HTLV-I, HIV-I, and Hepatitis B and C Viruses in Western Province, Papua New Guinea: A Serological Survey

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Seven hundred and twenty-three serum samples from individuals in 13 Gidra-speaking villages in Western Province, Papua New Guinea were tested for evidence of infection with human T-lymphotropic virus type I (HTLV-I), human immunodeficiency virus type I (HIV-I), hepatitis B virus (HBV) and hepatitis C virus (HCV). No samples were positive for antibodies to HIV-I. Antibodies to HTLV-I were found in 13 samples (1.8%), HBV surface antigens (HBsAg) were found in 86 samples (11.9%), and antibodies to HCV were found in 30 samples (4.1%). Six (46.2%) of 13 HTLV-I positive samples were positive for HCV or HBsAg. The seropositive rate varied in different villages and the incidence of HTLV-I and HCV was higher in coastal and riverine areas than inland.

Key words: HTLV-I — HIV-I — Hepatitis B virus — Hepatitis C virus — Papua New Guinea

Human T lymphotropic virus type I (HTLV-I) infection is causally associated with adult T cell leukemia (ATL), and a strong association has been shown between HTLV-I infection and HTLV-I associated myelopathy/ tropical spastic paraparesis (HAM/TSP) in endemic areas such as southwestern Japan and some parts of the Caribbean. In these areas, many HTLV-I antibody-positive, healthy individuals have been detected by seroepidemiologic analysis.

Recent reports have suggested relatively high densities of HTLV-I carriers in the northwest region in Papua New Guinea (PNG) and in part of Indonesian New Guinea.[1-6] However, little is known about whether HTLV-I infection is widespread in these countries.

The seroepidemiology of hepatitis B virus (HBV) infection has been studied in detail throughout South-East Asia and the Pacific.[7, 8] The prevalence rate determined in this paper is comparable with the findings in the Philippines, Micronesia, Polynesia, and some other parts of PNG. There has, however, been no previous seroepidemiological study of the hepatitis C virus (HCV) in this geographic area.

MATERIALS AND METHODS

Subject population The Western Province (District) is the most thinly populated area in PNG,[9] the population density in this area being less than one person per km². The average annual rainfall reaches 2,000 mm, with about 80% falling during the wet season from December to May, and the average monthly temperature fluctuates only from 25°C to 28°C.

Geographically, the thirteen Gidra-speaking villages whose inhabitants were the subjects of our study are situated in an oval-shaped territory of 4,000 km² of land that extends southeast and northwest, as shown in Fig. 1. The population of the Gidra group in these 13 villages numbered 1850 in 1980. One of these villages, called Dorogori, is located on the coast facing Daru (the capital of the province) across a small strait. Abam, Woigi, and Wuroi are located on the bank of the Oriomo River, and Ume is on the bank of the Ume River. Rual, Kapal, Wonie, Kuru, Iamega, Podare, Gamaeve, and Wipim are inland villages, while Rual and Kapal are situated fairly close to the tributaries of the Bituri River in the north. The geographical settings condition the way of living to some extent: for example, the people of the coastal village, Dorogori, depend on fishing, in contrast to the predominance of hunting among the inland people; coastal and riverine villagers travel mainly by canoe, while inland villagers travel exclusively on foot.

The Gidra linguistic group does not have a traditional chief or any political organization governing all of the members, and mutual communication among the people of the thirteen villages is infrequent.

Sera Permission to complete this work was obtained from the National Medical Research Advisory Committee of PNG. All subjects gave their informed consent. The blood was collected by venipuncture, and serum was separated and stored at –20°C. All subjects were ex-

