Han group from other parts of China had relatively low infection rates of KSHV before the migration but attracted the new infection after migrating to the region and exposing to the endemic population. This hypothesis is supported further by the results of genotyping. All the KSHV strains examined in this study were classified in the A clade (Fig. 2B). Co-migration of viruses with the human population is common. KSHV subtypes A and C are predominant in the Mediterranean, Middle Eastern and Asian regions [Zong et al., 2007], which were parts of the ancient Great Silk Road that went through the Xinjiang region. KSHV subtype C has

also been found in Xinjiang in another study [Dilnur et al., 2001]. Thus, it is tempting to speculate that KSHV might have been spread along the Silk Road with the human population. While related closely, the identified ORF-K1 sequences from Xinjiang differed from those of BC-1 and BCBL-1. Thus, it is unlikely that they were derived from contaminations of these common laboratory cell lines. Additional studies are needed to determine further the distribution of KSHV genotypes in this region.

The seroprevalence of KSHV in the general population in Xinjiang also increased with age. Subjects aged 20–50 and > 50 had 63.3% and 144.5% higher risk for KSHV than those aged < 20 ($P = 0.009$ and < 0.001 , respectively). These results are consistent with those of several other studies, which described an increase of seroprevalence of KSHV with age in different populations [Baillargeon et al., 2001; Sitas et al., 1999; Sitas et al., 2001; Stein et al., 2004; Zavitsanos et al., 2007].

Previous studies have shown that HIV-1 is a risk factor for KSHV infection [Gao et al., 1996a; Gao et al., 1996b; Kedes et al., 1996; Simpson et al., 1996]. Results from this study have shown that subjects infected with HIV-1 had a 220% higher risk for KSHV than those from the general population in Xinjiang ($P < 0.001$). These results, which are consistent with other studies, indicate that HIV-1 infection is also a risk factor for KSHV in this region.

In conclusion, the seroprevalence of KSHV is high in the Xinjiang region, and this high seroprevalence is associated preferentially with specific ethnic groups. Additional studies are needed to delineate further the risk factors for KSHV infection and the boundary of the endemic region of KSHV in Xinjiang. Results of these studies should be useful for the control and prevention of malignancies associated with KSHV infection, particularly in the era of worldwide HIV/AIDS epidemic.

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Fig. 1. Distribution of relative antibody titers reflected by absorbance values for serum samples from subjects of different populations examined with three KSHV serologic assays. Boxes represent the median, and the first and third quartile values while the bars represent the minimum and maximum values.

Fig. 2.

Genotyping of KSHV strains in Xinjiang. **A:** Comparison of the ORF-K1 VR1 sequences from three unique KSHV strains XJ1, XJ2, and XJ6 identified in this study with that of a subtype A sequence from the BC-1 cell line. **B:** Unrooted phylogenetic tree showing the relationship among the XJ1, XJ2, and XJ6, and 23 other KSHV strains available in the GenBank. The tree was constructed with the Neighboring-Joining (NJ) method using segments of the ORF-K1 VR1 region. The statistical reliability of the NJ tree was evaluated using 1,000 bootstrap samples. Branch lengths are drawn to scale, with the bar indicating 0.1-nt replacement per site. Numbers on the nodes indicate the percentage of bootstrap samples (1000) in which the cluster is supported.

TABLE I
Study Populations and Their Seroprevalence of KSHV

Population	Total	KSHV-seropositive subjects	
		N (%)	95% CI ^a
Classical KS (positive controls)	22	22 (100.0)	58.2–141.8
Healthy blood donors (negative controls)	50	2 (4.0)	1.1–13.5
Xinjiang general population	2,228	428 (19.2)	18.4–20.0
Xinjiang HIV-1-positive population	37	16 (43.2)	29.3–57.1
Hubei general population	560	53 (9.5)	8.7–10.3

^aConfidence interval.

TABLE II
Two-Way Comparisons of Three Serologic Assays for Detecting the Seroprevalence of KSHV in Different Populations^a

Xinjiang general population				P-value
ORF73 vs ORF65	ORF73+	ORF65+	ORF65-	Total
		84 (3.8)	83 (3.7)	167 (7.5)
	ORF73-	94 (4.2)	1967 (88.3)	2061 (92.5)
	Total	178 (8)	2050 (92)	2228 (100)
				< 0.0000
ORF73 vs K8.1	ORF73+	K8.1+	K8.1-	Total
		101 (4.5)	66 (3)	167 (7.5)
	ORF73-	81 (3.6)	1980 (88.9)	2061 (92.5)
	Total	182 (8.1)	2046 (91.9)	2228 (100)
				< 0.0000
K8.1 vs ORF65	K8.1+	ORF65+	ORF65-	Total
		62 (2.8)	120 (5.4)	182 (8.2)
	K8.1-	116 (5.2)	1931 (86.7)	2047 (91.8)
	Total	178 (8)	2051 (92.1)	2228 (100)
				< 0.0000
Xinjiang HIV-1-infected population				P-value
ORF73 vs ORF65	ORF73+	ORF65+	ORF65-	Total
		7 (18.9)	1 (2.7)	8 (21.6)
	ORF73-	6 (16.2)	23 (62.2)	29 (78.4)
	Total	13 (35.1)	24 (64.9)	37 (100)
				0.0005
ORF73 vs K8.1	ORF73+	K8.1+	K8.1-	Total
		4 (10.8)	4 (10.8)	8 (21.6)
	ORF73-	2 (5.4)	27 (73)	29 (78.4)
	Total	6 (16.2)	31 (83.8)	37 (100)
				0.0034
K8.1 vs ORF65	K8.1+	ORF65+	ORF65-	Total
		5 (13.5)	1 (2.7)	6 (16.2)
	K8.1-	8 (21.6)	23 (62.2)	31 (83.8)
	Total	13 (35.1)	24 (64.9)	37 (100)
				0.0069

