Epidemiol. Infect. (2017), **145**, 2330–2340. © Cambridge University Press 2017
doi:10.1017/S0950268817001054

Differential prevalence and correlates of whole blood Epstein–Barr virus DNA between HIV-positive and HIV-negative men who have sex with men in Shanghai, China

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Received 14 January 2017; Final revision 29 March 2017; Accepted 25 April 2017; first published online 5 June 2017

SUMMARY

This cross-sectional study aimed to examine and compare prevalence and correlates of whole blood Epstein–Barr virus (EBV) DNA between HIV-positive and HIV-negative men who have sex with men (MSM). Five hundred and four HIV-positive MSM and 504 age-matched HIV-negative MSM were recruited from an HIV counseling and testing clinic in Shanghai, China from November 2014 to November 2015 and were administered with a face-to-face questionnaire interview. Whole blood EBV DNA was tested by nested polymerase chain reaction assays on *EBNA-1*, *EBNA-2,* and *LMP-1* genes. The prevalence of whole blood EBV DNA was 56·0% (95% CI 51·7–60·3%) among HIV-positive MSM and 26·0% (95% CI 22·4–30·0%) among HIV-negative MSM. Whole blood EBV DNA positivity was significantly associated with HIV infection (adjusted odds ratio (aOR) 3·43, 95% CI 2·58–4·57) and frequent intake of pickled, smoked, or salty food (aOR 1·71, 95% CI 1·02–2·86) in the whole sample, and with <200 cells/μl CD4 cell counts (aOR 1·79, 95% CI 1·05–3·05) and pickled, smoked, or salty food intake (aOR 3·14, 95% CI 1·39–7·08) in HIV-positive group. HIV-infected MSM are at higher risk of active EBV replication than HIV-uninfected MSM, underscoring needs of surveillance and research on EBV-related carcinogenesis in this population.

Key words: Cancer, EBV, HIV, MSM, risk factors.

INTRODUCTION

Highly active antiretroviral therapy (HAART) or combination antiretroviral therapy (cART) has significantly increased life expectancy of people living with HIV/AIDS (PLWHA) in the past decades [1]. This has resulted in a shift in patterns of morbidities and mortalities in this population. A remarkable note is that both AIDS-defining cancers (ADCs) and non-AIDS-defining cancers (NADCs) are more often seen among PLWHA than among the general population [2–4]. PLWHA are at long-term status of immunodeficiency, immune activation and inflammation caused by HIV infection [5, 6], and have been observed to be at higher risks for both ADCs and

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NADCs than general population even after cART [7–9]. In addition to higher prevalence of infection with cancer-related viruses such as hepatitis C virus (HCV), human papilloma virus (HPV), Kaposi sarcoma associated herpes virus (KSHV) among PLWHA, higher prevalence of certain behavioral and psychological cancer risk factors such as tobacco use, heavy drinking and depression, etc. also plays important roles in carcinogenesis in this population. Thus, surveillance on cancer incidence and mortality as well as risk factors in this population are important for learning the disease burden and targeting people at high risk for cancer. Moreover, the associations between risk factors and carcinogenesis might be different in this immune compromised population, appealing for more detailed exploration.

The Epstein–Barr virus (EBV) is from the herpes virus family and persistently infects more than 90% of human adults. EBV infects B cells and epithelial cells with different mechanisms and replication patterns, and shows life-long latency after the initial lytic period [10]. Although the infection is ubiquitous and usually asymptomatic, EBV is constantly associated with certain types of human cancers including Hodgkin's lymphoma (HL) [11], non-Hodgkin's lymphoma (NHL) [12, 13] and nasopharyngeal cancer (NPC) [14, 15], among which NHL is one of the three ADCs. It is believed that genetic characteristics from both the viral and host sides, diet, behavioral, and environmental risk factors interplay in EBV-associated cancer development. In addition to EBV infection, risk factors for NPC include HPV infection, male, several dietary and behavioral factors such as salted fish intake and smoking, and family history of NPC [16, 17]; potential risk factors for HL include age, male, weakened immune system, and family history of the disease [18]; and risk factors for NHL vary by subtypes, including male, HIV infection, oncogene activation (*c-MYC*), malaria endemicity, family history, cigarette smoking, alcohol consumption, autoimmune diseases, and occupational factors [13, 19].

