

First Survey for Detecting the Presence of Human Herpesvirus 8 Infection (HHV-8) in Maputo, Mozambique

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Human herpesvirus 8 (HHV-8), the etiological agent of Kaposi's sarcoma (KS), is endemic in parts of the sub-Saharan, and KS has increased concomitantly with the HIV/AIDS epidemic. In Mozambique (MZ), no data concerning HHV-8 infection was available, thus the main of this work was to investigate, for the first time, the presence of HHV-8 infection in Maputo, MZ. Latent and lytic HHV-8-specific antibodies were assessed in blood samples from 189 individuals from the Central Hospital of Maputo, MZ, using "in-house" immunofluorescence assays conducted in São Paulo, Brazil. The results obtained were analyzed according to socio-demographic and clinical variables using the Chi-square test and logistic regression. An HHV-8 seropositivity of 1.8% and 9.7% was detected among 57 medical students and 31 individuals from the staff, respectively, in contrast to 16.4% detected among 67 out-patients. Concerning 34 hospitalized patients from the Dermatology Unit, 47.1% were HHV-8-seropositive overall, while the rate was 85.7% among KS patients. The present survey, conducted in Maputo, MZ, demonstrates great variation in HHV-8 infection frequencies depending on the group analyzed and epidemiological variables. An association between HHV-8 seropositivity and male gender (OR 5.72), the central origin of patients (OR 5.33), blood transfusions (OR 3.25), and KS (OR 24.0) was detected among hospitalized patients, and primary school (OR 7.18) and HIV-1 infection (OR 8.76) among out-patients.

Key-Words: Human herpesvirus 8 (HHV-8), serology, Mozambique, frequency, Kaposi's sarcoma.

Human herpesvirus 8 (HHV-8) is considered as the etiological agent of all forms of Kaposi's sarcoma (KS), a tumor that frequently occurs during HIV infection (epidemic KS [AIDS-KS]) transplant recipients (iatrogenic KS), elderly men of Mediterranean and Middle Eastern origin (classic KS), and inhabitants of eastern and central Africa (endemic KS) [1,2].

During the last decades on African continent, AIDS has had a profound impact on adult and child mortality, reducing life expectancy by 10-15 years, mostly in sub-Saharan countries [3]. This high rate of mortality is attributed to opportunistic infections and neoplasms, with an emphasis on tuberculosis and KS.

Although the incidence of KS depends on the prevalence of HHV-8, several factors have been associated with disease development and, in many African countries, KS has risen dramatically since the onset of the AIDS epidemic [4,5]. Interestingly, the introduction of highly active antiretroviral therapy (HAART) reverted the increase in KS in wealthier

countries [5], but because of limited access to antiretroviral therapies in Africa, KS continues to increase in this geographic area. The exact mechanism by which HAART controls KS progression is unknown, but it has been speculated that the control of HIV viral load by HAART and consequent low Tat protein production, which is thought to be involved in HHV-8 entry into the cell, leads to a decrease in virus spreading and HHV-8-associated diseases. In addition, reconstitution of the immune system after HAART could improve and/or prevent KS lesions [5,6]. Thus, knowledge of the prevalence of HHV-8, mostly among HIV-1-infected patients is necessary for the best control and management strategies for patients, as well as for controlling transmission/acquisition of stigmatizing and lethal AIDS-KS in Africa.

Until now, no study examining HHV-8 infection has been conducted in Mozambique (MZ), a country that is experiencing an AIDS epidemic (16.0% [14.0% to 17.0%] of individuals were living with HIV in 2007: 9.0% in the northern, 18.0% in the central, and 21.0% in the southern region of the country) [1], and is bordered in the northern and central regions by countries where KS is endemic [4]. Thus, an exploratory mission from Brazil supported by the Ministry of Science and Technology was conducted in Maputo, MZ with the aim of performing a preliminary survey on the occurrence of HHV-8 infection in this geographic area.

