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Seroprevalence of human T-lymphotropic virus type 1 in Papua New Guinea and Irian Jaya measured using different Western blot criteria

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Abstract

Background: Endemic foci of HTLV-1 carriers have been found in the world, however, the origin of HTLV-1 in humans is still unclear. Since a distinct type of virus strain was isolated from the Solomon Islands, detailed surveys on HTLV-1 prevalence in New Guinea are important to shed light on its history of dissemination. *Objective*: To clarify the seroprevalence of HTLV-1 in different regions of New Guinea Island. *Study design*: Sera from 1221 individuals (649 males, 454 females and 118 unknown) in New Guinea Island were studied for the presence of antibodies to HTLV-1 by a particle agglutination and the Western blot (WB) tests. Two different sets of criteria, proposed by WHO and Kiyokawa et al., were employed to interpret the WB test. Since the latter seemed to lack adequate specificity, the WHO criteria was used for the evaluation of the seroprevalence throughout the study. *Results*: Seroprevalence of HTLV-1 differed by the WB criteria. By the more stringent criteria, HTLV-1 carriers were found in Madang, Chimbu and one hinterland province, Enga, in Papua New Guinea. An overall seroprevalence rate in different regions ranged from 0 to 14.6%. No seropositive individuals were found in Irian Jaya. *Conclusions*: To avoid overestimating the seropositivity rates, the WHO criteria would be more appropriate to employ for WB test by using the samples obtained from tropical and/or malaria endemic areas. This study is the first to show HTLV-1 infected individuals in the hinterland of New Guinea Island. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: HTLV-1; New Guinea; Western blot criteria

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1. Introduction

Human T-lymphotropic retrovirus (HTLV-1) is a known causative agent of adult T-cell leukemia/

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lymphoma (ATL) (Hinuma et al., 1981; Yoshida et al., 1982) and HTLV-1 associated myelopathy (HAM)/tropical spastic paraparesis (TSP) (Gessain et al., 1985; Osame et al., 1986). HTLV-1 has been found in various regions in the world including endemic areas of ATL and HAM/TSP. Sporadic carriers have, however, been identified also in other regions (reviewed in Tajima and Hinuma, 1992; Ishida, 1995). Since the transmission of HTLV-1 requires the presence of HTLV-1 infected living cells (Yamamoto et al., 1982), natural transmission routes of HTLV-1 are sexual intercourse and breast feeding (Nakano et al., 1984; Yamanouchi et al., 1985). These restricted modes of transmission give rise to not only geographical but also familial and ethnical clustering of HTLV-1 carriers.

Discrete endemic foci for HTLV-1 carriers are located in Africa, Asia, Melanesia and Central and South America (reviewed in Tajima and Hinuma, 1992). Among Papua New Guineans and Australian aborigines, HTLV-1 carriers have been identified since the end of the 1980s (Kazura et al., 1987; Bastian et al., 1993). HTLV-1 among them must be original and it is highly conceivable that these indigenous people carried HTLV-1 before their arrival to New Guinea Island and to Australia because there were no animal reservoirs of HTLV-1/STLV-1 in this region. Another supporting evidence for the possible ancient origin of HTLV-1 in this region is that a distinct type of virus strain was isolated from the Solomon Islands (Yanagihara et al., 1991). Studies of detailed HTLV-1 prevalence in New Guinea are important to clarify HTLV-1 origin and its history of dissemination. Here we report results of seroepidemiological studies of HTLV-1 in New Guinea Island by the Western blot (WB) assay using two different sets of criteria.

Table 1
Prevalence of antibodies to HTLV-1 in New Guinea Island

Origin	Samples[a]	Positive no.[b]		
		PA	WB-1	WB-2
Papua New Guinea				
Madang	267 (27.4)	59	50	39
Enga	51 (28.3)	9	4	2
Chimbu	176 (33.0)	5	4	1
New Britain	197 (29.4)	12	9	0
Manus	99 (31.6)	2	2	0
Eastern Highlands	60 (23.9)	5	1	0
Morobe	25 (23.3)	2	0	0
Others[c]	143	9	7	4
Indonesia				
Dani group	142 (31.4)	0	0	0
Biak group	26 (27.0)	0	0	0
Sorong group	12 (24.7)	0	0	0
Asmat group	15 (18.1)	0	0	0
Unknown	8 (23.0)	0	0	0
Total	1221	103	77	46

[a] Figures indicate number of tested individuals and their mean age (in parenthesis).
[b] WB-1: WB test positivity determined according to the criteria of Kiyokawa et al. (1991); WB-2: WB test positivity according to the criteria of WHO (1992).
[c] Including subjects unrecorded and from other regions.

