Intrafamilial clustering and 4-year follow-up of asymptomatic human T-cell leukaemia virus type I (HTLV-I) infection in Benin (West Africa)

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**Background**
Few data exist concerning familial human T-cell leukaemia virus type I (HTLV-I) carrier states and transmission in African countries. Two previous surveys performed in Benin in 1989 and 1990 using a three-level cluster sampling method allowed us to identify HTLV-I positive subjects. The evolution of HTLV-I within the families of these subjects is described over a 4-year period, 1991–1995.

**Methods**
Since 1991, 37 HTLV-I seropositive subjects, six subjects with indeterminate Western-Blot pattern, and their relatives have been followed up once a year clinically and biologically.

**Results**
Twenty-three mothers in the study group gave birth to 27 children between 1991 and 1995. Among the 13 infants born to the 12 seropositive mothers, two seroconverted before their second birthday. One adult woman whose husband was seropositive developed seropositivity 4 years after marriage. In March 1992, a family case-control study (proband study) was conducted. A seroprevalence of 27.5% was found among 138 relatives of 32 infected subjects and 1.4% among 142 relatives of 32 control subjects.

**Conclusions**
There is clearly an intrafamilial clustering of HTLV-I in Benin. The annual incidence density of HTLV-I in this cohort is estimated at 6%.

**Keywords**
Africa, Benin, cohort study, HTLV-I, intrafamilial clustering

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Human T-cell leukaemia virus type I (HTLV-I) is commonly accepted as the cause of adult T-cell leukaemia (ATL) and of tropical spastic paraparesis/HTLV-I associated myelopathy (TSP/HAM). This retrovirus occurs in endemic proportions throughout Japan, the Caribbean, Sub-Saharan Africa and Melanesia.

Transmission routes include use of contaminated blood products, sexual contacts (principally from men to women) and vertically from mother to child (especially through breastfeeding). While the main risk of infection in most developed countries tends to come from the use of contaminated needles by drug users, in developing countries and Japan, transmission is predominantly intrafamilial. Familial clustering has been particularly well studied in Japan and to a certain extent in the Caribbean. However, little data exist concerning familial HTLV-I carrier states and transmission in African countries; the only studies available being limited to TSP/HAM familial clusters in Zaire. In 1989, we conducted a large seroepidemiological survey in Benin. We used a three-level cluster sampling method and found a global HTLV-I prevalence of 1.5% (39 positive subjects) among 2625 adults. No case of HTLV-II infection was detected. The study revealed that HTLV-I prevalence was geographically variable, being relatively high in several villages in the north of the country (up to 5.4% in ATACORA region). A second study conducted in 1990 in the ATACORA region allowed us to identify 31 HTLV-I seropositive individuals and six subjects with indeterminate Western blot (WB) patterns.

In the first part of this paper, we will present the follow-up study of the infected and non-infected subjects in order to study HTLV-I transmission. The second part will concern the study of intrafamilial clustering of HTLV-I in Benin.
Patients and Methods

Longitudinal survey

Among the 70 seropositive individuals identified during the two seroprevalence studies, 37 were included in the study in January 1991. The 33 remaining seropositive subjects were omitted for the following reasons: refusal (14 subjects); lost to follow-up between 1989 or 1990, and 1991 (8); subjects without family members available and therefore unsuitable for the assessment of intrafamilial transmission (8); and death (3—due to strangulated inguinal hernia, poisoning, and cause unknown). Of the 37 subjects included, there were 22 men and 15 women; the mean age was 42.6 ± 18.3 years.

Six subjects (two men and four women; mean age : 33.2 ± 17.3) with indeterminate WB patterns were also included in order to follow the variations in these WB patterns during the survey. Relatives (parents, children, siblings, husbands and wives living in the same household) of these 43 subjects were followed up once a year clinically and biologically until 1995. They were enrolled prospectively in the longitudinal survey. In total, 232 relatives were included, representing all the relatives living in the same household as the index subjects. At their inclusion, 40 of these relatives (12 men and 28 women; mean age : 30.6 ± 18.3) were already HTLV-I positive. The 192 negative relatives (103 men and 89 women; mean age : 19.4 ± 15.5) at their inclusion contributed 468 person-years (Figure 1).

The subjects originated from 26 villages, 23 of which were localized in the North Benin provinces where high HTLV-I prevalence has been recorded previously. In this cohort, several ethnic groups were represented, the largest being Otammaris (43.7%). Mean parity was three children per woman (±1.5); mean family size, was six (±2.5) and one father in four was polygamous.

