Seroprevalence of human T-cell lymphotropic virus type I among pregnant women in Accra, Ghana

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Infection with human T-cell lymphotropic virus type I (HTLV-I) occurs mainly in Japan, Central and West Africa and the Caribbean Basin. Although antibody to HTLV-I has been reported among pregnant women in several endemic countries, there is no information regarding the seroprevalence in pregnant Ghanaian women. The reported seroprevalence of HTLV-I among healthy Ghanaian blood donors is between 0.5 and 4.2 %. Therefore, this study was conducted to determine the seroprevalence of HTLV-I among pregnant women attending the antenatal clinic at the 37 Military Hospital, Accra, Ghana, between the months of January and December 2003. The presence of antibodies specific for HTLV-I/II was tested using a particle agglutination test (PAT) kit and confirmed by Western blotting (WB). Of the 960 sera tested, HTLV-I/II antibodies were detected in 24 samples using the PAT kit. WB results indicated that, of the 24 positive PAT specimens, 20 specimens (83.3 %) were HTLV-I positive, one (4.2 %) was HTLV-II positive, two (8.3 %) were HTLV positive and one (4.2 %) was indeterminate. Therefore, the overall seroprevalence of HTLV-I was 2.1 %. Seroprevalence increased with age, suggesting sexual contact as the primary mode of transmission among women of childbearing age, rather than breastfeeding during infancy. The seroprevalence of 2.1 % reported here for HTLV-I in pregnant women in Accra is comparable to that of human immunodeficiency virus among the same population. In conclusion, the results indicate that HTLV-I is prevalent among asymptomatic Ghanaian pregnant women and thus there is a need to consider introducing antenatal screening for HTLV-I in Ghana.

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INTRODUCTION

Human T-cell lymphotropic virus type I (HTLV-I) is known to be the pathogenic agent of adult T-cell leukaemia/lymphoma (ATLL) (Poiesz *et al.*, 1980; Yoshida *et al.*, 1982) and HTLV-I-associated myelopathy/tropical spastic paraparesis (Gessain *et al.*, 1985; Osame *et al.*, 1987). ATLL develops after a long incubation period, with an estimated lifetime risk of approximately 3–8 % in individuals infected before the age of 20 years (Cleghorn *et al.*, 1995). As the prognosis for patients with ATLL is extremely poor, with median survival after diagnosis of <6 months (Takatsuki *et al.*, 1996), the prevention of mother-to-child transmission of HTLV-I is of the utmost importance. HTLV-I is not ubiquitous, but is endemic in some geographical areas and among some ethnic groups. Population-based studies have reported the prevalence of antibody to HTLV-I to range from 3–6 %

in Jamaica, Trinidad and the Caribbean islands to 23.2 % in Nagasaki Prefecture in south-western Japan (Blattner *et al.*, 1990; Murphy *et al.*, 1991; Kishihara *et al.*, 2001). High HTLV-I seroprevalence rates (>2 % in the adult population) have been described in southern Japan, sub-Saharan Africa, the Caribbean Basin, parts of South America and some areas of Melanesia and the Middle East, where 15–20 million people are infected with this virus (Kazanji & Gessain, 2003). In every endemic population, there is an increase in HTLV-I seroprevalence with age, especially among women, reaching 40 % in women >50 years of age (Kazanji & Gessain, 2003).

HTLV-I is transmitted via infected lymphocytes by three main routes. Mother-to-child transmission, mainly due to ingestion of breast milk during breastfeeding, has been reported to be the predominant route (Tsuji *et al.*, 1990). The rates of HTLV-I transmission from mother to child are 2.7 % in formula-fed infants, 5 % after 3 months of breastfeeding and up to 39 % with prolonged breastfeeding

Abbreviations: ATLL, adult T-cell leukaemia/lymphoma; HIV, human immunodeficiency virus; HTLV-I, human T-cell lymphotropic virus type I; PAT, particle agglutination test; WB, Western blotting.