EBV DNA can be detected in both tumor tissues and blood samples from patients with EBV-related cancers, and circulating viral DNA level was proved to be an important biomarker in risk stratification, disease progression, and prognosis for NPC [15, 20, 21] and lymphoma [22, 23]. It has been reported that the EBV DNA were detected more often among HIV infected individuals than negative ones, suggesting a more active viral replication [24]. However, little has been explored for the association between non-viral risk factors and circulating EBV DNA in understanding risk and progression of EBV-related cancers especially in PLWHA.

Approximately 575 000 people were living with HIV/AIDS in China at the end of 2015. Higher prevalence and mortality of cancers have already been observed in this population [25]. Additionally, homosexual transmission of HIV between men has been markedly increasing in recent years, reporting a HIV prevalence of 8% in 2015 [26]. High prevalence of HIV infection and risky behaviors made it an urgent need to have close observation on cancer development in this population.

We hypothesize that HIV infection and other potential risk factors of EBV-related cancers may be associated with EBV DNA replication. This study was thus performed to compare whole blood EBV DNA positivity between HIV-positive and HIV-negative men who have sex with men (MSM), as well as to identify potential correlates with EBV DNA positivity in Shanghai, China.

METHODS

Study design

All HIV-positive MSM and 1:1 age-matched (±5 years) HIV-negative MSM were recruited from an HIV counseling and testing clinic in Shanghai from November 2014 to November 2015 and were administered with a questionnaire interview. The study was approved by the Institutional Review Board of Fudan University and written informed consent was obtained from each participant.

Study participants

Subjects who met the following inclusion criteria were enrolled: (1) male; (2) 18 years old or above; (3) can give oral or written informed consent in Mandarin; and (4) had oral or anal sex with men within the past 12 months.

Data collection

Participants were administered with a face-to-face questionnaire interview by trained and experienced health professionals to ascertain socio-demographic characteristics including age, residency, ethnic group, education level, marital status, and monthly income; and behavioral characteristics including lifetime

homosexual behaviors, number of male sexual partners, tobacco smoking, alcohol drinking, dietary habits especially on unhealthy food intake, and history of illicit drug use. HIV-related clinical characteristics such as $CD4^+$ T cell counts, diagnosis of AIDS and status of receiving ART were obtained from the Chinese National Information System for AIDS Prevention and Control (CNISAPC) given that all PLWHA in China have registered with and been routinely followed-up by CNISAPC.

Participants were asked to report their frequency of pickled, smoked, or salty food intake, and frequency of having fresh vegetables or fruits in the past 6 months. Frequencies were reported as 'never', 'occasionally', or 'frequently or daily'.

For the measurement of tobacco smoking, alcohol drinking and illicit drug use, the definitions were as following. Smoked in the past 6 months was defined as had smoked at least one cigarette every day during that period. Drank in the past 6 months was defined as had drunk alcohol at least once per week during the past 6 months. Types of alcohol consumed include beer, yellow wine, rice wine, Chinese white wine (a kind of distilled spirit), red wine, white wine, whisky, brandy, etc. Illicit drugs use in the past 6 months was defined as used methamphetamine, ecstasy, heroin, marijuana, opium, cocaine, sedatives, ketamine or poppers (amyl nitrites) during the past 6 months.

Participants were asked about sexual behaviors and the frequency of condom use. And the total number of sexual partners in life time was also collected.

EBV DNA extraction, amplification, and sequencing

For each participant, 5 ml venous blood withdrawn using an EDTA anticoagulation sterilized tube were further aliquoted in both whole blood and plasma, respectively. EBV-DNA was extracted from 200 µl whole blood in a nucleic acid-free room by High Pure Viral Nucleic Acid Kit (Roche, Germany) according to the manufacturer's instruction. The DNA was eluted into 50 µl elution buffer provided by the kit and stored at −20 °C until testing by nested polymerase chain reaction (PCR).

