

Epidemiological Report

Gender Differences in Kaposi's Sarcoma-Associated Herpesvirus Infection in a Population with Schistosomiasis in Rural China

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SUMMARY: Kaposi's sarcoma-associated herpesvirus (KSHV) is the causal agent of Kaposi's sarcoma (KS), a common cancer in patients with acquired immunodeficiency syndrome. The risk factors for KSHV infection have been extensively studied for Western countries but remain largely undefined for other parts of the world. Schistosomiasis, caused by *Schistosoma japonicum* infection, was recently identified as a cofactor for KSHV infection in rural Egypt. In this study, we examined the seroprevalence of KSHV in a population along the Yangtze River in China that has a high incidence of schistosomiasis. KSHV seroprevalence in subjects with schistosomiasis was slightly higher than that in subjects without schistosomiasis, but the difference was not statistically significant (8.4% versus 6.6%; $P = 0.204$). However, after adjusting for gender, KSHV seroprevalence in men with schistosomiasis was found to be significantly higher than that in men without schistosomiasis (8.4% versus 2.8%; odds ratio [OR], 3.170; 95% confidence interval [CI], 1.501–6.694; $P = 0.002$). Compared to men, women showed significantly higher seroprevalence of KSHV (5.9% versus 9.3%; OR, 1.621; 95% CI, 1.084–2.425; $P = 0.019$).

INTRODUCTION

Kaposi's sarcoma-associated herpesvirus (KSHV) was first identified in a human immunodeficiency virus (HIV) patient with Kaposi's sarcoma (KS) (1). KSHV has consistently been detected in lesions from all four clinical forms of KS, including acquired immunodeficiency syndrome (AIDS)-KS, classical KS, endemic KS, and transplantation KS. Furthermore, KSHV has been detected in primary effusion lymphoma and multicentric Castleman's disease. Seroprevalence of KSHV varies depending on the geographic regions and behavioral risk factors of the studied populations. KSHV seroprevalence in the general population is high (30–70%) in sub-Saharan Africa (2,3), and median (4–24%) in the Mediterranean and Eastern European regions (2,4,5). In most parts of mainland China, KSHV seroprevalence ranges from 0.5–7.3% (6,7); however, Xinjiang has a high incidence of KS and a KSHV seroprevalence of 19.2% (8).

Specific socioeconomic and environmental factors have been reported to be associated with KSHV infection. Bites from blood-sucking insects have been shown to be a cofactor in KSHV cases in some underdeveloped

countries (9). A recent study showed that the association of KSHV with antischistosomal antibodies was not significant for men but was marginally significant for women (10). As many as 65 million people in China are at risk for schistosomiasis; of these, 726,112 people are already infected with *Schistosoma japonicum* (11). Gonggan County in Hubei Province, which is located in the central Yangtze River region, is the area most severely affected by schistosomiasis, with 740,000 infected people. In this study, we examined KSHV seroprevalence in Gonggan County. Although infection by *S. japonicum* is not associated with KSHV seropositivity, we found that compared to men not infected by *S. japonicum*, men infected by *S. japonicum* had significantly higher KSHV seroprevalence.

MATERIALS AND METHODS

Diagnosis of schistosomal infections: Between July and December 2008, 1,398 serum samples were collected in Gonggan County, Hubei Province. Of these samples, 702 were obtained from patients with schistosomal infection and 696 were collected from a population that was negative for *S. japonicum*. Permission to conduct the study and informed consent of patients was obtained in accordance with a protocol approved by the Ethics Committee of Wuhan Institute of Virology, Chinese Academy of Sciences. Information on the age and gender was collected. Schistosomal infections were diagnosed by performing a standardized enzyme-linked immunosorbent assay (ELISA) to detect species-specific

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antibodies against *S. japonicum* (Shenzhen Kangbaide Biotech Co., Shenzhen, China). To exclude false seropositivity, seropositive patients were further confirmed by stool examination using Kato-Katz thick smear slides. Presence of eggs on the slides confirmed schistosomiasis.

