High prevalence of early childhood infection by Kaposi's sarcoma-associated herpesvirus in a minority population in China

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Abstract

In China, KSHV seroprevalence varies considerably among different regions and ethnicities. But in Xinjiang province, located in the northwestern China, there is a very high seroprevalence of KSHV in adults of Kazak and Ughur ethnicities. However, KSHV prevalence in children and the risk factors associated with the acquisition of infection are currently not known. The aim of this study was to investigate the prevalence of KSHV infection and identify associated socioeconomic or behavioural risk factors and the humoral immune response among children in this population. This is a cross-sectional study (N = 178) to screen children and their caregivers from Xinjiang for total KSHV antibodies, KSHV neutralizing antibodies and HIV infection. Structured questionnaires were utilized to investigate risk factors associated with KSHV prevalence. KSHV seroprevalence in children and caregivers in Xinjiang was 48.3% and 64.7%, respectively. Neutralizing antibody was detected in most seropositive caregivers (93.8%) but was detected in only 5.8% of the infected children. A significant association was observed between child KSHV seroprevalence and sharing of food among family members. These results suggest that similar to other endemic areas in Africa, KSHV infection in the minority populations of Xinjiang is likely to be occurring during early childhood, probably via horizontal transmission through saliva, and results in high seroprevalence in the adult population.

Keywords: China, Kaposi's sarcoma-associated herpesvirus, seroprevalence, Xinjiang
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Introduction

Kaposi sarcoma (KS)-associated herpesvirus (KSHV), or Human herpesvirus 8 (HHV8), is the aetiological agent associated with KS [1,2]. Global seroprevalence of KSHV varies in different geographical regions. It is generally low to moderate in Western countries (3–23%) but endemic in the general population (>50%) in sub-Saharan Africa and even higher in HIV-positive individuals [3–5].

As in most Asian countries [6], the incidence of KS and seroprevalence of KSHV is low in most provinces of China (7.3–16.1% in adults) [7–10]. Xinjiang province, situated in northwestern China, has a significantly higher incidence of KS (classic and AIDS-associated) and a higher seroprevalence of KSHV in adults [11]. The higher prevalence could be associated with the ethnic makeup of the population. In mainland China, Han is the major ethnic group but in Xinjiang, other ethnicities such as Uygur, Kazaks and Hui are in the majority [10,11]. Studies conducted in the Uygur and Kazak ethnic groups have reported KSHV seroprevalence in adults to be as high as 46.6% [10–12]. Interestingly, Xinjiang also has one of the highest prevalences of HIV infection in China, especially among injection drug users, in whom prevalence can be as high as 80% [13,14].

The exact routes of KSHV transmission are unclear and may differ by geographical region and risk group. Sexual transmission, organ transplant and blood transfusion in adults have been reported [15–18]. Saliva is considered to be the major route of transmission from infected adults to children in sub-

Saharan Africa, and early childhood infection could be contributing to the high prevalence of KSHV in the adult population [19,20]. The unique lifestyle and culture of the Uyghurs and the Kazakh ethnic groups in Xinjiang could facilitate salivary contact and thereby enhance early childhood KSHV infection, and subsequently high prevalence in the population, as seen in KS endemic regions.

Most reports published so far have investigated prevalence and risk factors in adults and not much is known about the prevalence and risk of KSHV infection in children in the Xinjiang region. We hypothesize that early childhood infection in Xinjiang is common and contributes to the high prevalence of KSHV in the population. Therefore, the goal of the current study is to investigate the serological profile of and immune response to KSHV in children and their caregivers, and determine the risk factors that may be associated with KSHV prevalence in children.

Material and Methods

Study cohort

Between March and October 2011, caregivers having children between 6 and 60 months of age, attending local clinics in Xinyuan and Jiashi Counties in Xinjiang province, were approached to participate in this study. Children over 6 months of age were recruited to avoid the detection of transplacental maternal antibodies. Recruitment occurred from at least three clinics representing different regions of the county to ensure random distribution of the study subjects and reflect the general population of the region, where the majority is of Uygur or Kazakh ethnicity. The caregivers were educated about the study and signed informed consent was obtained. This study was approved by the institutional review boards at the University of Nebraska and Hangzhou Normal University.

Sample collection

Blood samples were collected in EDTA tubes from children and their caregivers and plasma was separated. Specimens collected from children and caregivers were coded by a unique identification number. All specimens were stored at −70°C until testing.

Data collection

A standardized format was used to collect information on study participants and the data included socioeconomic status, home living conditions, lifestyle risk factors and child care. A trained interviewer conducted field-based intake interviews with the child's primary caregiver. Data collection instruments that were used in the study represent modified versions of the data forms used by our ongoing household study in Zambia, but were field tested and adapted to the local setting [21].

Serological testing

KSHV serological test. All plasma samples were tested using a monoclonal-enhanced immunofluorescence assay (mIFAs) as previously described [22]. Briefly, BC3 cells were stimulated with tetradecanoyl phorbol acetate (TPA), then fixed with 4% paraformaldehyde and incubated with patient plasma. The signal was enhanced using murine monoclonal anti-human immunoglobulin and then incubated with fluorescein-isothiocyanate conjugated anti-mouse antibody. Slides were read independently by two experienced laboratory workers. All positive plasmas were diluted further and tested by mIFA to estimate the KSHV antibody titre of each sample.

HIV-1 test. All plasma samples were tested by a standard HIV-1 test kit (1 + 2 type antibody diagnostic kits, Beijing Wantai Biological Pharmacy Enterprise Company, Beijing, China) and confirmed by a second kit (Colloidal Gold Device, Beijing Wantai Biological Pharmacy Enterprise Company).

KSHV neutralization assay. Neutralizing antibodies against KSHV were detected for all seropositive children and caregivers by a flow cytometry-based neutralization assay, as described earlier with minor modifications [23]. Plasma was diluted and mixed with recombinant KSHV expressing GFP (rKSHV.219) and incubated for 1 h at 37°C. The virus and plasma mixture was added to a 96-well plate seeded with 293 cells. After incubation for 72 h, green cells were counted by flow cytometry. Results were confirmed by counting at least 100 cells under a fluorescence microscope. A positive neutralizing antibody outcome was defined as ≥50% reduction in infectivity as compared with the sero-negative control serum. Positive plasma samples were diluted further to determine the neutralizing antibody titre.

Statistical analysis

All data were analysed using the statistical software packages, SPSS version 17 (SPSS, Chicago, IL, USA) and SAS v9.2 (SAS Institute, Cary, NC, USA). Descriptive analyses and distribution of all key variables were conducted. Risk factor analysis was conducted using logistic regression. Multivariable analysis included all variables that had a p-value ≤0.20 to control for possible confounders and identify independent associations. We also checked the correlation between all variables to identify interactions and multi-collinearity. Correlation analysis was evaluated by Pearson's correlation coefficient.