Nested PCR was performed to amplify the EBNA-1, EBNA-2, and LMP-1 genes of EBV. PCR reaction system was prepared in another nuclease-free room. The reaction mixture contained a final concentration of 1×PCR buffer, 200 µM of each dNTP, 0·4 µM of each primer, and 1·25 U of Taq polymerase. Outer and inner primers used for EBNA-2, EBNA-1, and LMP-1 genes in this study are shown in Table 1. About 1 µl of DNA (25–30 ng) was added into the mixture with aerosol-resistant pipette tips to avoid cross-pollution between samples in the nucleic acid extraction room. PCR amplification was performed in a different room to avoid pollution. The integrity of the extracted DNA and the exclusion of PCR inhibitors in samples were confirmed by amplifying the human gene. The first amplification round included an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 1 min, primers annealing at 50 °C (EBNA-2)/47 °C (EBNA-1)/52 °C(LMP-1) for 1 min, and an extension step at 72 °C for 1 min, followed by the final extension step at 72 °C for 10 min. For the second round, 1 µl of the first round PCR product was used as template and amplified under the same conditions. The second round of amplification products were tested in 2% agarose gel. The positive PCR products were purified and sequenced by the Beijing Genomics Institute (BGI), Beijing, China. The participant with any one of the above three target genes detected was defined as positive for EBV DNA.

Statistical analyses

Statistical analyses were performed using R (https://www.r-project.org/). The categorical variables were compared using χ^2 test or Fisher's Exact test when appropriate. Unconditional logistic regression was used to estimate the odds ratios and their 95% confidence intervals. The selection of variables into multiple logistic regression models was based on both its significance in the univariate regression and prior knowledge (for socio-demographic characteristics and potential known risk factors). A two-sided P-value <0·05 is considered as statistically significant.

RESULTS

Socio-demographic characteristics

A total of 1008 participants including 504 HIV-positive and 504 HIV-negative MSM were included in the study. Among them, 81·7% aged at 21–40 years (range 18–69 years), 62·4% were non-local Shanghai residents, 96·0% were ethnic Han, 66·8% received college or higher education, 76·5% were never married, and 50·9% reported to have monthly income more than 5000 RMB yuan (~US$735). The two groups were comparable in distributions of

Table 1. *Primers for amplification*

Gene	Sequences	Position	Product size	Reference
EBNA-2				
2.a	AGGGATGCCTGGACACAA	48 810–48 827	600	
2.b	GTGCTGGTGCTGCTGGTGG	49 410–49 391		
2.c	TCTTGATAGGGATCCGCTAGGATA	48 839–48 862	497	Aitken *et al.* [27, 28]
2.d	ACCGTGGTTCTGGACTATCTGGATC	49 335–49 311		
2.e	AGACTTAGTTGATGCCCTAG		140	
2.f	CATGGTAGCCTTAGGACA			
EBNA-1				
1.a	GTAGAAGGCCATTTTTCCAC	109 151–109 170	609	
1.b	CTCCATCGTCAAAGCTGCA	109 741–109 759		Wang *et al.* [29]
1.c	AGATGACCCAGGAGAAGKCCCAAGC	109 266–109 290	308	
1.d	CAAAGGGGAGACGACTCAATGGTGT	109 549–109 573		
LMP-1				
OF	AGACAGTGTGGCTAAGGGAGT	168 039–168 059	804/774	
OR	CTACAACAAAACTGGTGGACT	168 843–168 823		Van Kooij *et al.* [30]
IF	TGATTAGCTAAGGCATTCCCA	168 075–168 095	539/509	
IR	TGCTCTCAAAACCTAGGCGCA	168 609–168 589		

residency, ethnicity, and marital status, but were significantly different in age, education level, and monthly income (Table 2).

Lifetime homosexual behaviors

As shown in Table 2, 95·4% of the participants had homosexual behavior with a main male partner, 17·2% with a male sex worker, and 79·1% with a casual male partner in their lifetime. The majority (95·8%) of the participants reported having had two or more sexual partners and 41·6% having had $\geqslant 10$ partners so far. Compared with HIV-negative controls, HIV-positive MSM reported significantly higher proportion of homosexual activities including unprotected casual sex and having $\geqslant 5$ partners.

