



Journal of Medical Sciences

ISSN 1682-4474

science
alert

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JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued four times per year on paper and in electronic format.

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J. Med. Sci., 6 (1): 93-98
January-March, 2006

Prevalence of HHV-8 Infections Associated with HIV, HBV and HCV in Pregnant Women in Burkina Faso

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Abstract: We analyzed the prevalence of Human Herpes Virus-8 (HHV-8), Human Immunodeficiency Virus (HIV), Hepatitis C Virus (HCV), Hepatitis B Virus (HBV) infections among pregnant women in Burkina Faso, to estimate the seroprevalence of HHV-8 infection in this sub-Saharan country and to evaluate the co-infection rates for these viruses. Sera collected from pregnant women (n = 1420) were assayed to determine HIV prevalence. Subsequently, (n = 429) were tested to detect HHV-8, HCV and HBV infections. 108/1420 (7.61%) subjects were found HIV seropositive and the risk of being infected increases with age. Among the 429 women screened, 49 were HHV-8 infected (11.4%), 26 were seropositive for HCV (6.1%) and 40 were HBsAg positive (9.3%). Co-infection rates among HIV infected individuals were: 16/108 HHV-8 positive (14.8%), 14/108 HBV (12.9%) HBsAg positive, 8/108 (7.4%) HCV positive. Prevalence of HHV-8 infection in the analyzed population of Burkina Faso is lower than that found in other regions of Central Africa and this is in keeping with the low incidence of Kaposi's sarcoma observed in this country. Moreover, this research enabled us to estimate the prevalence of the co-infections of the HIV, hepatitis B and hepatitis C within our group of study. Considering only the three types of infections: HHV-8, HCV and HBV without taking into account the HIV, only 75.06% (322/429) of these women do not have any viral infection. This remark is very significant and it must be taken in consideration in the hospitals, at the time of the blood transfusions in emergency.

Key words: HHV-8, HIV, HCV, HBV, Burkina Faso

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INTRODUCTION

Kaposi's Sarcoma (KS) is a mesenchymal tumour, originally described in Eastern Europe, relatively rare in the general population^[1]. However, KS incidence showed a steep increase in the early 1980's in concomitance with the pandemy of human immunodeficiency virus (HIV) type I infection^[2]. Four epidemiological forms of KS have been identified: the classic KS is mostly present in the Mediterranean basin, shows a slow progression and is largely observed in the elderly; the endemic form, registered in Central Africa is more aggressive and affects younger individuals; the iatrogenic KS is described in post-transplanted individuals and is strongly related to the immunosuppressed status; the AIDS associated KS is by far the most frequent and aggressive form of this disease. In Western countries it has been shown that KS incidence rate among Human Immunodeficiency type-1 Virus (HIV) positive individuals is about 20,000 fold more frequent than in the general population^[3]. In the past decade two factors have changed the perspectives of this neoplastic disorder. The introduction of Highly Active Anti Retroviral Therapy (HAART) has strongly decreased KS incidence in AIDS patients, mostly due to an enhancement of immune response in HIV infected subjects^[4]. Furthermore, in 1994 a new herpes virus, indicated as human herpes virus-8 (HHV-8) has been found in KS lesions and has been invariably associated to all forms of KS, thus indicating a transmittable etiologic agent for this disease^[5].

Epidemiological surveys have shown that HHV-8 infection is not ubiquitous. The highest prevalence rates of HHV-8 infection in the general population have been registered in Africa, reaching in some areas 40-70%^[6,7]. Hot spots of HHV-8 infection have been described in some Mediterranean regions such as Sardinia, Sicily and Greece, 10 to 40% of healthy adult population^[8,9]. In US and North Europe, HHV-8 prevalence is quite low, ranging from 0.5-5%^[10]. However, among HIV positive subjects, HHV-8 prevalence rate is much higher: in US, different surveys have reported a HHV-8 prevalence in HIV positive subjects ranging around 35-49%^[11].

In Africa, HIV prevalence and AIDS-KS incidence reach the highest levels, although not uniformly distributed throughout the continent^[12]. In Ouagadougou, the capital city of Burkina Faso, the Centre Médical Saint Camille (SCMC) is a central institution for care of pregnant women and HIV infected people. In the period 1998-2003 more than a thousand of HIV infected subjects have received medical care at these hospitals, however less than ten cases of KS have been registered. This limited number of KS patients among HIV patients is in contrast with the reports of other African countries in the region.