(Tsuji *et al.*, 1990; Hino *et al.*, 1996; Takezaki *et al.*, 1997; Wiktor *et al.*, 1997). Antenatal screening for HTLV-I and the recommendation for formula feeding of infants of HTLV-seropositive mothers have been carried out in the Nagasaki prefecture of Japan since 1987 (Hirata *et al.*, 1992; Wiktor *et al.*, 1997) and have been proposed in Europe (Nightingale *et al.*, 1993; Weber & Taylor, 1996; Hale *et al.*, 1997; Otigbah *et al.*, 1997; Taylor *et al.*, 2005) and Jamaica (Hanchard, 1996). Sexual transmission, mainly from men to women, is the second most frequent route (Kajiyama *et al.*, 1986). The man-to-woman transmission rate of married couples was 60% in 10 years, in contrast to a woman-to-man transmission rate of 0.4% in 10 years (Kajiyama *et al.*, 1986). The third most prevalent route is blood transfusion, which includes HTLV-I-positive cellular components (Okochi *et al.*, 1984; Manns *et al.*, 1992). The importance of this route has decreased since the introduction of screening of blood products for antibodies to HTLV-I in Japan in 1987 (Inaba *et al.*, 1999) and subsequently in other countries (USA, UK, Canada, France, French West Indies, Portugal, Sweden, The Netherlands, Denmark and Finland) (Ades *et al.*, 2000).

In Ghana, studies of HTLV-I seroprevalence in pregnant women have not been done previously. However, recently we reported HTLV-I seroprevalence rates of 4.2 and 11.29% among blood donors and human immunodeficiency virus (HIV)/AIDS patients in Ghana, respectively (Adjei *et al.*, 2003a, b). Two earlier studies among blood donors presenting to blood banks in the late 1990s in Ghana reported an HTLV-I seroprevalence of 0.5–0.7% (Sarkodie *et al.*, 2001; Ampofo *et al.*, 2002). In a much earlier related study, Lal *et al.* (1994) reported the seroprevalence of HTLV-I among urban and rural dwellers in southern Ghana to be 1–2%. Currently in Ghana, neither blood donors nor pregnant women are screened routinely for HTLV-I antibodies. In view of the national public health policy in Ghana since 1990 encouraging prolonged breastfeeding (exclusive breastfeeding for the first 4–6 months of life and then breastfeeding supplemented with other feeds up to 2 years of age; Ministry of Health, 1990) and the increased risk of HTLV transmission after prolonged breastfeeding, it is necessary to determine the prevalence of antibodies to HTLV-I among pregnant Ghanaian women. The present study was conducted to determine this and to assess the implications for both antenatal and blood-donor screening.

METHODS

Subjects. All of the 960 pregnant women who attended the prenatal/antenatal care unit of the 37 Military Hospital, Accra, Ghana, between January and December 2003 were studied. The excess sera from blood samples drawn from these 960 pregnant women for their routine antenatal (syphilis, ABO and Rhesus) testing, with all identifiers removed except for age, were assayed for antibodies to HTLV-I. The 37 Military Hospital is a 600-bed national tertiary referral hospital of the Armed Forces of Ghana and serves Ghanaian military personnel and their families or dependants, other military personnel on peacekeeping missions in the West African subregion and part of the population of the city of Accra. Accra, the capital

city of Ghana, is a rapidly expanding city with a population of approximately 3 million. All HTLV-I-positive study subjects and their obstetricians were informed of the test result and counselled to bottle-feed their infants. The study was reviewed and approved by the Ethical and Protocol Review Committee of the University of Ghana Medical School, Accra, Ghana.