Cancer-related factors

The majority (87·5%) of the participants reported having had pickled, smoked, or salty food at least once in the past 6 months, and 7·5% had such food frequently or daily. Also 27·4%, 37·9%, and 30·5% of participants reported smoking tobacco, drinking alcohol and illicit drug use in the past 6 months. Forty percent had depressive symptoms when measured with the CES-D (Center for Epidemiologic Studies Depression Scale). Significantly higher proportions of HIV-positive MSM reported fresh vegetables or fruits intake, smoking, drugs use, and depression than HIV-negative group, but a lower proportion consumed alcohol (Table 2).

HIV infection-related characteristics

Among the 504 HIV-positive MSM, 142 (28·2%) had been diagnosed with AIDS, 300 (59·5%) were receiving ART, and 90 (17·9%) showed $CD4^+$ T cell counts <200 cells/μl at the time of interview.

Prevalence and correlates of whole blood EBV DNA

The whole blood EBV DNA positivity was 56·0% (95% CI 51·6–60·2%) for HIV-positive MSM and 26·0% (95% CI 22·4–30·0%) for HIV-negatives ($\chi^2 = 93{\cdot}53$, $P < 0{\cdot}001$).

In the univariate logistic regression analyses for all participants, positivity of whole blood EBV DNA was inversely associated with levels of education (crude odds ratio (cOR) = 0·55, 95% CI 0·38–0·82), but was positively associated with aged above 40 years old (cOR = 1·72, 95% CI 1·21–2·45), ever married (cOR = 1·43, 95% CI 1·07–1·92), ever had commercial sex with a male sex worker without consistent condom use in life time (cOR = 1·59, 95% CI 1·09–2·31), ever had sex with a casual male partner without consistent condom use in lifetime (cOR = 1·55, 95% CI 1·13–2·14), had five or more male partners in life time (cOR = 1·53, 95% CI 1·12–2·10), frequent or daily intake of pickled, smoked, or salty food in the past 6 months (cOR = 1·98, 95% CI 1·23–3·17), used illicit drugs in the past 6 months (cOR = 1·45, 95% CI 1·10–1·90), had depression symptoms (cOR = 1·31, 95% CI 1·01–1·69) and HIV infection (cOR = 3·62, 95% CI 2·77–4·72) (Table 3). In the multiple logistic regression

Table 2. *Distribution and comparison of characteristics between HIV-positive and HIV-negative MSM in the study (N = 1008)*

	HIV negative		HIV positive		Total			
Characteristics	n_1	%	n_2	%	n	%	χ^2	P
Socio-demographic characteristics								
Age (years)								
18–20	20	4·0	19	3·8	39	3·9	18·205	0·003
21–30	278	55·1	238	47·2	516	51·1		
31–40	156	31·0	152	30·2	308	30·6		
41–50	31	6·1	50	9·9	81	8·0		
51–60	15	3·0	36	7·1	51	5·1		
61–69	4	0·8	9	1·8	13	1·3		
Permanent legal residency								
Local	201	39·9	178	35·3	379	37·6	2·237	0·135
Non-local	303	60·1	326	64·7	629	62·4		
Ethnic group								
Han	482	95·6	486	96·4	968	96·0	0·614	0·433
Other	22	4·4	18	3·6	40	4·0		
Level of education								
Primary school	3	0·6	7	1·4	10	1·0	18·336	<0·001
Middle school	33	6·6	70	13·9	103	10·2		
High school	108	21·4	114	22·6	222	22·0		
College or above	360	71·4	313	62·1	673	66·8		
Marital status								
Never married	402	79·7	369	73·3	771	76·5	5·994	0·052
Currently married	75	14·9	99	19·6	174	17·2		
Divorced/widowed	27	5·4	36	7·1	63	6·3		
Monthly income (Yuan, RMB)								
<1000	39	7·8	49	9·7	88	8·7	23·188	<0·001
1000–2999	47	9·3	83	16·5	130	12·9		
3000–4999	126	25·0	151	30·0	277	27·5		
⩾5000	292	57·9	221	43·8	513	50·9		
Lifetime homosexual behaviors								
Ever had sex with a main male partner								
No	16	3·2	31	6·2	47	4·6	7·120	0·028
Yes, with consistent condom use	37	7·3	25	5·0	62	6·2		
Yes, without consistent condom use	451	89·5	448	88·9	899	89·2		
Ever had commercial sex with a male sex worker								
No	424	84·1	411	81·5	835	82·8	1·254	0·534
Yes, with consistent condom use	23	4·6	25	5·0	48	4·8		
Yes, without consistent condom use	57	11·3	68	13·5	125	12·4		
Ever had sex with a casual male partner								
No	136	27·0	74	14·7	210	20·9	24·953	<0·001
Yes, with consistent condom use	50	9·9	45	8·9	95	9·4		
Yes, without consistent condom use	318	63·1	385	76·4	703	69·7		
Number of male partners								
<5	145	28·8	81	16·1	226	22·4	23·362	<0·001
⩾5	359	71·2	423	83·9	782	77·6		
Cancer-related factors								
Frequent or daily intake of pickled, smoked, or salty food in the past 6 months								
No	471	93·5	461	91·5	932	92·5	1·423	0·233
Yes	33	6·5	43	8·5	76	7·5		
Frequent or daily intake of fresh vegetables or fruits in the past 6 months								
No	179	35·5	123	24·4	302	30·0	14·826	<0·001
Yes	325	64·5	381	75·6	706	70·0		