2. Materials and methods

As a part of the population survey, venous blood samples were collected from 1221 individuals in Irian Jaya, Indonesia, and 16 provinces of Papua New Guinea after obtaining informed consent. They were 649 males, 454 females and 118 unknown aged between 0 and 69 years (Table 1 and Fig. 1). To represent the population, unrelated individuals were sampled. Sera were separated and stored at −30°C or lower before use.

Sera were initially screened for the presence of antibodies to HTLV-1 using a particle agglutination (PA) test (Serodia HTLV-1, Fujirebio, Tokyo). Sera giving positive patterns in the screening test at a dilution of 1:32, together with the selected PA negative samples, were then subjected to the WB test. We used a commercial WB kit (Problot, Fujirebio, Tokyo) to identify specific antibodies to HTLV-1 antigens. To confirm specific reactions, especially the demonstration of an *env* band, the WB test was performed in triplicate. When a specific band was recognized more than twice, it was considered to be positive. Interpretation of the WB test positivity was based on two different sets of criteria, i.e. (1) more than one *env* and one *gag* band (WHO, 1992); and (2) one *env* or plural *gag* including p53 bands (Kiyokawa et al., 1991).

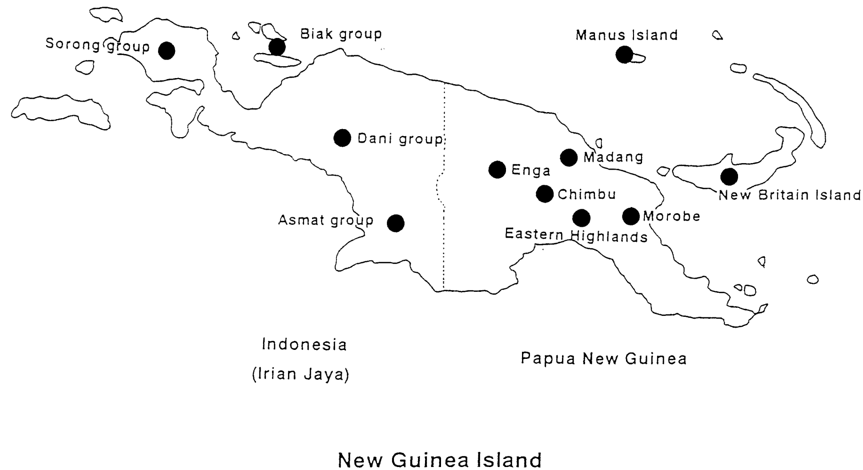

Fig. 1. Closed circles represent the geographical origin of the tested individuals in New Guinea Island.

3. Results

Out of 1221 New Guinean samples, a total of 103 (8.4%) sera showed positive reaction in the first screening PA test. They were then tested by the WB assay. Among them, 46 and 77 were regarded as seropositive for HTLV-1 according to WHO (WHO, 1992) and Kiyokawa's (Kiyokawa et al., 1991) criteria, respectively (Table 1). A total of 12 PA negative samples that showed negative or indeterminate results at a dilution of 1:32 were all negative in WB test by any criteria. Table 1 shows the geographical distribution of HTLV-1 antibody carriers. Kiyokawa's criteria-based WB positive subjects originated from the following provinces: Madang, Enga, Chimbu, East New Britain, West New Britain, Manus and Eastern Highlands, whereas WHO criteria-based WB positive subjects were restricted to Madang, Enga and Chimbu. None of the Irianese, West New Guineans, were seropositive. Seroprevalence of HTLV-1 ranged from 0 to 14.6% and 0 to 18.7% in eight major Papua New Guinean provinces studied when the results of the WB test were interpreted by the WHO criteria and Kiyokawa's, respectively. The WHO criteria was much stringent than that of Kiyokawa et al. in the interpretation of the WB test.

In the Madang Province, 39 out of 267 sera were interpreted as positive by the WHO WB criteria. The seropositive cases were found not only coastal regions but in the inlands of this Province. Higher seropositivity rates were observed among aged groups (Fig. 2).