Specimen collection and serological tests. Venous blood samples were taken and sera were separated and kept frozen at -20°C before being sent to our laboratory for testing. Fully informed consent was obtained from each study subject. When study subjects were younger than 18 years, informed consent was obtained from their parents. Samples were anonymous for the patient's name and hospital number, but data on age were retained. All of the sera were screened in duplicate for antibodies to HTLV-I/II by a gelatin particle agglutination test (PAT) (Serodia HTLV-I kit; Fujirebio) in accordance with the manufacturer's instructions. All samples that were repeatedly positive by PAT were confirmed by Western blotting (WB) (HTLV Blot 2.4 kit; Gene Labs Diagnostics). WB results were interpreted according to the manufacturer's instructions as follows: (i) HTLV-I positive, reactivity to GAG p19 (with or without p24) and two ENV (GD21 and rgp46I); (ii) HTLV-II positive, reactivity to GAG p24 (with or without p19) and two ENV (GD21 and rgp46II); (iii) HTLV positive, reactivity to GAG p19 and p24 and ENV GD21; (iv) indeterminate, reactivity to HTLV-specific bands detected but does not meet the criteria for HTLV-I, HTLV-II or HTLV seropositivity; and (v) HTLV negative, no reactivity to HTLV-specific bands.

Statistical analysis. The Statistical Analysis System version 9.1 (SAS Institute) was used to complete all data analyses. We divided the pregnant women into five categories of age: ≤ 20 , 21–25, 26–30, 31–35 and ≥ 36 years. Serum results were classified as positive or negative. In the univariate analysis, the frequency for each of the age categories and the mean, median and maximum and minimum age for the overall sample were determined, as well as SD. We repeated the univariate analysis of age after having stratified the data by serum analysis results and compared the mean ages for a statistically significant difference using Student's *t*-test. We also obtained the frequency of seropositive and seronegative women. In the bivariate analysis, we evaluated the relationship between age and serum results categories using Pearson's χ^2 test. Logistic regression analysis was used to model the relationship between age categories and serum results. The logistic model with a maximum-likelihood estimate was fitted to the ordinal response of age categories and 95% confidence intervals for the odd ratios were calculated with the age category of ≤ 20 years as the reference group. A χ^2 test for trend over increasing age categories was also performed.

RESULTS

A total of 960 pregnant women was screened for HTLV-I antibodies. Their ages ranged from 15 to 41 years, with a mean age $\pm$ SD of 25.6 ± 5.8 years. The median and modal ages of all of the pregnant women studied were 26 years. All of the patients were found to be healthy on routine antenatal medical examination. In the primary screening, 24 (2.5%) samples tested positive by gelatin PAT. All of the samples were assayed in duplicate and repeatedly positive samples were confirmed by WB analysis. WB results indicated that, of these 24 repeatedly PAT-positive specimens, 20 specimens (83.3%) were HTLV-I positive, one (4.2%) was HTLV-II positive, two (8.3%) were HTLV positive and one (4.2%) was indeterminate. In order to address the issue of false negatives during the PAT screening, 24 repeatedly PAT-negative specimens were randomly selected and analysed by

WB. All of these 24 PAT-negative specimens were found to be HTLV negative by WB (no reactivity to HTLV-specific bands).

Therefore, the overall HTLV-I seroprevalence rate among pregnant women in Accra over the 12 month period was 2.1%. The age distribution of pregnant women seropositive for HTLV-I ranged from 18 to 38 years and their median and modal ages were 35 and 37 years, respectively. The age distribution of pregnant women seronegative for HTLV-I ranged from 15 to 41 years and both their median and modal ages were 26 years. The mean age ($\pm$ SD) of the seropositive pregnant women (32.4 ± 6.0 years) was significantly higher ($P < 0.0001$) than that of the mean age of the seronegative pregnant women (25.6 ± 5.8 years). A highly significant correlation existed between increasing age and HTLV-I seropositivity ($P < 0.0001$; Table 1).