Previous studies carried out in Burkina Faso have revealed that the prevalence of infection by HIV, Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) are in line with those registered in other countries of Western Africa^[13,14]. So far, no data are available on HHV-8 infection in Burkina Faso. In the present report we analyzed the diffusion of this infection in sera obtained from pregnant women attending antenatal visits at SCMC in Ouagadougou in a period of about one year. Furthermore we evaluated HHV-8 prevalence and its correlation with the HIV infection status. At the same time, samples were also assayed for HBV and HCV antibodies, to determine the co-infection rates in the screened population.

MATERIALS AND METHODS

The sample: The sera assayed in the present survey were collected at the SCMC in Ouagadougou, Burkina Faso, from April 20, 2003 to May 25, 2005. Overall, 1420 pregnant women, aged 18-44 years, average 25.92±5.82, were enrolled in the study. All the women had less than 32 weeks of amenorrhea at time of sampling. Each subject issued the informed consent before being blooded for this survey and the study accomplishment was approved by the Ethics Committee of the SCMC. For the HHV-8, HCV and HBV tests we invited 429 pregnant women (321 HIV negative, 108 HIV positive) who freely agreed to be screened for these viral infections. Among these 108 HIV positive pregnant women, 33 were in HIV/AIDS state.

Laboratory studies: From each patient 10 mL venous blood was drawn. Within 3 h after drawing test, a tube of plasma was separated by centrifugation at 3,000 rpm, for 10 min and frozen at -40°C, to be used for the tests to reveal HIV, HCV, HBV and HHV-8 infections.

HIV antibodies test was made by Enzyme Immuno Assay (EIA) technique, using France Abbott Laboratories S.A Determine HIV-1 and France Bio-Rad Laboratories S.A Genie II HIV-2 test. The EIA was performed as described previously^[15]. HHV-8 serostatus was determined by screening antibodies to viral lytic antigens in the collected samples. The analysis was carried out by means of the two tests most widely used in HHV-8 serodiagnosis: an Immunofluorescence Assay (IFA) and an EIA using the HHV-8 K8.1 protein as antigen. IFA was performed on BC-3 cells, an HHV-8 positive cell line, treated with 20 ng mL⁻¹ of 12-tetradecanoylphorbol 13-acetate to induce HHV-8 lytic cycle^[15]. EIA was undertaken using bacterially produced K8.1 protein purified by chromatography as previously described^[15]. Sera were diluted 1:10 and 1:20, respectively, for IFA and EIA.

To analyze HCV infection, we screened sera for antibodies to HCV antigens, whereas for HBV, we tested sera to detect the viral marker Hepatitis B Surface Antigen (HBsAg) to identify subjects with an active infection by HBV. In both these cases, sera were assayed by an Inter Second Antibody Immunoassay (ISAI) (Huma-Tech House rapid test, Germany).

Statistical analysis: Demographic and clinical profiles were recorder on computer file and analyzed by standard software SPSS-10 and EpiInfo-6. Statistical significance was set at $p < 0.05$.

RESULTS

Prevalence of HIV infection: Initially, we evaluated the HIV serostatus in 1420 women attending an antenatal visit at the SCMC. In this group we found that 108/1420 were HIV positive with a prevalence of 7.61% (Table 1). HIV type-1 infection was largely predominant in the seropositive individuals: indeed 103/108 seropositive subjects were infected by HIV-1, 3/108 by HIV-2, only 2/108 by HIV-1 and HIV-2. As shown in Table 2, HIV rate of infection significantly increases with age, ranging from 1.89% among 15-19 years old women to 11.81% in 25-29 years old patients ($p = 0.002$). However, HIV rate of infection decreases but not significantly with age, ranging from 11.81% among 25-29 years old women to 5, 40% in 35-44 years old patients ($p = 0.117$).

