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Gender Difference of Kaposi's sarcoma-associated Herpesvirus Infection in Population with Schistosomiasis in Rural China

Bishi Fu[1,2], **Fan Xia**[3], **Baolin Li**[1,2], **Xinxing Ouyang**[1,2], **Shou-Jiang Gao**[4], **Rongge Yang**[2,*], and **Linding Wang**[1,2,*]

[1]Department of Microbiology, Anhui Medical University, Hefei 230032, China

[2]State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China

[3]College of Biological Science, Yangtze University, Jingzhou 434025, Hubei, China

[4]Department of Molecular Microbiology and Immunology, Keck School of Medicine, University of Southern California, Los Angeles, California, USA

Summary

Kaposi sarcoma-associated herpesvirus (KSHV) is the causal agent of (KS), a common cancer in AIDS patients. The risk factors for KSHV infection have been extensively studied in Western countries but remain largely undefined in other parts of the World. Schistosomiasis caused by infection of Schistosoma japonicum is recently identified as a cofactor for KSHV infection in rural Egypt. In this study, we examined the seroprevalence of KSHV in a population with high incidence of Schistomasis along the Yangtze River in China. The seroprevalence of KSHV is slightly higher in subjects with than without Schistosomiasis but it is not statistically significant (8.4% vs 6.6%, p = 0.204). The seroprevalence of KSHV is significantly higher in female than male subjects (9.3% vs 5.9%, OR: 1.621, 95% CI: 1.084–2.425, p = 0.019). When adjusted for gender, the seroprevalence of KSHV is significantly higher in subjects with than without Schistosomiasis in male subjects (8.4% vs 2.8%, OR: 3.170, 95% CI: 1.501–6.694, p = 0.002).

Keywords

associated herpesvirus; schistosomiasis; seroprevalence; risk factor

Introduction

Kaposi sarcoma -associated herpesvirus (KSHV) was initially identified in a human immunodeficiency virus (HIV) patient with Kaposi's sarcoma (KS) (1). KSHV was consistently detected in lesions of all four clinical forms of KS, including AIDS-KS, classical KS, endemic KS and transplantation KS. KSHV has also been found in primary

*Address correspondence and reprint request to: Dr. Linding Wang, Department of Microbiology, Anhui Medical University, Hefei, 230032, China. Tel: +86-551-5123422. Fax: +86-551-5123422. wanglinding@hotmail.com.

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Authors' contributions

BF carried out the immunoassay and drafted the manuscript. FX, BL, XO participated in design and coordination of study, data and sample collection. SJG revised manuscript for important intellectual content. RY and LW conceived study, provided guidance to all aspects of study, and performed quality assessment of data, data analysis, data preparation, and drafted manuscript. All authors read and approved final manuscript.

effusion lymphoma and multicentric Castleman's disease. KSHV seroprevalence varies according to geographic regions and behavioral risk factors of the studied populations. KSHV seroprevalence in the general population is high in sub-Saharan Africa ranging 30–70% (2, 3) and median in Mediterranean and East European regions ranging 4–24% (2, 4, 5). In most parts of mainland China, KSHV seroprevalence is in the range of 0.5–7.3% (6, 7); however, in Xinjiang where there is high incidence of KS, it is 19.2% (8).

Specific socioeconomic or environmental factors have been associated with KSHV infection. Exposure to bites from blood-sucking insects has been proposed as a cofactor in some undeveloped countries (9). A recent study has shown that KSHV association with antischistosomal antibodies was not significant for men but marginal for women (10). As many as 65 million people in China are threatened by schistosomiasis among which 726,112 people are already infected by *S. japonicum* (11). Gongan County in Hubei Province, located in the middle of Yangtze River, is the most severe region threatened by schistosomiasis with 740,000 people afflicted. In this study, we examined KSHV seroprevalence in the Gongan County. While infection by *S. japonicum* is not associated with KSHV seropositivity, we found that male subjects infected by *S. japonicum* had significantly higher KSHV seroprevalence.

Materials and Methods

Schistosomal infections definition

A total of 1,398 serum samples were collected from July to December of 2008 in Gongan County, Hubei Province, of which 702 were from patients with schistosomal infection, while 696 were collected form general population which was negative for *S. japonicum*. 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 Scineces. A questionaire on age, gender was collected. Schistosomal infections were first diagnosed with a standardized enzyme-linked immunosorbent assay (ELISA) to detect species-specific antibodies against *S. japonicum* (Shenzhen Kangbaide Biotech Co., Shenzhen, People's Republic of China). To exclude false seropositivity, seropositive persons were further confirmed by stool examination using Kato-Katz thick smear slides. Persons with eggs on the slides were confirmed as patients with schistosomiasis who has definitely schistosomal infections.