Table 2 (*cont.*)

Characteristics	HIV negative		HIV positive		Total			
	n_1	%	n_2	%	n	%	χ^2	P
Smoked in the past 6 months								
No	385	76·4	347	68·8	732	72·6	7·205	0·007
Yes	119	23·6	157	31·2	276	27·4		
Drank in the past 6 months								
No	289	57·3	337	66·9	626	62·1	9·712	0·002
Yes	215	42·7	167	33·1	382	37·9		
Used illicit drugs in the past 6 months								
No	383	76·0	318	63·1	701	69·5	19·789	<0·001
Yes	121	24·0	186	36·9	307	30·5		
Depressive symptoms								
No	331	65·7	269	53·4	600	59·5	15·828	<0·001
Yes	173	34·3	235	46·6	408	40·5		

analysis with adjustment for potential confounding variables, positivity of whole blood EBV DNA was positively associated with HIV infection (adjusted odds ratio (aOR) = 3·43, 95% CI 2·58–4·57) and with frequent intake of pickled, smoked, or salty food (aOR = 1·71, 95% CI 1·02–2·86) (Table 3).

The associations were further examined stratified by status of HIV infection and were shown in Table 4. For HIV-infected MSM, 282 were tested positive for whole blood EBV DNA. Positivity of whole blood EBV DNA was positively associated with $CD4^+$ T cell counts <200 cells/μl (aOR = 1·79, 95% CI 1·05–3·05) and with frequent intake of pickled, smoked, or salty food (aOR = 3·14, 95% CI 1·39–7·08). For HIV-negative MSM, 131 were tested positive for whole blood EBV DNA and the positivity was only significantly associated with monthly income (3000–4999 *vs.* <1000 RMB yuan: aOR = 3·75, 95% CI 1·21–11·65; ⩾5000 *vs.* <1000 RMB yuan: aOR = 3·61, 95% CI 1·21–10·71) in the multiple logistic regression model.

DISCUSSION

In this cross-sectional study, we reported a higher prevalence of whole blood EBV DNA among HIV-positive participants and its positive association with dietary factors and immune-suppressed status in a MSM population from Shanghai, China. These results suggested an active EBV replication status among PLWHA, and appealed for further explorations in the risk factors and their interaction in EBV-related carcinogenesis.

The prevalence of whole blood EBV DNA was found to be 56·0% (95% CI 51·6–60·2%) for HIV-positive MSM and 26·0% (95% CI 22·4–30·0%) for HIV-negative MSM in this study. Among limited literature in Chinese PLWHA, Wu *et al.* reported an overall prevalence of peripheral EBV DNA as 32·3% in 257 HIV/AIDS patients from a hospital in Shenzhen [31]. Wang *et al.* reported higher prevalence among 73 HIV-infected participants when compared with 80 healthy controls in Henan (35·6% *vs.* 5·0%) [32]. The prevalence for HIV-infected people found in the current study was higher than the above mentioned studies, probably because it targeted HIV-positive MSM population specifically. Evidence from a study performed in Amsterdam showed a prevalence of 67% for EBV DNA from peripheral blood among HIV-positive MSM, higher than the prevalence of 39% among HIV-negative MSM and the prevalence of 6% among HIV-negative heterosexual men [24]. Our results added to the evidence that HIV-positive homosexual men were at higher risk of having positive whole blood EBV DNA.