Xinjiang general population					P-value
Hubei general population					P-value
ORF73 vs ORF65	ORF73+	ORF65+	ORF65-	Total	< 0.0000
	ORF73-	15 (2.7)	529 (94.4)	544 (97.1)	
	Total	19 (3.4)	541 (96.6)	560 (100)	
ORF73 vs K8.1	ORF73+	K8.1+	K8.1-	Total	< 0.0000
	ORF73-	28 (5)	516 (92.1)	544 (97.1)	
	Total	38 (6.8)	522 (93.2)	560 (100)	
K8.1 vs ORF65	K8.1+	ORF65+	ORF65-	Total	< 0.0000
	K8.1-	13 (2.3)	509 (90.9)	522 (93.2)	
	Total	19 (3.4)	541 (96.6)	560 (100)	

^aShow in number (%).

TABLE III
Demographics of the General Population in Xinjiang

Subject	Number	Percentage
Overall	2,228	100
Gender		
Male	1,090	48.9
Female	1,138	51.1
Ethnic group		
Han	431	19.3
Uygur	878	39.4
Hazakh	705	31.6
Hui	173	7.8
Xibo	27	1.2
Others	14	0.6
Age		
< 20	314	14.1
20–50	1,195	53.6
> 50	719	32.3

TABLE IV
Seroprevalence of KSHV and Univariate Chi-Square Test in Different Groups in the General Population in Xinjiang

Group	KSHV-positive subjects		Chi-square test		
	N (%)	95% CI ^d	OR ^b	95% CI	P-value
Gender					
Male	199 (18.3)	17.8–20.4	1.00 ^c	-	-
Female	228 (20.0)	19.6–22.2	1.14	0.92–1.41	0.249
Ethnic group					
Han	68 (15.8)	14.3–17.3	1.00 ^c	-	-
Uygur	182 (20.7)	19.3–22.1	1.62	1.18–2.22	0.003
Hazakh	140 (19.9)	18.4–21.4	1.64	1.18–2.28	0.004
Hui	29 (16.8)	14.3–19.3	1.30	0.80–2.11	0.286
Xibo	9 (33.3)	20.7–45.9	2.80	1.2–6.56	0.018
Others	0 (0)	0	0	0	0.999
Age					
< 20	37 (11.8)	8.7–15.8	1.00 ^c	-	-
20–50	214 (17.9)	15.8–20.2	1.63	1.12–2.37	0.009
> 50	177 (24.6)	21.6–27.9	2.45	1.67–3.59	< 0.001

^aConfidence interval

^bOdds ratio

^cReference category.

TABLE V
Seroprevalence of KSHV and Univariate Chi-Square Test in Different Populations from Hubei and Xinjiang

Population	KSHV-seropositive subjects N (%)	Chi-square test		
		OR ^a	95% CI ^b	P-value
Hubei vs Xinjiang general population				
Hubei general population	53 (9.5)	1.00 ^c	-	-
Xinjiang general population	428 (19.2)	2.28	1.68–3.08	< 0.001
Hubei vs Xinjiang Han population				
Hubei Han population	53 (9.5)	1.00 ^c	-	-
Xinjiang Han population	68 (15.8)	1.67	1.19–2.33	< 0.003
Xinjiang general vs HIV-1 population				
Xinjiang general population	428 (19.2)	1.00 ^c	-	-
Xinjiang HIV-1 population	16 (43.2)	3.20	1.66–6.19	< 0.001

^a Odds ratio

^b Confidence interval

^c Reference category.

TABLE VI
Seroprevalence of KSHV and Univariate Chi-Square Test in Different Populations from Hubei and Xinjiang Based on a Single or Two Serologic Assays

	ORF73 & ORF65		ORF73 & K8.1		K8.1 & ORF65		ORF65		ORF73		K8.1	
	+	-	+	-	+	-	+	-	+	-	+	-
Hubei vs Xinjiang general population												
Hubei ^a	4	556	10	550	6	554	19	541	16	544	38	522
Xinjiang	84	2144	101	2127	62	2166	178	2050	167	2061	182	2046
P-value	0.0004		0.004		0.0000		0.002		0.0001		n.s.	
OR ^b	5.5		2.61		40		2.47		2.75			
95% CI ^c	1.9–17.9		1.3–5.3		16.7–104		1.5–4.1		1.6–4.8			
Hubei vs Xinjiang Han population												
Hubei ^a	4	556	10	550	6	554	19	541	16	544	38	522
Xinjiang	14	54	16	52	10	58	28	40	26	42	29	39
P-value	0.0000		0.0000		0.0000		0.0000		0.0000		0.0000	
OR	36.06		16.9		15.9		19.9		21.0		10.2	
95% CI	10.8–153.6		6.8–43.6		5.0–54.8		9.7–41.0		9.9–45.1		5.4–19.0	
Xinjiang general vs HIV-1 population												
General ^a	84	2144	101	2127	62	2166	178	2050	167	2061	182	2046
HIV-1	7	30	4	33	5	32	13	24	8	29	6	31
P-value	0.0000		0.072		0.0001		0.0000		0.0014		0.0784	
OR	5.96		2.6		5.46		6.2		3.4		2.2	
95% CI	2.1–14.3		0.64–7.3		1.6–14.8		2.8–13.0		1.3–7.8		0.73–5.4	

^aReference category

^bOdds ratio

^cConfidence interval.