Detection of KSHV: ELISAs were performed to detect specific antibodies against three KSHV antigens: latent nuclear antigen (encoded by open reading frame 73 [ORF73]) and the lytic antigens (encoded by ORF-K8.1 and ORF65) (12). These assays have been previously used in epidemiologic studies of KSHV (8,12–14). All antigens were expressed as 6X-His-tagged recombinant proteins, purified using a nickel column, and used in ELISA as described previously (13). Briefly, purified recombinant ORF73, ORF65, and ORF-K8.1 proteins were diluted in 0.05 M sodium carbonate/bicarbonate buffer (pH 9.6) to obtain concentrations of 1, 0.5, and 1 µg/ml, respectively. Next, recombinant proteins were 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. Each well was washed 3 times with 250 µl of PBS containing 0.05% Tween-20 and blocked with 200 µl of blocking solution (5% dried skim milk, 1% normal goat serum, and 0.05% Tween-20 in PBS). The plates were incubated for 2 h at 37°C and again washed 3 times. Serum samples were 1:200 diluted in blocking solution, and 50 µl of this solution was added to each well. The plates incubated for 2 h at 37°C and then washed 5 times. An alkaline phosphatase-conjugated goat anti-human-IgG (Vector Laboratories Inc., Burlingame, Calif., USA) was diluted at 1:3,000 in blocking solution and was added to each well (50 µl per well). After incubation at 37°C for 2 h, the plates were again washed 5 times, and 50 µl of substrate solution containing 1 mg/ml of para-nitrophenyl phosphate in 10% diethanolamine (pH 9.8) was added to each well. After 30 min of reaction at 37°C, 50 µl of stop solution containing 3 N NaOH was added to each well. The absorbance of the plates was measured at 405 nm using an automated ELISA Plate Reader. A serum sample (S558) obtained from an AIDS-KS patient having high titers for antibodies to KSHV latent and lytic antigens was used as a positive control. A serum sample (H14) obtained from a healthy blood donor without any KSHV antibodies was used as a negative control. Both the positive and negative controls used in this study were characterized in a previous study (15). Each sample was tested 3 times. A serum sample with an absorbance value more than the average value plus five standard deviations of the negative control was considered positive for the assay. If a serum sample scored positive in any one of the three serologic assays, it was considered KSHV seropositive.

Diagnosis of hepatitis B: Hepatitis B virus (HBV) infection was diagnosed using a commercial HBV kit by measuring anti-HBV antibodies (Wuhan IND Biotechnology Co., Wuhan, China). The blood samples were tested for IgG against hepatitis B surface antigen (HBsAg) and hepatitis B core (HBc). Individuals who were positive for antibodies against HBc or HBsAg were considered HBV positive.

Statistical analysis: KSHV seroprevalence and the corresponding 95% confidence intervals (CI) were cal-

culated using standard epidemiologic methods. Risk factors associated with the presence of KSHV antibodies were assessed separately by univariate and multivariate logistic regression analyses. Odds ratios (OR) and the 95% CI were determined to quantify the relationships between estimates, while *P* values were calculated to determine statistical significance. CI was calculated based on coefficients and standard errors from the logistic model. A *P* value less than 0.05 was considered statistically significant.

RESULTS

To examine the serostatus of our subjects for KSHV, a combination of three ELISAs to detect specific antibodies to one KSHV latent antigen (encoded by ORF73) and two KSHV lytic antigens (encoded by ORF65 and ORF-K8.1) was used. Overall, the KSHV serological assay had a combined sensitivity of 100% and a specificity of 96% (8).

Of the 1,398 subjects, 741 (53%) were men and 657 (47%) were women. The seroprevalence of antigens encoded by ORF73, ORF-K8.1, and ORF65 were 5.8%, 6.3%, and 6.2%, respectively. The assays were highly consistent, and an 89.5% concordance was obtained for serum samples detected to be positive and negative by the three assays. The overall KSHV seroprevalence was 7.4%, which is consistent with the results of previous studies (6,8). Although previous studies have shown higher KSHV infection in men (2,12), we found that women had higher KSHV seroprevalence than men (9.3% versus 5.9%; *P* = 0.019) (Table 1). Univariate logistic regression analyses showed that female subjects had 62.1% increased risk for KSHV seropositivity (OR, 1.621; 95% CI, 1.084–2.425; *P* = 0.019). In univariate analysis, KSHV seroprevalence was not associated with age; the seroprevalence of KSHV in subjects aged <20, 20–50, and >50 were 7.4%, 7.6%, and 7.4%, respectively (Table 1). Further, KSHV seroprevalence was not associated with schistosomal infection (8.4% in subjects with schistosomal infection versus 6.6% in subjects without schistosomal infection; OR, 1.297; 95% CI,