The higher prevalence of EBV DNA among PLWHA indicated that people with suppressed immune system were at higher risk for active EBV viral replication, which was also suggested by our finding that having $CD4^+$ T cell counts <200 cells/ul were positively associated with detectable EBV DNA. Wu's study also found higher EBV DNA prevalence among inpatients who were at advanced stage of AIDS than outpatients (44·0% *vs.* 25·9%, $\chi^2 = 8{\cdot}7605$, $P < 0{\cdot}005$) [31]. Chronic HIV infection causes antigen simulation of B cells, breaks the

Table 3. *Association between characteristics and whole blood EBV DNA positivity among MSM in the study*

Characteristics	EBV DNA positivity		Crude OR (95% CI)	*P*-value	Adjusted OR[a] (95% CI)	*P*-value
	n	%				
Age (years)						
⩽40	337	39·0	1·00		1·00	
>40	76	52·4	1·72 (1·21–2·45)	0·003	1·20 (0·76–1·89)	0·430
Permanent legal residency						
Local	157	41·4	1·00		1·00	
Non-local	256	40·7	0·97 (0·75–1·26)	0·821	0·86 (0·64–1·15)	0·310
Ethnic group						
Han	399	41·2	1·00		1·00	
Other	14	35·0	0·77 (0·40–1·49)	0·434	0·82 (0·41–1·66)	0·584
Level of education						
Middle school or below	61	54·0	1·00		1·00	
High school or above	352	39·3	0·55 (0·38–0·82)	0·003	0·84 (0·53–1·34)	0·457
Marital status						
Never married	300	38·9	1·00		1·00	
Ever married	113	47·7	1·43 (1·07–1·92)	0·017	1·11 (0·76–1·61)	0·592
Monthly income (Yuan, RMB)						
<1000	37	42·0	1·00		1·00	
1000–2999	62	47·7	1·26 (0·73–2·17)	0·412	1·10 (0·61–2·01)	0·747
3000–4999	122	44·0	1·09 (0·67–1·76)	0·742	1·13 (0·66–1·93)	0·658
⩾5000	192	37·4	0·82 (0·52–1·31)	0·410	1·04 (0·63–1·73)	0·876
Ever had commercial sex with a male sex worker in lifetime						
No	326	39·0	1·00		1·00	
Yes, with consistent condom use	24	50·0	1·56 (0·87–2·80)	0·134	1·59 (0·85–2·96)	0·147
Yes, without consistent condom use	63	50·4	1·59 (1·09–2·31)	0·016	1·26 (0·83–1·91)	0·271
Ever had sex with a casual male partner in lifetime						
No	71	33·8	1·00		1·00	
Yes, with consistent condom use	31	32·6	0·95 (0·57–1·59)	0·840	0·80 (0·45–1·41)	0·435
Yes, without consistent condom use	311	44·2	1·55 (1·13–2·14)	0·007	1·09 (0·74–1·61)	0·654
Number of male partners in lifetime						
<5	75	33·2	1·00		1·00	
⩾5	338	43·2	1·53 (1·12–2·10)	0·007	1·09 (0·75–1·58)	0·645
Frequent or daily intake of pickled, smoked, or salty food in the past 6 months						
No	370	39·7	1·00		1·00	
Yes	43	56·6	1·98 (1·23–3·17)	0·005	1·71 (1·02–2·86)	0·044
Frequent or daily intake of fresh vegetables or fruits in the past 6 months						
No	129	42·7	1·00		1·00	
Yes	284	40·2	0·90 (0·69–1·19)	0·462	0·78 (0·57–1·05)	0·102
Smoked in the past 6 months						
No	287	39·2	1·00		1·00	
Yes	126	45·7	1·30 (0·99–1·72)	0·064	1·03 (0·75–1·42)	0·849
Drunk in the past 6 months						
No	257	41·1	1·00		1·00	
Yes	156	40·8	0·99 (0·77–1·28)	0·946	1·08 (0·80–1·44)	0·628
Used illicit drugs in the past 6 months						
No	268	38·2	1		1·00	
Yes	145	47·2	1·45 (1·10–1·90)	0·008	1·26 (0·93–1·71)	0·136