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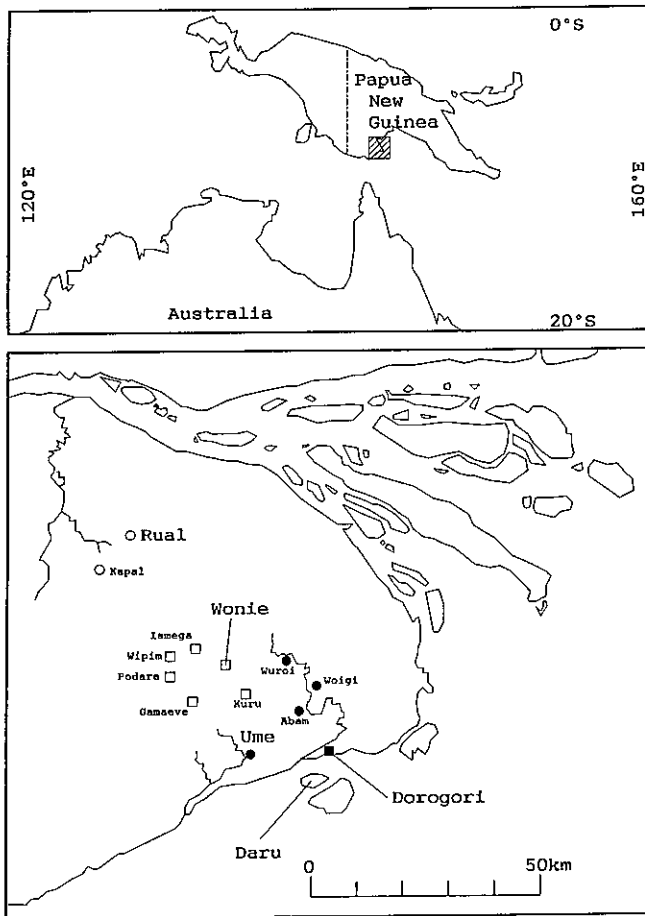[6] To whom correspondence should be addressed.

Fig. 1. The location of 13 Gidra-speaking villages in Western Province, Papua New Guinea. The map covers the area approximately between 8° 20′ and 9° 20′ S in latitude and 142° 40′ and 143° 40′ E in longitude.

amined by a PNG physician carrying out this survey and, when appropriate, medical treatment was given or referrals were made to the area hospital. Between July and September 1989, a total of 723 serum samples (333 males and 390 females) were collected in the 13 Gidra-speaking villages in Western Province, PNG. The subjects, whose ages ranged approximately from 18 to 65 years, amounted to about 60% of the adults as a whole, though the stated ages of most individuals were probably incorrect.

Detection of viruses HTLV-I: All sera were initially screened for HTLV-I antibodies, using the gelatin particle agglutination test (PA, Serodia HTLV-I, Fujirebio, Tokyo).[10] Sera that caused agglutination at a final dilution of 1:16 or more in the screening were then subjected to enzyme-linked immunosorbent assay (ELISA, Eitest-ATL, Eisai, Tokyo)[11] and immunofluorescence testing

(IF),[12] using HTLV-I-producing MT-2 cells and Western blot analysis (WB, ED-005, Eisai), to confirm antibody specificity. In the WB, we judged antibody in a given serum to be positive when it gave at least two bands with molecular weights of 19, 24, 28, 46, or 53 kDa.[13]

HBV: All sera were screened for hepatitis B surface antigen (HBsAg) by ELISA (Enzygnost HBsAg micro, Behringwerke AG, Marburg, Germany) and reversed passive hemagglutination (RPHA, Auscell, Abbott, North Chicago). The results of these assays were in complete correspondence.

HCV: All sera were tested using two recombinant ELISA (Ortho Diagnostic Systems, Raritan, New Jersey and Abbott).[14]

HIV-I: All sera were tested using the PA (Serodia HIV, Fujirebio).[15]

RESULTS

Antibodies to HTLV-I Serum samples from 333 males and 390 females were tested for antibodies to HTLV-I (Table I). Of these samples, 17 (2.4%) were seropositive as determined with the PA. Fifteen of 16 sera with ELISA absorbance higher than the cut-off level were defined as positive. Fourteen of 17 were reactive at 1:10 dilution, determined by indirect IF on MT2 cells. Two showed non-specific reaction. As verified by Western blotting, IgG antibodies against HTLV-I-specific pro-

Table I. Frequency of Positive Results for the Stated Serological Markers of Virus Infection

Village name	No. of serum samples tested	No. of positive cases (%)		
		HTLV-I	HBsAg	HCV
1. Kapal	66	0	6 (9.1)	0
2. Rual	55	1 (1.8)	9 (16.4)	2 (3.6)
Subtotal	121	1 (0.8)	15 (12.4)	2 (1.6)
3. Wonie	52	0	7 (13.5)	0
4. Kuru	48	0	7 (14.6)	1 (2.1)
5. Iamega	80	1 (1.3)	5 (6.3)	0
6. Podare	46	0	5 (10.9)	1 (2.2)
7. Gamaeve	21	0	3 (14.3)	1 (4.8)
8. Wipim	61	0	3 (4.9)	3 (4.9)
Subtotal	308	1 (0.3)	30 (9.7)	6 (1.9)
9 Ume	100	2 (2.0)	19 (19.0)	7 (7.0)
10. Abam	65	0	5 (7.7)	1 (1.5)
11. Wuroi	36	2 (5.5)	4 (11.1)	1 (2.8)
12. Woigi	28	0	1 (3.6)	2 (7.1)
13. Dorogori	61	7 (11.5)	10 (16.4)	11 (18.0)
Subtotal	290	11 (1.7)	29 (12.7)	11 (4.8)
Others	4	0	2 (50.0)	0
Total	723	13 (1.8)	86 (11.9)	30 (4.1)