DISCUSSION

The HTLV-I seroprevalence of 2.1% in Ghanaian pregnant women reported here agrees well with what is known about the endemicity of HTLV-I in West Africa and among ethnic groups of African origin (Kazanji & Gessain, 2003; Carles *et al.*, 2004). For example, in French Guyana, the overall seroprevalence in pregnant women is 4.4%, but it is more prevalent among ethnic groups of African origin, such as the Noir Marron population (5.5%) and Haitians (6.3%) (Carles *et al.*, 2004). Our observed rapid increase in seroprevalence with age and the low seroprevalence in the youngest age groups (Table 1) point to sexual contact as the primary mode of transmission among women of child-bearing age, with only a small fraction attributable to breastfeeding during infancy. This is also consistent with reports from other endemic countries (Murphy *et al.*, 1991; Kazanji & Gessain, 2003). Indeed, this suggests the importance of man-to-woman transmission in these pregnant women, as the duration of their sexual activity increases with age, whilst mother-to-child transmission would be the predominant route in the younger women/girls through breastfeeding during infancy. This is supported further by our observation that the mean age of the seropositive pregnant women was

significantly higher ($P < 0.0001$) than that of the seronegative women.

The HTLV-I seroprevalence of 2.1% among the healthy Ghanaian pregnant women in the current study was somewhat lower than the seroprevalence of 4.2% reported recently in healthy Ghanaian blood donors (Adjei *et al.*, 2003a). This difference is probably due to the fact that most blood donors in Ghana are replacement donors (usually relatives of hospitalized patients who need transfusion) rather than non-compensated repeat voluntary donors and to the reported significantly higher prevalence of viral markers of HIV-1/2, hepatitis B virus, hepatitis C virus and HTLV-I/II in replacement blood donors compared with voluntary blood donors in Ghana (Sarkodie *et al.*, 2001). In sharp contrast, the mean seroprevalence of HTLV-I in Western Europe was sixfold higher among pregnant women than among blood donors, probably because the vast majority of blood donors in Western Europe are non-compensated repeat voluntary donors rather than replacement donors (Taylor *et al.*, 2005).

The epidemiology of sexually transmitted diseases (including HTLV and HIV) in pregnant women is often used to approximate the epidemiology within the general population, as pregnant women are generally considered to be the most sexually active segment of the population. Therefore, excess sera from blood samples drawn from pregnant women for their routine antenatal testing are used for sentinel surveillance of HIV in Ghana (Ministry of Health, 2004) and in several other countries. The HTLV-I seroprevalence of 2.1% among Ghanaian pregnant women reported here is comparable to the 3.1% seroprevalence of HIV-1 and -2 among pregnant women in Ghana (Ministry of Health, 2004). A comparison of our results with similar studies around the world (Table 2) confirms the known endemicity of HTLV-I in Japan, sub-Saharan Africa, the Caribbean Basin and parts of South America, but not in Western Europe (Kazanji & Gessain, 2003).

The policy of not screening for HTLV antibody in pregnant women and in blood and organ donors in most countries is based partly on its perceived low prevalence and on the low lifetime risk of its associated diseases, although the cost of antenatal and blood-donor screening could be limited by selecting those thought to be at high risk. With appropriate counselling, screening for HTLV should be accepted in the same light as testing for HIV, which recently has been recommended as part of the routine antenatal screening programme in several countries (Nightingale *et al.*, 1993; Hanchard, 1996; Weber & Taylor, 1996; Hale *et al.*, 1997; Otigbah *et al.*, 1997; Taylor *et al.*, 2005; Wiktor *et al.*, 1997). However, unlike HIV infection, infection with HTLV is less likely to become clinically apparent and the factors that confer a high risk of developing associated disease have not been defined. In the meantime, antenatal screening and the promotion of bottle-feeding for children of seropositive mothers could help limit vertical transmission, as its cost-effectiveness has been proven in Japan (Kashiwagi *et al.*,

Table 1. Odds ratios for HTLV-I seropositivity and corresponding 95% confidence intervals (CI) by age of pregnant women in Accra, Ghana

χ^2 test for trend (d.f. = 1): $\chi^2 = 30.36$; $P < 0.0001$.