Table 3 (*cont.*)

Characteristics	EBV DNA positivity		Crude OR (95% CI)	*P*-value	Adjusted OR[a] (95% CI)	*P*-value
	n	%				
Depressive symptoms						
No	230	38·3	1·00		1·00	
Yes	183	44·9	1·31 (1·01–1·69)	0·039	1·08 (0·82–1·43)	0·580
HIV infection status						
Negative	131	26·0	1·00		1·00	
Positive	282	56·0	3·62 (2·77–4·72)	<0·001	3·43 (2·58–4·57)	<0·001

[a] Adjusted for all the other variables in the table.

equilibrium that newly infected and differentiating B cells were controlled by cytotoxic T cell responses, and thus leads to uncontrolled lymphoproliferation and cancer development [12]. It has been observed that the standardized incidence rates for most NADCs were greater than doubled in HIV population, and lower $CD4^+$ T cells and taking HAART were found to be two major risk factors [33]. Moreover, it has been believed that the EBV DNA load and HIV viremia are correlated [34] and could serve as the diagnostic and prognostic marker for lymphoma in HIV-infected patients [35–38]. Based on these evidence and our finding, we suggest closer surveillance of EBV-related cancer incidence, known risk factors, as well as biomarkers including EBV DNA especially among PLWHA with lower CD4 counts or with HIV viremia.

One major finding of the current study was that participants who frequently consume pickled, smoked, or salty food were at higher risk of having positive whole blood EBV DNA. Among known risk factors for NPC, salted fish intake was a well-established one and was firstly reported in Chinese population [39, 40]. Chinese-style salted fish was defined as Group I carcinogen by IARC (International Agency for Research on Cancer) since high levels of NDMA (N-nitrosodimethylamine) was generated during making the fish. In this study, we were the first to investigate participants' habit of pickled, smoked, or salty food intake, which was a broad range of food processed using salt or other sources, and found a positive association with circulating EBV DNA in MSM. In our observation, the association was only significant among HIV-positive MSM in stratified analyses. The possible explanations for the heterogeneity of the association include that HIV infection could be a stronger risk factor for active EBV replication, making those under immunosuppressed condition more vulnerable to other potential cancer risk factors to play a role. This is consistent with our observation that lower CD4 T cell counts also showed to be a risk factor. Once confirmed, the association between pickled, smoked or salty food and active EBV replication would suggest a population at higher risk of EBV-related cancers for intervention and cancer prevention.

There are several limitations in the current study. First, by nature of a cross-sectional design, we may only describe the distribution of risk factors and associations identified in this study can be further investigated using other study designs. However, the associations found between positive whole blood EBV DNA and HIV infection and frequent pickled, smoked, or salty food intake still calls attention not only because it was the first time examining the association in this population, but also for the strong associations discovered. Second, residual confounding may be present and partly explain the association we found between EBV DNA and cancer risk factors. For example, HPV infection is a risk factor for NPC and has not been measured in this study. We collected information on sexual behaviors as a proxy, since HPV infection is associated with active and unsafe sex. A more comprehensive collection of information on exposures for known risk factors such as viral co-infection is needed in future research. Third, most of the exposure measurements from this study were based on self-reported data. Thus the information bias may exist especially for the validity of answers to sensitive questions such as on sexual behaviors and illicit drug use. Also, the measurements on tobacco and alcohol use, dietary patterns, and quantities of food can be improved for better estimates of the associations. However, the validity of the major finding on HIV infection and unhealthy food intake

Table 4. *Separate multiple logistic regression analyses of associates with whole blood EBV DNA positivity within the stratum of HIV-positive and HIV-negative MSM, respectively*