Table II. Seroprevalence of HTLV-I, HIV-I, and Hepatitis B and C Viruses in Western Province, Papua New Guinea

	HTLV-I Ab		HIV-I Ab		HBs Ag		HCV Ab	
	Male	Female	Male	Female	Male	Female	Male	Female
Number tested	333	390	333	390	333	390	333	390
Positive	9 (2.7%)	4 (1.0%)	0	0	45 (13.5%)	41 (10.5%)	14 (4.2%)	16 (4.1%)
Indeterminate	0	4 (1.0%)	0	0	0	0	1 (0.3%)	0

Table III. HTLV-I Western Blot Band Patterns of 17 PA-positive Sera

Sera	PA (Serodia)	ELISA (Eisai) cut-off 0.483	IF	Western blot band pattern	Hepatitis virus infection
433	1:16	1.168	+	Negative	—
727	1:16	>2.5	+	p19, 24	—
75	1:32	0.401	±	Negative	—
456	1:32	1.403	NS	p19, 28, 68	—
472	1:32	2.404	+	p19 Indeterminate	—
679	1:32	>2.5	+	p19, 53	—
619	1:128	>2.5	+	p19, 24, 28, 46, 53	—
625	1:128	>2.5	+	p19, 53	—
667	1:128	>2.5	+	p19, 53	HCV
665	1:256	1.011	+	p19, 24, 28, 53	—
694	1:256	1.419	+	p19, 24, 53	HCV
707	1:256	NT	+	p19, 53	HCV
88	1:512	>2.5	+	p19, 24, 28	—
462	1:512	0.763	+	p19, 24, 28, 32, 46	HBsAg
705	1:512	>2.5	+	p19 Indeterminate	—
236	1:1024	1.065	+	p19, 24, 28, 32, 46	HBsAg
711	1:8192	0.716	NS	p19, 53	HBsAg

IF: indirect immunofluorescence test. NT: not tested. NS: non-specific reaction.

teins were positive in 13 of the 17 PA- and ELISA-positive serum samples and WB had indeterminate results in 2 serum samples. No significant difference was found between the incidence of HTLV-I antibodies in the two sexes. Table III shows the geographic distribution of HTLV-I-seropositive subjects. The seropositivity rate of HTLV-I varied depending on birthplace. The incidence in the coastal and riverine areas was higher than inland, and was especially high in the coastal village (Dorogori) (Table I).

Antibodies to HIV-I None of the samples tested was positive for antibodies to HIV-I (Table II).

HBsAg Eighty-six (11.9%) of the 723 serum samples were positive for HBsAg; no significant difference was found between the incidence of HBsAg in males and females (Table II) and there was no significant difference related to geographic distribution (Table I).

Antibodies to HCV Thirty (4.1%) of the 723 serum samples were positive for HCV, as determined by using the two ELISA kits; no significant difference was found between the incidence in males and females. Table II shows the geographic distribution of HCV-seropositive subjects. Like the incidence of HTLV-I, the incidence of HCV was high in the coastal and riverine regions, especially in the coastal village (Dorogori).

Double infection of viruses Three (0.4%) of the 723 serum samples were positive for HTLV-I and HCV. Another three samples were positive for HTLV-I and HBsAg. Six (46.2%) of 13 HTLV-I positive samples were positive for HCV or HBsAg (Table III). Two of the 723 serum samples were positive for HCV and HBsAg.

DISCUSSION

Outside the endemic areas in Japan and the Caribbean, cases of HTLV-I infection have been reported sporadically in the US, South America, and elsewhere. Accurate diagnosis of HTLV-I infection has become important. In general, PA (Serodia) and ELISA assays are used for screening, whereas IF and WB assays are used as confirmatory assays. A high rate of false-positives in sera obtained from tropical areas such as West Africa and

South Asia has been reported. Weber *et al.* criticized a previous report on seropositivity in PNG.[16]

Controversy has surrounded the question of HTLV-I infection and disease in Melanesia, including PNG. This study showed the average incidence of confirmed HTLV-I seropositivity in Western Province, PNG to be 1.8%, which is similar to that in other areas of PNG,[3] but lower than that reported in endemic areas in Japan and the Caribbean basin. We also observed some false-positive results not only on PA and ELISA, but also on IF and WB. We therefore judged real seropositivity to be positive serum detection by all four assays.