Data collection was done using a standardized questionnaire, which included general demographic information and risk factors for HTLV-I infection (sexual transmitted diseases (STD), transfusion history, intravenous drug use ...). The questionnaires were administered by trained personnel who were blinded to the serological status of the subjects.
Table 1 Demographic characteristics and HTLV-I seroprevalence in the study groups of the family case-control study, Benin, 1992

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Prevalence (%)</td>
</tr>
<tr>
<td>0-9</td>
<td>60</td>
<td>20.0</td>
</tr>
<tr>
<td>10-19</td>
<td>30</td>
<td>13.0</td>
</tr>
<tr>
<td>20-29</td>
<td>16</td>
<td>37.5</td>
</tr>
<tr>
<td>30-39</td>
<td>14</td>
<td>50.0</td>
</tr>
<tr>
<td>40-49</td>
<td>6</td>
<td>66.7</td>
</tr>
<tr>
<td>50-59</td>
<td>9</td>
<td>44.4</td>
</tr>
<tr>
<td>&gt;60</td>
<td>3</td>
<td>33.3</td>
</tr>
<tr>
<td>Total</td>
<td>138</td>
<td>27.5</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>17.9 ± 16.4</td>
<td>17.6 ± 15.8</td>
</tr>
<tr>
<td>Age range</td>
<td>0-62</td>
<td>1-65</td>
</tr>
</tbody>
</table>

Note: Group I = relatives of HTLV-I seropositive index cases. Group II = relatives of HTLV-I seronegative subjects (matched index controls).

Family case-control study

A family case-control study was conducted in 1992. It was similar to the classic case-control design, but differed in that the statistical analysis was performed only in relatives of each group (proband study).

The index cases were the 32 individuals examined in 1992 and identified as HTLV-I positive. Five subjects among the 37 seropositive for HTLV-I, enrolled in the longitudinal survey, were not found in 1992 and therefore did not participate to this family case-control study.

The index controls were 32 HTLV-I negative subjects matched by age (±5 years), sex, ethnic origin and neighbourhood to the index cases. Neighbourhood was defined as the houses surrounding the house of the index case. If several subjects corresponded to the control definition, one control subject was randomly selected.

Relatives were defined as the family members (parents, children, siblings, husbands/wives) of either the index cases or the index controls, who were living in the same household. The 32 index cases allowed us to identify 150 relatives (group I). The 32 matched controls allowed the identification of 157 relatives (group II). Due to refusals, 12 relatives from group I and 15 from group II did not participate in the serological testing. The study was then completed in 138 relatives from group I and 142 from group II. The demographic characteristics of the two groups are shown in Table 1.

Statistical methods

Proportions were compared by using the χ² test with or without Yates correction, and means were compared by using Student t test. For all tests, a P-value of <0.05 was considered to have statistical significance. Incidence data were estimated using incidence density (new cases reported to person-years followed up). Vertical transmission (mother-to-child) was investigated by measuring parental HTLV-I infection proportion and comparing it with HTLV-I seropositivity in subsequent offspring.

Biological tests

The serological screening was conducted with an HTLV-I whole virus lysate EIA (HTLV-I EIA, Abbott laboratories, North Chicago). Positive or doubtful samples were then tested with a commercial WB assay (HTLV 2-3 blot, Diagnostic Biotechnology, Singapore) for confirmation of seropositivity as well as HTLV-I/II differentiation. Food and Drug Administration and Centers for Diseases Control recommendations were followed in determining sample positivity, which was judged by reactivity against major gag (p19 and p24, p53) and env proteins, i.e. native (gp46) and/or recombinant (rgp21, rgp46) proteins. Discrimination between HTLV-I and HTLV-II infection was done by 'rgp 46' reactivity i.e. rgp 46 for HTLV-I and rgp 52 for HTLV-II infection).

Samples exhibiting other patterns (i.e. p19 without p24 or p19/p24 without env proteins) were considered as indeterminate. Any sample leading to indeterminate WB patterns was tested with a home radioimmuno precipitation assay (RIPA), using HUT 102 B2 cells.

Antibody titres were quantitatively determined for HTLV-I seropositive spouses by means of a passive agglutination test (Serodia ATLA, Fujirebio, Tokyo), using sensitized gelatin particles as per manufacturer's recommendations. The sera were tested in serial dilutions from 1/8 to 1/4096 and the inverse of the last positive dilution determined its titre.

In 1995, we obtained peripheral mononuclear blood cells from three individuals with indeterminate WB patterns for a preliminary molecular study. A standard HTLV polymerase chain reaction (PCR) was done with SK 110-111 primers followed by a southern blot and hybridization with SK 112 (HTLV-I) and SK 188 (HTLV-II) DIG labelled probes.

Human immunodeficiency virus (HIV) seroprevalence was also tested by EIA (Abbott recombinant HIV-I/HIV-2 EIA, Wiesbaden, Germany) and confirmed if necessary by double HIV-1 and HIV-2 WB (new lav blot I and II, Pasteur Diagnostic, France).

Results

Longitudinal survey

Among the 232 relatives, 136 were children. Among these children, 86 were born to seropositive mothers (13 of them were born during this 4-year study) and 50 were born to seronegative mothers (14 of them were born during this study) (Figure 2). In our cohort, all children were breastfed. Two (15.4%) children (one girl and one boy), among the 13 born in 1991 to seropositive mothers seroconverted (one in 1992, i.e. in her first year of life, and the other in 1993, i.e. in his second year after birth), whereas none of the 14 born to seronegative mothers seroconverted (Figure 2).