Table 1. Prevalence and risk factors for KSHV infection among study subjects by univariate statistical analysis

Group	KSHV-positive subjects, no. (%)	OR	95% CI	<i>P</i>
Gender				
Male	44 (5.9)	1.00 ¹⁾	—	—
Female	61 (9.3)	1.621	1.084–2.425	0.019
Age				
<20	9 (7.4)	1.00 ¹⁾	—	—
20–50	54 (7.6)	1.040	0.499–2.165	0.917
>50	42 (7.4)	0.999	0.473–2.110	0.997
Schistosomiasis				
Negative	46 (6.6)	1.00 ¹⁾	—	—
Positive	59 (8.4)	1.297	0.869–1.935	0.204
HBV				
Negative	49 (7.0)	1.00 ¹⁾	—	—
Positive	56 (8.1)	1.177	0.790–1.753	0.423

¹⁾ Reference category.

KSHV, Kaposi's sarcoma-associated herpesvirus; HBV, hepatitis B virus; OR, odds ratio; CI, confidence interval.

Table 2. Prevalence and risk factors for KSHV infection among study subjects using multivariate statistical analysis

Risk factor	B	S.E.	Wald	P	OR	95% CI
Gender	0.532	0.208	6.543	0.011	1.703	1.132–2.560
Age <20 ¹⁾	—	—	0.025	0.988	—	—
20–50	–0.053	0.378	0.019	0.889	0.949	0.453–1.989
>50	–0.060	0.384	0.024	0.876	0.942	0.443–2.000
Schistosomiasis	0.322	0.209	2.379	0.123	1.380	0.917–2.077
HBV	0.54	0.205	0.569	0.451	1.167	0.781–1.743
Constant	–2.987	0.390	58.554	0.000	0.050	—

¹⁾: Reference category.

B, regression coefficient; S.E., standard error of arithmetic mean.

Table 3. Prevalence and risk factors for KSHV infection among study subjects analyzed by gender by univariate statistical analysis

Group	KSHV-positive subjects, no. (%)	OR	95% CI	P
Male				
Age				
<20	4 (5.9)	1.00 ¹⁾	—	—
20–50	24 (6.7)	1.150	0.386–3.426	0.802
>50	16 (5.1)	0.856	0.277–2.646	0.787
Schistosomiasis				
Negative	9 (2.8)	1.00 ¹⁾	—	—
Positive	35 (8.4)	3.170	1.501–6.694	0.002
HBV				
Negative	22 (6.1)	1.00 ¹⁾	—	—
Positive	22 (5.8)	0.952	0.518–1.752	0.875
Female				
Age				
<20	5 (9.3)	1.00 ¹⁾	—	—
20–50	35 (10.2)	0.925	0.342–2.497	0.877
>50	28 (10.8)	1.113	0.407–3.041	0.835
Schistosomiasis				
Negative	37 (9.9)	1.00 ¹⁾	—	—
Positive	24 (8.5)	0.844	0.493–1.446	0.537
HBV				
Negative	27 (7.9)	1.00 ¹⁾	—	—
Positive	34 (10.8)	1.421	0.836–2.415	0.194

¹⁾: Reference category.

0.869–1.935; $P = 0.204$) (Table 1). Similar results were obtained by multivariable analysis; KSHV seroprevalence was only associated with gender (OR, 1.703; 95% CI, 1.132–2.560; $P = 0.011$) (Table 2). In multivariate analysis, KSHV seroprevalence was not associated with schistosomal infection (OR, 1.380; 95% CI, 0.917–2.077; $P = 0.123$) (Table 2). However, univariate logistic regression analyses for different genders showed that KSHV seroprevalence in men with schistosomal infection was higher than that in men without the infection (8.4% versus 2.8%; OR, 3.170; 95% CI, 1.501–6.694; $P = 0.002$), but this trend was not observed in women (OR, 0.844; 95% CI, 0.493–1.446; $P = 0.537$) (Table 3). Multivariable analysis yielded results similar to that of univariate analysis; KSHV seroprevalence in men with schistosomal infection was higher than that in men without this infection (OR, 3.213; 95% CI, 1.516–6.810; $P = 0.002$) (Table

4). These results are in contrast to those of a previous study showing a marginal association of KSHV seroprevalence with schistosomal infection in women, but not in men, in rural Egypt (10). KSHV seroprevalence among subjects with or without HBV infection was not different (OR, 1.177; 95% CI, 0.790–1.753; $P = 0.423$) (Table 1).

