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Seroepidemiology of HTLV-I in relation to that of HIV-1 in the Gauteng region, South Africa, using dried blood spots on filter papers

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SUMMARY

The seroprevalence of human T-lymphotropic virus type I (HTLV-I), in relation to that of human immunodeficiency virus type 1 (HIV-1), was determined in a comparative unlinked anonymous antenatal and neonatal (for indirect measurement of maternal antibodies) sero-survey in the Gauteng region of South Africa, using dried blood spots (DBS) and modified particle agglutination assays. Samples were confirmed to be antibody positive by western blot. A total of 2582 DBS collected during 1993 and 1994 from subjects of African, European and coloured origin were tested. Ten were confirmed as positive for HTLV-I and 128 for HIV-1. No antibodies to HTLV-I or HIV-1 were demonstrated in the 221 women of European and coloured origin who were screened. The HTLV-I seroprevalence rate in subjects of African origin appeared to increase from 0% in 1993 to 0·49% in 1994, while HIV-1 seroprevalence rates of 5·2% and 5·4% were recorded for 1993 and 1994 respectively. No significant differences in HTLV-I and HIV-1 seroprevalence rates were noted for the two areas investigated. These results indicate that HTLV-I should be included in infection control programs, and provide baseline data for monitoring the possible spread of HTLV-I in the heterosexual population in this region.

INTRODUCTION

Human T-lymphotropic virus type I (HTLV-I) has been associated aetiologically with adult T-cell leukemia/lymphoma (ATL) [1, 2], the chronic degenerative neurological disease termed tropical spastic paraparesis (TSP) [3] or HTLV-I associated myelopathy (HAM) [4, 5], uveitis [6] and infective dermatitis [7]. In regions where HTLV-I is endemic, ATL has been estimated to occur in 2–4% of infected individuals, with a latency period of a few decades, while TSP/HAM occurs in < 1% of infected people, with a latency shorter than that for ATL [8]. Endemic foci of HTLV-I infection occur mainly in south-western Japan [9, 10], the Caribbean basin [11], central and southern Africa [12–16] and the south-eastern parts of the USA [17]. Infection has also been recorded in migrants from endemic to non-endemic regions [18, 19]. The occurrence of TSP/HAM and ATL in South Africa has been well documented [20–23]. Several seroprevalence surveys of HTLV-I antibodies have been carried out in selected South African communities, with seroprevalence rates ranging from 0% among blood donors to 5·2% among black

female staff in the Kruger National Park [15, 24–31]. A high prevalence of HTLV-I antibodies has also been reported in human immunodeficiency virus type 1 (HIV-1) positive patients with neurologic disease [32].

HTLV-I and HIV-1 share common risk factors, consequently the pattern of spread of HTLV-I within a community may be similar to that observed in the HIV epidemic. Unlinked anonymous testing in pregnant women and of newborn babies has proved invaluable in monitoring the HIV epidemic in a heterosexual population [33, 34]. The presence of HIV-1 antibody in neonatal serum reflects maternal infection, and seroprevalence surveys among sexually active women represent a large stable heterosexual population in which to monitor the spread of the virus in a community [33]. The same would therefore apply to HTLV-I infection.

With the recent political changes, South Africa has been subdivided into nine geographic regions with the industrialized Pretoria-Witwatersrand-Vereeniging complex of the old Transvaal province forming one of these new provinces, namely Gauteng, with an estimated population of 6850000, and 195000 live births in 1994 [35, 36]. An increase in the number of cases of TSP in the Gauteng region has been noted [37]. Whether this observed increase is a true increase or due to an increased awareness of the clinical condition and availability of diagnostic assays is not known, but the potential importance of HTLV-I infection appears to have been overlooked with the dramatic rise in HIV infection in South Africa [34]. We report a sero-survey of HTLV-I infection, in relation to that of HIV-1 infection, in an anonymous unlinked antenatal and neonatal survey, in this densely populated region of South Africa.

MATERIALS AND METHODS

Dried blood spot (DBS) collection

Neonatal specimens

DBS specimens on filter paper were prepared from cord blood as soon after the infant's birth as possible. Specimens were collected from infants born to mothers of African (black), European (white) and coloured (of mixed descent) origin at the Kalafong and HF Verwoerd Hospitals, Pretoria during 1993 and 1994. Testing was carried out within 2 months of sample collection.

Antenatal specimens

DBS specimens on filter paper were prepared from venous blood from women, of African origin, presenting for the first time during pregnancy at Baragwanath Hospital, Soweto in 1994.