KSHV diagnosis

ELISA assays were used to detect specific antibodies to three KSHV antigens: latent nuclear antigen encoded by ORF73 and the lytic antigens ORFK8.1 and ORF65 (12). These assays have been used previously in epidemiologic studies of KSHV(8, 12–14). All the antigens were expressed as 6×His-tagged recombinant proteins, purified with a nickel column, and used in the 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 solution, pH 9.6, at 1, 0.5 and 1 μg/ml, respectively, and coated onto 96-well ELISA plates (Greiner Bio One, 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 blocking solution containing 5% dried skimmed milk, 1% normal goat serum, and 0.05% Tween-20 in PBS. The plates were further incubated for 2 h at 37°C, and again washed three times. Serum samples diluted at 1:200 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 again washed 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 at 50 μl per well was added. The plates were read at 405 nm on an automated ELISA Plate Reader. A serum named S558 from an AIDS-KS that has high antibody titers to both KSHV latent and lytic antigens, and a serum named H14 from a healthy blood donor without any KSHV antibodies were used as positive and negative controls. Both positive and negative controls were characterized in a previous study (15). Each sample was tested three times. 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. If a serum sample is positive in any one of the three serologic assays, it is considered KSHV-seropositive.

HBV diagnosis

HBV infection was diagnosed with commercial HBV kit by measuring anti-HBV antibodies (Wuhan IND Biotechnology Co., Ltd, Wuhan, China). The blood samples were tested for HBsAg, anti-HBc IgG. Individuals who were positive for antibodies to HBc, or HBsAg were considered to have HBV infection.

Statistical analysis

KSHV seroprevalence and corresponding 95% confidence intervals were calculated using standard epidemiologic methods. Risk factors associated with the presence of KSHV antibodies were assessed by univariate and multivariable logistic regression analyses separately. Odds ratios (OR) and the 95% confidence intervals (CI) were used to quantify the relationships in estimates while p-values were calculated to indicate the statistical significance. CI was calculated based on coefficients and standard errors from the logistic model. A p-value less than 0.05 were considered to be significant.

Results

To examine KSHV serostatus in the studied subjects, a combination of three ELISA assays was used, which detected specific antibodies to one KSHV latent antigen (ORF73) and two KSHV lytic antigens (ORF65 and ORF-K8.1). Overall, the KSHV serological assays had a combined sensitivity of 100% and specificity of 96% (8).

Of 1,398 subjects, 741 (53%) were males and 657 (47%) were females. The KSHV seroprevalence detected by ORF 73 was 5.8%, the KSHV seroprevalence detected by ORF-K8.1 was 6.3%, and the KSHV seroprevalence detected by ORF 65 was 6.2%, the assays were highly consistent in at least 89.5% of the combined concordant positive and negative serum samples in the studied population. The overall KSHV seroprevalence was 7.4%, which is consistent with results of previous studies (6, 8). While previous studies have shown a preferential KSHV infection in males (2, 12), we found that females had higher KSHV seroprevalence than males (9.3% *vs* 5.9%, p = 0.019) (Table 1). Logistic regression analysis showed that female subjects had 62.1% increase in the risk for KSHV-seropositivity (OR: 1.621, 95% CI: 1.084–2.425, p=0.019). KSHV seroprevalence was not associated with age. Subjects at age <20, 20–50, and >50 had 7.4%, 7.6% and 7.4% KSHV seroprevalence, respectively (Table 1). KSHV seroprevalence was also not associated with schistosomal infection (8.4% vs 6.6%, OR: 1.380, 95% CI: 0.869–1.935, p = 0.204) (Table 1). In a multivariable analysis, similar results were obtained. KSHV seroprevalence was also not associated with schistosomal infection (OR: 1.380, 95% CI: 0.917–2.077, p = 0.123) (Table 2). However, when analyzed by gender, KSHV seroprevalence was higher in subjects with than without schistosomal infection in males (8.4% *vs* 2.8%, OR: 3.170, 95% CI: 1.501–

6.694, p=0.002) but not in females (OR: 0.844, 95% CI: 0.493–1.446, p = 0.537) (Table 3). These results are in contrast to those of a previous study showing marginal association of KSHV seroprevalence with schistosomal infection in females but not males in rural Egypt (10). In a multivariable analysis, similar results were also obtained. When analyzed by gender, KSHV seroprevalence was higher in subjects with than without schistosomal infection in males (OR: 3.213, 95% CI: 1.516–6.810, p=0.002) (Table 4).No difference was observed among subjects with or without HBV infection (OR: 1.177, 95% CI: 0.790–1.753, p=0.423) (Table 1).