Age (years)	Women (n)	HTLV-I-positive women [n (%)]	Odds ratio	95% CI
≤ 20	220	1 (0.45)	1	—
21–25	251	2 (0.80)	1.76	0.16–19.53
26–30	311	3 (0.96)	2.13	0.22–20.64
31–35	113	5 (4.42)	10.14	1.17–87.83
≥ 36	65	9 (13.85)	35.19	4.37–283.53

Table 2. Comparison of HTLV-I seroprevalence in pregnant women worldwide

Country	Seroprevalence (%)	Reference(s)
Sub-Saharan Africa		
Ghana	2·1	This study
The Gambia	1·2	Del Mistro <i>et al.</i> (1994)
Guinea-Bissau	2·3	Andersson <i>et al.</i> (1997)
Mozambique	0·7	Melo <i>et al.</i> (2000)
Congo	0·7	Tuppin <i>et al.</i> (1996)
Gabon	6·8–10·5	Delaporte <i>et al.</i> (1988); Schrijvers <i>et al.</i> (1991)
Zaire	2·4–14·8	Goubau <i>et al.</i> (1993); Delaporte <i>et al.</i> (1995)
South America		
Brazil	0·84–0·88	dos Santos <i>et al.</i> (1995); Bittencourt <i>et al.</i> (2001)
Peru	2·3–2·5	Zurita <i>et al.</i> (1997); Sanchez-Palacios <i>et al.</i> (2003)
Caribbean Basin		
Martinique (French West Indies)	1·93	Mansuy <i>et al.</i> (1999)
Jamaica	2·0	Dowe <i>et al.</i> (1998)
French Guyana	4·4	Carles <i>et al.</i> (2004)
Asia Pacific		
Japan	3·7	Kashiwagi <i>et al.</i> (2004)
Western Europe		
Germany	0·007	Taylor <i>et al.</i> (2005)
Spain	0·010	Taylor <i>et al.</i> (2005)
Portugal	0·013	Taylor <i>et al.</i> (2005)
Italy	0·017	Taylor <i>et al.</i> (2005)
Belgium	0·020	Taylor <i>et al.</i> (2005)
UK	0·047	Taylor <i>et al.</i> (2005)
France	0·115	Taylor <i>et al.</i> (2005)

2004). Therefore, it has been recommended that HTLV-I should be screened for during pregnancy in women living in and originating from highly HTLV-I-endemic countries; in cases of HTLV-I seropositivity, mothers should be informed of the risks of transmission and the promotion of bottle-feeding of their children should be strongly proposed (Carles *et al.*, 2004). Additionally, determination of the HTLV-I serostatus of pregnant women would ensure that doctors could take further precautions to protect against nosocomial infection and to ensure that newborns do not swallow blood at the time of delivery from HTLV-I-seropositive mothers, in order to minimize perinatal HTLV-I transmission in non-breastfed children of HTLV-I-seropositive mothers (Carles *et al.*, 2004).

The argument for antenatal HTLV testing in Ghana is compelling, as breastfeeding is prolonged and widespread among Ghanaian women. Our findings re-emphasize the suggestion that targeting high-risk women or universal testing in high-prevalence areas, which includes Ghana,

could identify most women infected with HTLV at a relatively low cost (Ades *et al.*, 2000). Antenatal HTLV testing is likely to be less beneficial economically and clinically than antenatal HIV testing, but should be evaluated fully, as the risks of infection and disease after contaminated transfusion are less with HTLV than with HIV (Ades *et al.*, 2000). This study highlights the need for screening of pregnant women for circulating antibodies to HTLV-I. A further larger-scale prospective survey of HTLV-I infection in Ghana should be conducted to verify the results of the present study and to analyse in more detail the epidemiological features of this retroviral infection and evaluate the cost-effectiveness of antenatal HTLV screening in Ghana.

In conclusion, the results of this study and our recent results in healthy blood donors (Adjei *et al.*, 2003a) demonstrate a high prevalence of HTLV-I infection in Ghana. Therefore, preventative measures to decrease the spread and transmission of HTLV in Ghana are warranted. These measures should include the systematic HTLV-I screening of blood donors and pregnant women in order to counsel them about the risk of HTLV-I transmission by prolonged breastfeeding, and the prevention of sexual transmission of HTLV-I by educational programmes emphasizing the importance of using condoms to prevent all sexually transmitted diseases, including HIV and HTLV infection.

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