Material and Methods

The mission consisted of a visit by two Brazilian physicians to the Central Hospital of Maputo, University Eduardo Mondlane, Maputo, MZ, who joined with local physicians and together established a plan for selecting individuals who could be included in this preliminary survey,

Received on 6 April 2009; revised 8 June 2009.

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as well as more appropriate demographic, clinical and laboratorial variables for analysis. A questionnaire containing information on socio-demographic characteristics of individuals such as gender, age, geographic origin, house location, number of household persons, number of siblings, household electricity, household tap water, marital status, occupation, and years of education was generated. Information regarding prior blood transfusions, number of sexual partners in the last months, prior exposure to sexually transmitted diseases (STDs), partners with AIDS, hepatitis, use of illicit drugs, HIV status, and KS were included. All participants were interviewed, answered the questionnaire, and signed the informed consent form before blood collection was performed. The study was approved by the Ethics Committees of Brazilian Institutions, and by the Ethics Committees of the Ministry of Health of Mozambique and Brazil.

Blood samples were collected on filter paper from 189 individuals: 57 students from the medical school (Group 1), 31 individuals from the staff (Group 2), 67 out-patients (in ambulatory assistance at the Dermatology Department, Group 3), and 34 hospitalized patients from the Dermatology Unit (Group 4). Subsequently, one physician from MZ came to Brazil in order to update her knowledge of AIDS-KS at a Reference Centre of AIDS in São Paulo. Latent and lytic HHV-8 antibodies were assessed in eluted blood using "in house" indirect immunofluorescence assays (IFA-LANA and IFI-Lytic) at the Instituto Adolfo Lutz, São Paulo, Brazil as previously described. Briefly, cells of the BCBL-1 line latently infected with HHV-8 or stimulated with tetradecanoyl phorbol ester acetate (TPA) were used to prepare slides for IFA-LANA and IFA-Lytic, respectively. Samples were eluted from dried blood

punches of 6 mm diameter in 200 μ L of PBS (dilution 1:40), and then adjusted to 1:50, 1:100, and 1:200 in PBS/skim milk. Samples were considered HHV-8 seropositive when the results showed reactivity in at least one IFA (LANA or Lytic) in three dilutions employed. The sensitivity and specificity of the assays were confirmed in KS-AIDS patients and healthy persons from São Paulo [8]. Statistical analysis of socio-demographic and clinical variables, and HHV-8 serological results in each Group were conducted using the Chi-square test and a p level ≤ 0.05 was considered statistically significant. Logistic regression was employed for calculating the odds ratio (OR) and 95% confidence intervals (CI).

Results

Table 1 presents the HHV-8 serological results from each Group analyzed, and Table 2 shows statistically significant associations. The results obtained showed great variation in HHV-8 frequencies of infection in Maputo (south of MZ), with low frequencies among healthy individuals (Group 1 and 2) and high frequencies among hospitalized patients (Group 4). However, the best Groups for analysis were Group 3 and 4, comprising individuals of several ages and different socio-demographic statuses (data not shown); among them, significant associations between HHV-8 seropositivity and male gender, the central origin of patients, primary school, prior blood transfusions, KS and HIV-1 infection were detected (Table 2).

Discussion

The results obtained could suggest that Maputo is not an endemic area of HHV-8, but we cannot exclude bias in these results since the individuals analyzed were not representative

Table 1. HHV-8 serological results using IFA-LANA and IFA-Lytic according to the group analyzed.

Group	HHV-8+	HHV-8-	Total
N=189	N (%)	N (%)	N
G1 - students	1 (1.8)	56 (98.2)	57
G2 - staff	3 (9.7)	28 (90.3)	31
G3- out-patients	11 (16.4)	56 (83.6)	67
G4- hospitalized patients	16 (47.1)	18 (52.9)	34

N: number of individuals; G: Group; + denote sera that gave a positive result at least once. IFA-LANA or IFA-Lytic; - denote sera that were negative for HHV-8 serology. Chi-square test with statistical significance: G1 vs G3 $p=0.006$; G1 vs G4 $p=0.000$; G2 vs G4 $p=0.001$; G3 vs G4 $p=0.001$.