Prevalence of HHV-8 infection: As shown in Table 3, 49/429 (11.4%) subjects were HHV-8 seropositive and no significant difference in the mean age was found among

seropositive and seronegative subjects ($p = 0.497$). Furthermore, the prevalence rate tends to increase from 7.7% in the group aged 15-19 years to 13.6% in the 35-44 years old subjects, although the risk of being infected during adulthood does not seem particularly high. Moreover, as described in Table 4, HHV-8 infection rate was poorly affected by the HIV status of the subjects: 10.3% HHV-8+/HIV- compared to 12% HHV-8+/HIV+. In the group of AIDS patients HHV-8 prevalence was higher: 21.2% of the individuals screened were found HHV-8 seropositive, however the difference registered with the HIV negative women was not statistically significant ($p = 0.109$).

Prevalence of HBV and HCV infection: As reported in Table 3, 40/429 (9.3%) subjects were actively infected by HBV at the moment of collecting the sample, as documented by the presence of HBsAg in the serum. Once again, no difference of the mean age was found among HBsAg positive and negative individuals. An increase of prevalence rate was registered when comparing the group aged 15-19 years to the group 30-34 years old subjects 2.6% vs. 10.1%, although the difference is not statistically significant. As shown in Table 4, HIV serostatus had a low effect on the rate of HBV active infection: in the present survey, 8.1% of the HIV negative subjects were found infected by HBV compared to 12.0% of the HIV positive individuals. A higher HBV infection rate was observed among the AIDS patients 15.1%, however also in this case the difference was not statistically significant.

26/429 (6.1%) of the assayed population was found to be HCV seropositive, with no statistical difference in the mean age and a constant prevalence rate in the different age groups. As reported in Table 5, HIV serostatus seemed to affect the HCV prevalence rate: the percentage of HCV seropositive subjects almost doubled in the HIV positive individuals compared to the HIV negative (5.6 vs. 10.7%), however this difference was not statistically significant ($p = 0.182$). It should be noted that none of the 33 AIDS patients was found HCV infected. It is likely that the limited number of AIDS patients analyzed in this survey does not reflect HCV seroprevalence rate in the population affected by AIDS in Burkina Faso.

Co-infection by HIV, HHV-8, HBV and HCV: As shown in Table 5, when considering the HIV positive individuals screened in the present survey, co-infection frequencies were higher than that registered in the entire cohort of pregnant women tested. Indeed, 16/108 (14.8%) was found HHV-8 seropositive, similar to HBsAg positive subjects 14/108 (13.0%) whereas HCV co-infected individuals were a smaller fraction 8/108 (7.4%).

Table 1: HIV serostatus on 1420 pregnant women in Ouagadougou

	HIV		HIV types in seropositive subjects		
	HIV-	HIV+	HIV-1	HIV-1/2	HIV-2
N	1312	108	103	2	3
%	92.39%	7.61%	95.37%	1.85%	2.78%

Table 2: Prevalence of HIV infection for age groups

Age group (year)	Subjects	HIV positive	%
15-19 ¹	211	4	1.89
20-24 ²	459	29	6.32
25-29 ³	381	45	11.81
30-34 ⁴	221	22	9.95
35-44 ⁵	148	8	5.40
Total	1420	108	7.61

P values of prevalence of HIV infection for age groups comparisons were obtained by χ^2 test
 15-19 yrs¹ - 20-24 yrs²: $p = 0.018$; 15-19 yrs¹ - 25-29 yrs³: $p < 0.0001$
 15-19 yrs¹ - 30-34 yrs⁴: $p < 0.001$; 15-19 yrs¹ - 35-44 yrs⁵: $p = 0.079$ (NS)
 20-24 yrs² - 25-29 yrs³: $p = 0.011$;
 25-29 yrs³ - 30-34 yrs⁴: $p = 0.120$ (NS)
 25-29 yrs³ - 35-44 yrs⁵: $p = 0.703$ (NS)
 30-34 yrs⁴ - 35-44 yrs⁵: $p = 0.147$ (NS)
 (NS) = not significant