	HIV positive				HIV negative			
	EBV DNA positivity				EBV DNA positivity			
Characteristics	*n*	%	Adjusted OR[a] (95% CI)	*P*-value	*n*	%	Adjusted OR[a] (95% CI)	*P*-value
Age (years)								
⩽40	224	54·8	1·00		113	24·9	1·00	
>40	58	61·1	1·12 (0·62–2·00)	0·713	18	36·0	1·27 (0·61–2·66)	0·520
Permanent legal residency								
Local	101	56·7	1·00		56	27·9	1·00	
Non-local	181	55·5	0·91 (0·60–1·36)	0·629	75	24·8	0·77 (0·50–1·19)	0·240
Ethnic group								
Han	275	56·6	1·00		124	25·7	1·00	
Other	7	38·9	0·55 (0·20–1·46)	0·228	7	31·8	1·46 (0·55–3·86)	0·451
Level of education								
Middle school or below	47	61·0	1·00		14	38·9	1·00	
High school or above	235	55·0	0·86 (0·49–1·53)	0·617	117	25·0	0·66 (0·30–1·47)	0·313
Marital status								
Never married	205	55·6	1·00		95	23·6	1·00	
Ever married	77	57·0	0·88 (0·53–1·45)	0·607	36	35·3	1·46 (0·83–2·57)	0·190
Monthly income (Yuan, RMB)								
<1000	33	67·3	1·00		4	10·3	1·00	
1000–2999	51	61·4	0·80 (0·36–1·76)	0·574	11	23·4	2·42 (0·68–8·63)	0·175
3000–4999	85	56·3	0·70 (0·34–1·44)	0·336	37	29·4	3·75 (1·21–11·65)	0·022
⩾5000	113	51·1	0·57 (0·29–1·14)	0·110	79	27·1	3·61 (1·21–10·71)	0·021
Frequent or daily intake of pickled, smoked, or salty food in the past 6 months								
No	247	53·6	1·00		123	26·1	1·00	
Yes	35	81·4	3·14 (1·39–7·08)	0·006	8	24·2	0·87 (0·37–2·04)	0·749
Frequent or daily intake of fresh vegetables or fruits in the past 6 months								
No	77	62·6	1·00		52	29·1	1·00	
Yes	205	53·8	0·75 (0·48–1·16)	0·195	79	24·3	0·78 (0·50–1·20)	0·250
Smoked in the past 6 months								
No	186	53·6	1·00		101	26·2	1·00	
Yes	96	61·1	1·18 (0·77–1·81)	0·437	30	25·2	0·85 (0·51–1·43)	0·542
Drank in the past 6 months								
No	183	54·3	1·00		74	25·6	1·00	
Yes	99	59·3	1·12 (0·74–1·69)	0·597	57	26·5	1·09 (0·71–1·69)	0·694
Used illicit drugs in the past 6 months								
No	171	53·8	1·00		97	25·3	1·00	
Yes	111	59·7	1·36 (0·91–2·01)	0·132	34	28·1	1·21 (0·75–1·95)	0·440
$CD4^+$ T cell count (cells/μl)								
⩾350	104	50·5	1·00		–	–	–	–
200–349	119	57·2	1·31 (0·88–1·95)	0·189	–	–	–	–
<200	59	65·6	1·79 (1·05–3·05)	0·031	–	–	–	–

[a] Adjusted for all the other variables in the table.

and their associations with EBV DNA would not be biased seriously since HIV infection was determined by laboratory tests and questions on dietary were not sensitive. Last but not least, although we included all the HIV-infected MSM attending the clinic with matched controls during the study period, the prevalence and the association may not be representative for all MSM in Shanghai or in China.

In conclusion, this study reported a prevalence of 56% for positive whole blood EBV DNA among the

HIV-infected MSM, higher than the prevalence of 26% among the HIV-negative MSM in Shanghai, China. It also reported significant association between HIV infection, frequent pickled, smoked, or salty food intake, and lower $CD4^+$ T cell counts with detectable whole blood EBV DNA. These results suggest that HIV-positive MSM in Shanghai, China are at higher risk for EBV-related cancers and further conformational studies are warranted.

ACKNOWLEDGEMENTS

This study was supported by Natural Science Foundation of China (grant no. 81361120385), Shanghai Municipal Health and Family Planning Commission (grant numbers GWTD2015S05, 15GWZK0101, 15GWZK0801, and 201440328), and the Chinese National Major Science & Technology Project of Infectious Diseases (grant no. 2012ZX10001007–006).

DECLARATION OF INTEREST

None.

ETHICAL STANDARDS

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

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