Table 2. Significant associations between socio-demographic and clinical variables and HHV-8 positivity in each Group analyzed.

Variable	vs	Group	Chi-square	OR (95% CI)
Gender	G4	male	$p=0.017$	5.72 (1.06-34.02)
Geographic origin	G4	central	$p=0.160$	5.33 (0.71-49.11)
Education	G3	primary	$p=0.019$	7.18 (1.47-37.52)
Blood transfusion	G4	yes	$p=0.036$	3.25 (0.59-19.08)
Kaposi's sarcoma	G4	yes	$p=0.000$	24.0 (2.99-257.38)
HIV	G3	yes	$p=0.009$	8.76 (1.51-65.78)

G3: out-patients; G4: hospitalized patients. Chi-square test ($p \leq 0.05$ was considered statistically significant). Odds ratio (OR) and 95% Confidence Intervals (CI) were calculated using logistic regression.

of the general population of Maputo or other regions of the country.

Nevertheless, concerning gender, the majority of studies worldwide have demonstrated more cases of KS among males, and female hormones have been suggested to be protective factors in HHV-8 transmission/acquisition [1,2,4,5]. With regard to the central origin of HHV-8-infected individuals detected in the present study, this result is not surprising because of the vicinity of this region to HHV-8- and KS-endemic countries, such as Tanzania, Malawi, Zambia, and Zimbabwe [4]. Interestingly, HIV was introduced and spread in MZ via the northern and central regions, and is now increasing in the south [7,9]; thus we can speculate that an increase in the epidemic form of KS could occur in the south of MZ in the future. In support of this hypothesis, the highest rate of HHV-8-seropositivity in the present study was detected among out-patients and hospitalized patients, most of which were HIV-1-infected, and had originated in the central region of MZ.

The association between HHV-8 infection and a low education level (primary school) of the individuals could suggest that poor household conditions facilitate virus transmission/acquisition. In support of this finding, some studies conducted in Africa demonstrated poor sanitary and hygienic practices as well as lack of tap water and low socioeconomic status as risk factors for HHV-8 transmission/acquisition [10,11]. In addition, segments of DNA/HHV-8 were detected in saliva and urine, which implicate these specimens as potential routes of virus transmission [5,12]. Of note, although not statistically significant, several individuals enrolled in the present study mentioned lack of electricity and tap water at home. Thus, we can speculate that HHV-8 infection may be preventable if sanitary conditions and the population education level were improved in Africa.

The association between HHV-8 infection and previous blood transfusions are in agreement with the finding of the presence of virus in blood samples [5]. No association between HHV-8 and exposure to STDs, use of illicit drugs, hepatitis, or other variables was detected, but we could not exclude participant omission or lack of knowledge regarding this information. Concerning the significant association between KS and HIV, the results are in accordance with the literature as well as our previous studies confirming HHV-8 infection in KS patients, most of whom were HIV/AIDS patients [6,8,12,13]. Although the number of individuals analyzed was small, this preliminary study emphasizes the need for extending and confirming these data in order to properly prevent and treat individuals from this geographic area. Extraordinary hope for antiretroviral therapy in South Africa was observed in HIV-1 subtype C-infected patients who were co-infected with either *Mycobacterium tuberculosis* or HHV-8 [14,15].

Another study approved by the institutions and governments of Brazil and MZ on HHV-8 infection that includes individuals representing different risk-factors from the northern, central and southern regions of MZ is currently in progress. Owing to Brazilian experience in the fight against AIDS and KS [13] along with the results obtained from prior and present studies, we hope to provide information to the government of MZ regarding how to manage and control this emerging infectious disease.

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