Table 3: HHV-8, HCV, HBV serostatus and correlation with age groups

Serostatus	HHV-8		HCV		HBV		
	-	+	-	+	-	+	
N	380.0	49.0	403.0	26.0	389.0	40	
Mean age	25.8±5.8	26.4±5.8	25.9±5.9	25.6±5.0	25.9±5.9	25.6±4.6	
Variance	33.9	33.6	34.5	25.4	35.2	21.4	
t-Test : p =	0.497 (NS)		0.799 (NS)		0.757 (NS)		
Age group (year)	N	HHV-8+	N %	HCV+	N %	HBV+	N %
15-19 ¹	39	3	7.7	2	5.1	1	2.6
20-24 ²	162	17	10.5	11	6.8	19	11.7
25-29 ³	121	16	13.2	7	5.8	11	9.1
30-34 ⁴	69	8	11.6	4	5.8	7	10.1
35-44 ⁵	38	5	13.6	2	5.3	2	5.3
Total	429	49	11.4	26	6.1	40	9.3

NS = not significant

Table 4: Correlation between HIV serostatus and HHV-8, HCV, HBV infections

	HIV(-)	HIV(+)	AIDS	HIV+/AIDS	Total
HHV-8 (-) ¹	289/321 (89.7%)	65/75 (88.0%)	26/33 (78.8%)	91/108 (84.3%)	380
HHV-8 (+) ²	33/321 (10.3%)*	9/75 (12.0%)	7/33 (21.2%)**	16/108 (14.8%)	49
HCV (-) ³	304/321 (94.4%)	66/75 (89.3%)	33/33 (100%)	99/108 (91.7%)	403
HCV (+) ⁴	18/321 (5.6%)°	8/75 (10.7%)°°	0/33 (0%)	8/108 (7.4%)	26
HBV (-) ⁵	296/321 (91.9%)	65/75 (88.0%)	28/33 (84.8%)	93/108 (86.1%)	389
HBV (+) ⁶	26/321 (8.1%)^	9/75 (12.0%)	5/33 (15.1%)^^	14/108 (12.9%)	40

p-values of correlation between HIV serostatus and HHV-8, HCV, HBV infection comparisons were obtained by Fishers' exact test.

X² : * → ** : p = 0.062 (NS); X² : ° → °° : p = 0.095 (NS); X² : ^ → ^^ : p = 0.148 (NS),

Table 5: Co-infections frequencies

HIV+ (n = 108)			HHV-8+ (n = 42)		
HHV-8	HBV	HCV	HIV	HBV	HCV
16 (14.8%)	8 (7.4%)	14 (13.0%)	9 (21.4%)	3 (7.1%)	6 (14.3%)
HCV+ (n = 26)			HBV+ (n = 35)		
HIV	HBV	HHV-8	HIV	HCV	HHV-8
8 (30.8%)	1 (3.8%)	3 (11.5%)	9 (25.7%)	1 (2.9%)	6 (17.1%)

p-values of co-infections frequencies comparisons were obtained by Yates' corrected X² test, or ° by Fishers' exact test (when needed)

HIV/HHV8 → HHV8/HIV: p = 0.464 (NS) °HCV/HBV → HBV/HCV: p = 0.675 (NS)

Conversely, excluding the 33 women affected by AIDS, 42/396 (10.6%) was HHV-8 positive. Among them, the possibility of being co-infected by HIV was rather high: 9/42 (21.4%). Prevalence of co-infection by HCV and HHV-8 was much lower, 3/42 cases (7.1%), whereas 6/42 (14.3%) individuals were found HHV-8 and HBsAg seropositive. Finally, in the non-AIDS women that were evaluated, subjects found either HCV or HBV seropositive showed a high possibility to be co-infected by HIV, 30.8 and 25.7%, respectively.

Infection by one of these virus: HHV-8, HCV or HBV:

24.9% (107/429) of these pregnant women are infected by one of these viruses: HHV-8, HCV or HBV. 4.1% (2/49), 12.2% (6/49) and 2.5% (1/40) of these women have respectively a co-infection: HCV+/HHV-8+, HBV+/HHV-8+, HCV+/HBV+.

DISCUSSION

In the sub-Saharan Africa, Burkina Faso is one of the countries less characterized for prevalence of viral

infections. The epidemiological surveys carried out so far have described that HIV, HBV and HCV infections rates are quite similar to those registered in bordering regions of Western Africa^[13,16-18].