DISCUSSION

KSHV seroprevalence among the general population of sub-Saharan Africa is reported to be 40–60% and that in South Africa is 20–40% (2,3). The seroprevalence in countries such as Italy, Greece, and Spain is mid-range; these countries show high incidences of classical KS (2,4,5,12). However, KSHV seroprevalence is low in Northern Europe and the United States (2,4,5,8,12,13). Few studies have examined KSHV seroprevalence and the risk factors for KSHV infection in mainland China.

We found that KSHV seroprevalence in Gonggan County was 7.5%, which is consistent with the results of previous studies performed on a general population from mainland China (6,7). Interestingly, we observed that women had higher KSHV seroprevalence than men; this is in contrast to the findings of most of the studies on homosexual and/or HIV-infected subjects (2,5,8,13). These data suggest that sexual behavior is an unlikely risk factor in this population. Although previous studies have observed an association of KSHV infection with age and HBV infection, we did not observe this association in our study.

A recent study conducted in rural Egypt showed that schistosomal infection increased susceptibility to KSHV infection at relatively low viral exposure (10). That study reported a 2-fold increase in KSHV seroprevalence in subjects with schistosomal antibodies. Another study reported that the association between anti-KSHV antibodies and anti-schistosomal antibodies was not significant for men but was marginally significant for women (15).

In this study, we retested the hypothesis that schistosomal seropositivity is associated with KSHV seropositivity. Our results showed that subjects infected with *S. japonicum* had a higher KSHV infection rate than those negative for schistosomal infection (8.4% versus 6.6%), but the association of KSHV seroprevalence with schistosomal infection was not significant. However, univariate analysis showed significant associ-

Table 4. Prevalence and risk factors for KSHV infection among study subjects analyzed by gender using multivariate logistic regression analysis

Risk factor	B	S.E.	Wald	P	OR	95.0% CI
Age < 20 ¹⁾	—	—	0.847	0.655	—	—
20–50	–0.018	0.564	0.001	0.975	0.982	0.325–2.969
> 50	–0.314	0.583	0.291	0.590	0.730	0.233–2.289
Schistosomiasis	1.167	0.383	9.270	0.002	3.213	1.516–6.810
HBV	–0.096	0.314	0.093	0.760	0.909	0.491–1.681
Constant	–3.376	0.594	32.321	0.000	0.034	—

¹⁾: Reference category.

Abbreviations are in Table 2.

ation between KSHV seroprevalence and schistosomal infection in men (OR, 3.170; 95% CI, 1.501–6.694; $P = 0.002$). In the rural regions of China, compared to women, men are more likely to spend more time in the farm with a concomitant increase in risk of attracting *S. japonicum*. Our results indicate that the modes of KSHV infection in men and women may be different in this population, and schistosomal infection is a risk factor for men but not women.

Certain species of blood-sucking arthropods have been suggested to promote KSHV transmission at the site of the bite when KSHV-infected relatives rub their saliva on the bite sites on child to relieve the child's itching (16). Further, aggressive, non-anthropophilic species that are mainly encountered outdoors have been reported to have the greatest potential to be promoter insects. These insects include Culicinae mosquitoes, sand flies, black flies, and biting midges; bites of all these insects elicit strong skin reactions. Although *S. japonicum* is not a blood-sucking arthropod, its bite can also elicit strong skin reactions. Our results showed that schistosomal infection among men was associated with KSHV infection, suggesting that schistosomal infection may promote KSHV infection in this population. Thus, our results support the promoter arthropod hypothesis of KSHV infection (16).

Our results showed that subjects infected with *S. japonicum* had a higher KSHV seroprevalence than those negative for schistosomal infection. However, the association of KSHV seroprevalence with schistosomal infection was only significant among male patients, highlighting a gender difference in KSHV infections in this rural Chinese population.

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Conflict of interest None to declare.

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