Elution of serum

A 5·5 mm diameter spot was punched out of each DBS and eluted in 100 μl elution buffer (phosphate-buffered saline, pH 7·2, containing 0·05% 'Tween 20' and 0·05% sodium azide) as described by Peckham and colleagues [38].

Assay for the detection of antibodies to HTLV-I

Eluates were tested for antibodies to HTLV-I using the Serodia HTLV-I gelatin particle agglutination (PA) assay (Fujirebio Inc., Tokyo, Japan) as described previously [39]. A 1/6 dilution of each eluate was made in TPHA buffer (Mast Diagnostics, Bootle, Merseyside, UK) in a V-well microtitre plate. HTLV-I gelatin particles, reconstituted according to manufacturer's instructions, were diluted 1/10 in TPHA buffer immediately before use. Twenty-five microliters of diluted particles were added to the diluted eluate and the plate incubated at room temperature for 15 min. After incubation the plates were spun at 200 g for 3 min and then placed on a lightbox at a 70° angle. Results were read after 10 min and considered to be positive for anti-HTLV-I if the particles formed a discrete agglutination pattern, whereas in the absence of antibodies the gelatin particles streamed to form a 'teardrop' pattern.

Assay for the detection of antibodies to HIV-1

The eluates were tested for anti-HIV-1 in a modified gelatin PA assay as described previously [33, 38].

Confirmatory testing

Specimens found to be reactive repeatedly on the modified PA screening assays for HTLV-I and HIV-1 were confirmed using western blot analysis (WB), i.e. HTLV BLOT 2·3 (Diagnostic Biotechnology, Singapore) and HIV BLOT 2·2 (Diagnostic Biotechnology, Singapore) respectively. A 1/50 dilution of reconstituted serum was tested according to the manufacturer's recommendations. Specimens were considered to be positive for HTLV-I antibodies if the envelope proteins rgp21, rgp46-I and core proteins

p19 or p24 bands were present. HIV WBs were interpreted according to the criteria of the Centers for Disease Control where sera demonstrating two of the following three bands, p24, gp41, gp120/160, were considered to be HIV positive [40].

Ethical approval

This project was approved by the ethical committee of the Faculty of Medicine, University of Pretoria and the Committee for Research on Human Subjects – Medical, University of the Witwatersrand.

RESULTS

A total of 2582 DBSs, collected between October 1993 and December 1994, were included in the study. Of these, 1323 were prepared from cord blood from neonates delivered in the Pretoria area. The remaining 1259 were obtained from pregnant women attending an antenatal clinic at Baragwanath hospital in Soweto. The HTLV-I and HIV-1 seroprevalence rates are summarised in Table 1. No HTLV-I antibody positive specimens were detected in the 309 DBSs collected from subjects of African ethnic origin in the Pretoria area in 1993, while a seroprevalence rate of 5·2% was demonstrated for HIV-1. In 1994, seroprevalence rates of 0·38% and 5·5% were recorded for HTLV-I and HIV-1 respectively in neonates of African origin from the Pretoria area, with HTLV-I and HIV-1 seroprevalence rates of 0·56% and 5·4% being recorded in antenatal specimens in Soweto during the same period. When the HTLV-I and HIV-1 seroprevalence rates for 1994 for subjects of African origin from the two areas within the Gauteng region are compared, no significant differences were noted and seroprevalence rates of 0·49% and 5·4% were recorded for HTLV-I and HIV-1 respectively for black subjects from the region. No HTLV-I or HIV-1 positive DBSs were detected from white or coloured subjects, and the overall HTLV-I and HIV-1 seroprevalence rates demonstrated for the region for 1994 were 0·44% and 5·0% respectively. No dual infections were detected in any of the specimens tested.

DISCUSSION

DBSs on filter papers/Guthrie cards have proved to be suitable for large scale seroprevalence studies on HIV [33], HTLV-I [41], and for the detection of both HTLV-I and HIV-1 proviral DNA [41, 42]. This method of blood specimen collection has the advantage that specimens can be collected, stored and transported without prior processing, and minimizes the risk of laboratory acquired infection. The cost-effective modified PA assays used in this study for the detection of antibodies to both HTLV-I and HIV-1 have been shown to be highly sensitive, specific and suitable for epidemiological studies [33, 38, 39].