The present study has been an attempt to evaluate more thoroughly HHV-8 prevalence in Burkina Faso. The SCMC is a great medical institution for the population of Ouagadougou, the capital city of Burkina Faso. In this centre, in the past ten years, a low incidence of KS patients even in subjects affected by AIDS has been observed. This is in keeping with a retrospective study carried out in another major hospital in Ouagadougou, which reported 29 KS cases in patients admitted in the 5 years 1992-1996^[19]. It should be noted that only 1/429 women that took part to the present survey developed KS during the year of sampling: this case was a woman affected by AIDS with 178 CD4+ T cell μL⁻¹.

In the pregnant women not affected by AIDS tested in this screening, HHV-8 seroprevalence was 10.6%. The prevalence for HIV, HBV and HCV infections in the same cohort was 7.8, 8.8 and 6.6%, respectively, in line with the above mentioned reports. Thus, the present

epidemiological survey seems to suggest that Burkina Faso is a country with an intermediate level of HHV-8 infection in the population, certainly lower than that reported in other sub-Saharan countries, such as Uganda, Zambia, Malawi, South Africa, Lesotho^[12]. Since HHV-8 is a necessary co-factor in KS development, the relative low HHV-8 prevalence presumably plays an important role in determining the low KS incidence observed in Ouagadougou, if compared to other sub-Saharan countries. In fact, the concordance at local level between HHV-8 prevalence and KS incidence is an established factor already pointed out in epidemiological studies carried out in different areas.

Thus far, the reasons for the uneven geographical distribution of HHV-8 infection and KS worldwide are largely unknown and environmental, genetic, lifestyle factors have been suggested to be involved. In Africa, HHV-8 infection is characterized by the highest rates in the general population, particularly in the Equatorial regions and by the early acquisition of HHV-8 infection during childhood or adolescence, thus suggesting that routes of transmission, alternative to the sexual contact, are frequently involved in the spreading of the infection. Interestingly, it has been shown that infectious HHV-8 is released in saliva in healthy seropositive individuals, although it is unknown the mechanism through which the virus, shed in the saliva, might subsequently infect a seronegative individual^[20,21]. A rather suggestive theory for an alternative pathway of transmission from mother to child of viral infections through saliva has been recently proposed^[22]. In this hypothesis, the infection takes place during childhood and involves the bite of hematophagous arthropods as a promoting factor and the application of saliva by the parents to heal the itching and scratching at the site of the bite. If this transmission route actually plays a role in HHV-8 infection, then the local density of biting arthropods would be a relevant factor. At this purpose, it should be considered that the Ouagadougou area has a relatively dry climate if compared to the Equatorial African countries characterized by higher rates of HHV-8 infection. The difference in the climate reflects also the diffusion of insects, which is sensibly lower in the Ouagadougou area if compared with the above mentioned countries. Of course, further epidemiological studies still need to be carried out to validate this intriguing hypothesis.

The present survey has also evaluated the rates of co-infection by the four viruses assayed. Considering all the women that were screened, the possibility to be infected by one of these 4 viruses is 31.0% and the probability to be co-infected by two viruses was about 4.0% in the more frequent cases which are HHV-8/HIV and

HBV/HHV-8. Moreover, among the subjects seropositive for HIV, the chance to be co-infected by HHV-8 is less than 14.8% (16/108) and also for co-infections with HCV (8/108 = 7.4%) and HBV (14/108 = 13.0%), the risk is not much greater than that found for the general population. On the other hand, among the subjects seropositive either for HHV-8, HBV or HCV, the risk to be co-infected by HIV is higher than that found in the assayed population overall. However, no causative-effect link could be established since this survey does not indicate the moment the infections were acquired and whether this happened through common routes or behaviours. Therefore, epidemiological studies aimed to confirm this finding and the potential co-transmission are needed to better clarify these issues.

Furthermore, the present study has been performed on a group of pregnant women and 49 of them were found to be HHV-8 seropositive before delivery. It should turn out interesting to analyze the serostatus of their infants, in particular comparing the prevalence rate of the newborns from HHV-8 seropositive mothers versus those seronegative and at the same time, how this correlates with the transmission of HIV or HBV and HCV from seropositive mothers.

ACKNOWLEDGMENTS

We are grateful to the pregnant women of the Centre Médical de Saint Camille 01 BP 364 Ouagadougou 01 Burkina Faso. We are deeply grateful to the personnel of the Centre Médical Saint Camille and particularly to Madame Justine Yara and Monsieur Robert Bakamba for their skilful technical assistance.

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