Discussion

KSHV seroprevalence in the general population in sub-Saharan Africa is in the order of 40–60% and 20–40% in South Africa (2, 3). It is in the medium range in countries such as Italy, Greece and Spain, where there are higher incidence of classical KS (2, 4, 5, 12). However, KSHV seroprevalence is low in Northern Europe and US (2, 4, 5, 8, 12, 13). In mainland China, limited studies have been done to examine KSHV seroprevalence and the risk factors for KSHV infection.

We have found that KSHV seroprevalence in Gongan County is 7.5%, which is consistent with the results of previous studies performed in the general population in mainland China (6, 7). Interestingly, we found that women had higher seroprevalence of KSHV than men, which is in contrast to most of the studies performed in homosexual and/or HIV-infected subjects (2, 5, 8, 13), suggesting that sexual behaviors are unlikely risk factors in this population. While previous studies have observed an association of KSHV infection with age and HBV infection, we failed to confirm this observation in this population.

It was shown in a recent study conducted in rural Egypt that schistosomal infection could increase susceptibility to KSHV infection at relatively low exposure to the virus (10). This work also found a 2-fold increase in KSHV seroprevalence in persons who had schistosomal antibodies. The association of anti-KSHV antibodies with anti-schistosomal antibodies was not significant for men, but marginal for women (15).

In this study, we sought to retest the hypothesis that schistosomal seropositivity is associated with KSHV seropositivity. Our results showed that subjects infected with *S. japonicum* had higher KSHV infection rate than those negative for schistosomal infection (8.4% *vs* 6.6%), but the association of KSHV seroprevalence with schisotosomal infection was not significant. However, among men, the association of KSHV seroprevalence with schisotosomal infection was significant (OR: 3.170, 95% CI: 1.501–6.694, p=0.002). In the rural region of China, men are more likely to have longer exposure time in the farm with increasing risk of attracting *S. japonicum* than women. Our results showed that men and women in this population might have different modes of KSHV infection, and schistosomal infectionis a risk factor for men but not women.

It has been suggested that certain species of blood-sucking arthropods could promote KSHV transmission at the bite site when KSHV-infected relatives rub their own saliva on the sites to relieve a child's scratching (16). It has also been suggested that more-aggressive species that bite mainly outdoors and that are not primarily anthropophilic could have the greatest potential to be promoter insects. These insects include Culicinae mosquitoes, sand flies, black flies and biting midges, all of which could elicit strong skin reactions. Although *S. japonicum* is not blood-sucking arthropod, its biting can also elicit strong skin reactions. Our results showed that schisosomal infection among males was associated with KSHV infection, suggesting a promotion effect of schisotosomal infection on 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 higher KSHV infection ratio than those negative for schistosomal infection, the association of KSHV seroprevalence with schistosomal infection was only significant among male patients, showing gender difference of KSHV infection in population with schistosomiasis in rural China.

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List of Abbreviations

KSHV	associated herpesvirus; KS
ELISA	enzyme-linked immunosorbent assay
OR	Odds ratios
CI	confidence intervals

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Table 1

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

Group	KSHV-positive subjects, N (%)	OR	95% CI	P-value
Gender				
Male	44 (5.9)	1.00	-	-
Female	61 (9.3)	1.621	1.084–2.425	0.019
Age				
< 20	9 (7.4)	1.00	-	-
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	-	-
Positive	59 (8.4)	1.297	0.869–1.935	0.204
HBV				
Negative	49 (7.0)	1.00	-	-
Positive	56 (8.1)	1.177	0.790–1.753	0.423

Table 2

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

Risk factor	B	S.E.	Wald	P-value	OR	95% CI
Sex	0.532	0.208	6.543	0.011	1.703	1.132–2.560
Age <20			0.025	0.988		
Age 20–50	−0.053	0.378	0.019	0.889	0.949	0.453–1.989
Age >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	

B: Regression coefficient, S.E: standard error of arithmetic mean, SEM: 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, N (%)	OR	95% CI	P-value
Male				
Age				
< 20	4 (5.9)	1.00	-	-
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	-	-
Positive	35 (8.4)	3.170	1.501–6.694	0.002
HBV				
Negative	22 (6.1)	1.00	-	-
Positive	22 (5.8)	0.952	0.518–1.752	0.875
Female				
Age				
< 20	5 (9.3)	1.00	-	-
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	-	-
Positive	24 (8.5)	0.844	0.493–1.446	0.537
HBV				
Negative	27 (7.9)	1.00	-	-
Positive	34 (10.8)	1.421	0.836–2.415	0.194

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-value	OR	95.0% C.I
Age <20			0.847	0.655		
Age 20–50	−0.018	0.564	0.001	0.975	0.982	0.325–2.969
Age >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	

B: Regression coefficient, S.E: standard error of arithmetic mean, SEM: standard error of arithmetic mean;