HTLV-I and human T-lymphotropic virus type II (HTLV-II) are closely related but distinct oncoviruses [43]. Approximately one half of blood donors in the USA, seropositive for HTLV-I/II, are infected with HTLV-II [8]. HTLV-I sero-surveys in South Africa have focussed mainly on selected communities [15, 24–26, 28–30], with little data from the general population. Caution must however be exercised when interpreting HTLV-I serological results on serum samples of African origin as unrecognized but related retroviruses may be present [26, 30, 44]. The relatively high HTLV-I seroprevalence rates reported in early studies [24, 25, 45] may therefore be overestimates as no WB confirmatory testing was done on sera found to be positive by enzyme immunoassay. In this investigation no evidence of HTLV-I infection was detected in DBSs collected in 1993, while in 1994 a seroprevalence rate of 0·38% (95% confidence interval [CI] = 0·016–0·60%) was recorded in the same community, with an overall seroprevalence rate of 0·44% (95% CI = 0·17–0·71%) for the region. In community-based studies, an HTLV-I seroprevalence rate of 0·2% (95% CI = 0·01–1·3%) was reported in sera from 428 black South African pregnant women from the Pretoria area [44], and Maaga and co-workers [31] reported a seroprevalence rate of 0·2% in sera collected in 1989 from 509 healthy black family volunteers from the Ga-Rankuwa area (north-west of Pretoria) of the Gauteng region. To date only one case of HTLV-II seropositivity has been recorded in South Africa, i.e. in KwaZulu/Natal [15].

Although the apparent increase in the HTLV-I seroprevalence rate reported in this study for the Gauteng region was small (0–0·44%), it should be noted that the rate of 0·44% is similar to the HIV seroprevalence rate (0·53%) reported for women attending antenatal clinics in the Transvaal province in 1990 [34]. The concurrent HIV-1 seroprevalence rate for our subjects for 1994 was 5·0% while an overall seroprevalence rate of 6·44% (95% CI = 5·63–7·25%) was reported for the Gauteng region for 1994 [36]. It is not known whether this observed increase in HTLV-I seropositivity is due to changing

Table 1. *Seroprevalence of HTLV-I and HIV-1 antibodies in the Gauteng region, South Africa, by area, ethnic group and year*

	Seroprevalence								
	1993				1994				
	HTLV-I		HIV-1		HTLV-I			HIV-1	
Ethnic group	Number tested*	%† (95% CI)‡	Number positive§	% (95% CI)	Number tested	Number positive	% (95% CI)	Number positive	% (95% CI)
By area									
Pretoria									
Black	309	0	16	5·2 (2·71–7·65)	789	3	0·38 (0·16–0·60)	43	5·5 (3·87–7·03)
Coloured	21	0		0	107		0		0
White	0	n/a¶		n/a	73		0		0
Unspecified‖	0	n/a		n/a	24		0	1	4·2 (0·0–12·17)
Soweto									
Black	0	n/a		n/a	1259	7	0·56 (0·15–0·97)	68	5·4 (4·15–6·65)
By ethnic group									
Black	309	0	16	5·2 (2·71–7·65)	2048	10	0·49 (0·34–0·64)	111	5·4 (4·44–6·40)
Coloured	21	0		0	107		0		0
White	0	n/a		n/a	73		0		0
Unspecified	0	n/a		n/a	24		0	1	4·2 (0·0–12·17)
Total	330	0	16	4·8 (2·53–7·17)	2252	10	0·44 (0·17–0·71)	112	5·0 (4·07–5·87)

* Number of dried blood spot specimens tested.
† Percentage seropositivity.
‡ 95% confidence interval.
§ Number of dried blood spot specimens which tested positive.
‖ Ethnic group not specified.
¶ Not applicable.

population dynamics or an increase in HTLV-I infection rate, but our data should inform the decision about screening of blood, plasma and organ donations, for anti-HTLV-I/II in South Africa. When they isolated the HTLV-I virus from an indigenous black South African, Becker and colleagues [46] recommended then that routine testing of blood donors should be instituted in South Africa, while Maaga and colleagues [31] indicate that the routine screening of blood for HTLV-I/II may be premature, except in Kwazulu/Natal. The results of our investigation suggest that there is an urgent need for larger serosurveys, and support the recommendations that HTLV-I should be included in infection control programs [29]. The selective screening of blood donations from endemic regions such as Kwazulu/Natal, as well as from risk groups that are otherwise not excluded from blood and organ donations, such as migrants from endemic regions, could possibly be introduced prior to the introduction of universal anti-HTLV-I/II screening.

Sentinel sero-surveillance plays an important role in monitoring the course of a specific disease in a community. The use of DBSs and the modified PA assay as described in this study would be a safe and cost-effective method for screening large numbers of specimens. In addition, the data presented here provide not only a useful baseline for further serosurveys in these communities but also the basis for the formulation and monitoring of local counselling and intervention programmes.

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