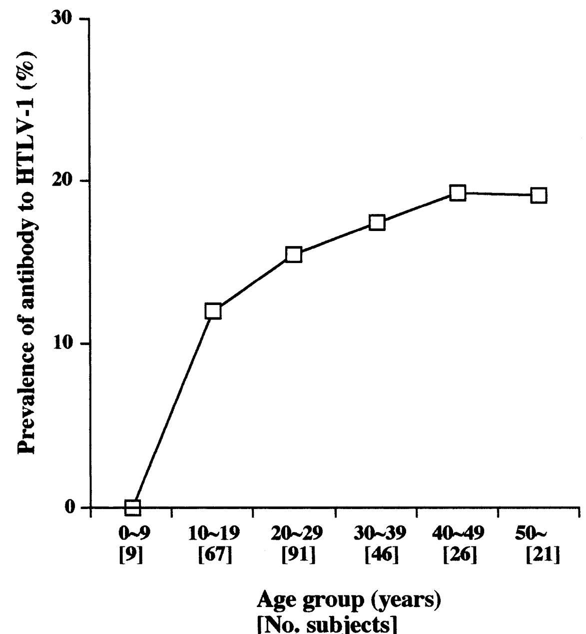

Fig. 2. Seroprevalence of HTLV-1 by age in the Magang Province.

4. Discussion

Since a distinct sub-type of HTLV-1 has been isolated in Melanesia, this region bears an important role in the HTLV-1 research (Yanagihara et al., 1991). To understand more about the origin and dissemination of HTLV-1, we studied its seroprevalence in New Guinea Island by the WB test. During these studies we have encountered a discrepancy in the interpretation between the WB results based on the two types of criteria used. Seropositivity according to Kiyokawa's criteria relies on the presence of at least two positive bands regardless of origin, whereas the WHO criteria requires the presence of positive bands for at least one *env* product and for any *gag* product. Results interpreted by the former criteria showed a good correlation with the PCR test in Japanese subjects (Fukuoka et al., 1992). Many sera from tropical and/or malaria endemic areas gave positive reaction in the screening tests, such as the PA and EILSA, however, they turned to be seronegative for *env* product in the WB test (Anthony et al., 1993; Ishida et al., in preparation). In New Guinea Island and the Malay Peninsula, detection of HTLV-1 proviral genome failed in subjects with WB results showing positive bands for *gag* products only (Nerurkar et al., 1992; Ishida et al., unpublished data). In fact, DNA samples isolated from peripheral blood lymphocytes of several seropositive individuals were subjected to the PCR test and the WB result interpreted by the WHO criteria showed a better correlation with the PCR test (data not shown). Thus the presence of a positive band for *env* seems essential for the establishment of seropositivity in these areas. It may therefore be appropriate to follow the WHO criteria to evaluate HTLV-1 seropositivity in these regions to avoid overestimation of its prevalence.

Substantial numbers of seropositive subjects were found only in the Madang Province, Papua New Guinea, where the overall seroprevalence rate was 14.6% (Table 1). This is similar to the rate estimated by Brabin et al. (1989). We found that the seroprevalence of HTLV-1 ranges from 0 to more than 10% in New Guinea Island. The heterogeneity of New Guinean populations which consist of so many isolated tribes of Papuans and Austronesians may partially account for the different prevalence of HTLV-1. It has been reported that an age dependent increase in HTLV-1 carriers is absent in Papua New Guinea (Imai et al., 1990). However, we found higher seropositivity rates among aged groups (Fig. 2) similarly as has been observed in other HTLV-1 endemic areas, such as Japan and Caribbean basin (Hinuma et al., 1982; Clark et al., 1985).

Using the stringent WB criteria, HTLV-1 seropositive subjects were found in one of the hinterland provinces, Enga Province, where the presence of HTLV-1 had previously not been reported. It has been pointed out that seropositive individuals are distributed more in the coastal regions than in the hinterlands in HTLV-1 endemic areas (Hinuma, 1985). This phenomenon was also reported in Papua New Guinea (Imai et al., 1990; Sanders et al., 1993). In these reports, however, the apparent absence of HTLV-1 seropositive subjects in several hinterland provinces in Papua New Guinea could possibly be explained by the fact that only limited numbers of subjects were tested.

The origin and the dissemination of HTLV-1 in New Guinea Island is still a mystery because of (1) the occurrence of a distinct sub-type of HTLV-1 in the region (Yanagihara et al., 1991); and (2) the presence of virus carriers isolated from other endemic foci. To shed light on this, it is worth investigating HTLV-1 distribution in Irian Jaya and eastern Indonesian Islands in detail.

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