Recently, Yanagihara *et al.* reported the detection of HTLV-I genomic sequences by polymerase chain reaction (PCR) in lymphocyte cultures of three unrelated native Solomon Islanders (including a patient with HTLV-I myeloneuropathy) residing in widely separated regions.[17] These investigators detected virus-specific proteins of 15, 19, 24, 46, and 53 kDa on WB. Amplification of HTLV-I *gag, pol,* and *env* sequences by PCR confirmed that the viral isolates were HTLV-I, not HTLV-II. They also reported that the Solomon Islands viral isolates represented variants of HTLV-I, and they analyzed the nucleotide sequences of these viral strains. The isolates of HTLV-I from Japan and the Caribbean are genetically very closely related; however, HTLV-I from PNG and that from the Solomon Islands are different from the strains isolated in Japan and the Caribbean.[4]

Due to the antigenic similarities between HTLV-I and HTLV-II, definitive differentiation can be made only by using molecular techniques such as gene amplification by PCR. However, reliable differentiation between HTLV-I and -II can be made by analyzing the relative reactivity of a given serum to p19 and p24 by WB. Sera from individuals with PCR-proven HTLV-I infection react either more strongly to p19 than to p24 or equally strongly to both proteins. By contrast, sera from PCR-proven HTLV-II-infected individuals react more strongly to p24 than to p19 or not at all to p19.[18] In our series, the pattern on WB showed a predominant trend toward p19 rather than toward p24 (data not shown). We believe that HTLV-I or/and variant forms of HTLV-I infection also exist in Western Province, PNG; however, the mode of transmission of HTLV-I in PNG is unknown at the present time.

The high incidence (46.2%) of double infection of HTLV-I and HCV/HBsAg suggests that the transmission of those viruses was through the blood.

The persons whose sera were collected manifested no ATL or HAM/TSP-like diseases. HTLV-I is clearly associated with these diseases in HTLV-I endemic areas, so the significance of HTLV-I or HTLV-I variant virus

infection in PNG in relation to other clinical manifestations should be studied further.

Concerning HBV, we found no differences in the incidence of HBsAg in the villages in Western Province. Iga *et al.* reported that traditional lifestyles are conducive to the maintenance and spread of this virus, which is much more prevalent in the less-modernized provinces of PNG than in Port Moresby, the capital.[8] Woodfield *et al.,* using an immunoelectroosmophoresis method in 1972,[19] reported that leprosy patients and healthy Papua New Guineans (blood donors) showed HBV prevalence of 6.8% and 7.5%, respectively.

It has recently been demonstrated that HCV is a major cause of non A-non B hepatitis.[14] Most cases of non A-non B hepatitis and HCV infection are associated with blood transfusion and blood products, but the frequent occurrence of non A-non B hepatitis and HCV infection in the absence of any obvious parenteral exposure has been well documented. In our study in Western Province, PNG, the HCV seroprevalence in most villages, except for Dorogori (coastal), was comparable with that in Western developed countries. The villagers have virtually no experience of blood transfusions; however, they do have a traditional custom of venipuncture for which they use shells.[20]

Our study showed HTLV-I and HCV seropositive subjects in the coastal and riverine areas of the Western Province, but not inland, even though there is no difference in HBsAg incidence in those areas. Although the exposure to these viruses via blood for the villagers living inland may be less than that in the coastal and riverine areas, the seroconversion from HBsAg to HBs antibody may be less frequent because of undernutrition. The relationship of seropositivity in specific populations with regard to communication between tribes, habits, sexual behavior, and various customs should be studied further from the anthropological viewpoint.

The differences among the villages in the seroprevalence of HTLV-I and HCV are probably due to social and cultural stratification of relatively recent date and to the migration to the capital of more acculturated persons who tend to adopt a Westernized lifestyle. A corollary of this is the obvious conclusion that village life is still conducive to the maintenance and spread of the carrier state, and that control of the condition requires vigorous public action.

A vaccination campaign such as has lately been projected will produce results only after a number of years, and it is plain that health education should play a major part in the control of the spread of the HBV. Fortunately, HIV-I infection has not spread in this area.

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