The serum titre of HTLV-I antibodies was quantitatively determined from the seropositive mothers of the 13 children born during the study. Nine mothers (including those of the two seroconversion cases) out of the 13, exhibited a titre in excess of the last dilution (>4096).

A cross-sectional analysis of the 86 children born to seropositive mothers showed that 20 children (23.2%) were HTLV-I positive (Figure 2). Serological status of the mother appeared to be the key factor in determining transmission of the infection: when the mother alone was seropositive, 30% of children were seropositive (17.4% when both father and mother were seropositive).
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... (..., HTLV-I positive children; ..., HTLV-I negative children)

Figure 2 Description of the children surveyed during the cohort study, Benin, 1991–1995

Table 2 Influence of parent’s serological status on HTLV-I transmission to children, Benin, 1991–1995

<table>
<thead>
<tr>
<th>Father HTLV-I +ive/Mother HTLV-I +ive</th>
<th>Father HTLV-I +ive/Mother HTLV-I –ive</th>
<th>Father HTLV-I –ive/Mother HTLV-I +ive</th>
</tr>
</thead>
<tbody>
<tr>
<td>No parent pairs</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No children</td>
<td>27</td>
<td>28</td>
</tr>
<tr>
<td>No HTLV-I + children (%)</td>
<td>4 (14.8)</td>
<td>0</td>
</tr>
<tr>
<td>Girls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No children</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>No HTLV-I + children (%)</td>
<td>4 (21.0)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>48</td>
</tr>
<tr>
<td>No HTLV-I + children (%)</td>
<td>8 (17.4)*</td>
<td>0*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 (30.0)*</td>
</tr>
</tbody>
</table>

* P < 0.001 by \( \chi^2 \) test.


positive), whereas none were positive when the father alone was HTLV-I positive (P < 0.001) (Table 2). Neither the sex of the children (18.7% HTLV-I +ive for boys and 28.9% HTLV-I +ive for girls, P > 0.05), nor rank in sibling order seemed to influence the mother-to-child transmission of HTLV-I (seropositivity proportion was 18.2% in last born children and 12.1% in other children, P > 0.05). In addition, the mean age of mothers responsible for transmitting the virus was similar to that of non-transmitting seropositive mothers (23 and 25 years old respectively).

The case of a seronegative 20 year old woman who married a seropositive man in 1990 confirmed the existence of a sexual transmission pathway in this particular population. This woman had no risk factor for HTLV-I infection other than marriage to her seropositive husband. By 1994, her serum antibody titre had risen to a high level (>4096) and she seroconverted. Among 28 husband and wife pairs in which only one member was seropositive (discordant couples), this was the only example where one of the spouses seroconverted (out of the 15 pairs in which the husband was seropositive [6.7%]). It should be added that in this instance, HTLV-I was sexually transmitted from male to female.

Considering the three cases of seroconversion, the annual incidence density of HTLV-I in this cohort is estimated at 6‰ (95% confidence interval: 0–14).

Among the seropositive subjects, WB patterns were unequivocal revealing the presence of gag (p19, p24 and p53) and env (rgp21, rgp46 and sometimes native gp46) proteins. No reactivity against HTLV-II or HIV was observed. Among the six sera which exhibited indeterminate HTLV WB patterns (gag protein: p19, p26/28, p53), four were tested using RIPA and were found negative; three of these sera were also found negative by PCR. The indeterminate WB patterns remained unchanged throughout the study and relatives of these subjects displayed no sign of seropositivity.
Table 3. Seroprevalence of HTLV-I in Group I (relatives of seropositive cases) according to parentage link with index cases, family case-control study. Benin, 1992

<table>
<thead>
<tr>
<th>Family Link</th>
<th>Wives</th>
<th>Husbands</th>
<th>Children</th>
<th>Siblings</th>
<th>Parents</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTLV-I +</td>
<td>13</td>
<td>4</td>
<td>17</td>
<td>1</td>
<td>3</td>
<td>38</td>
</tr>
<tr>
<td>(%)</td>
<td>(56.5)</td>
<td>(36.4)</td>
<td>(17.5)</td>
<td>(33.3)</td>
<td>(75.0)</td>
<td>(27.5)</td>
</tr>
<tr>
<td>HTLV-I -</td>
<td>10</td>
<td>7</td>
<td>80</td>
<td>2</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>11</td>
<td>97</td>
<td>3</td>
<td>4</td>
<td>138</td>
</tr>
</tbody>
</table>

Family case-control study

Among group I, 21.7% (30/138) were found to have a history of STD and 2.4% (4/138) had received blood transfusions. Among group II, 19% (27/142) were found to have had STD and 2.1% (3/142) had received blood transfusions. Intravenous drug use and homosexual activity were absent in both groups.

Thirty-eight subjects were confirmed to be seropositive for HTLV-I among the 138 relatives from group I (27.5%). Only two seropositive subjects were found among the 142 relatives of group II (1.4%). The prevalence ratio was 19.5. The difference was statistically significant (P < 0.001).

Among group I, the seroprevalence among males was 19.4% (13/67), against 35.2% (25/71) in females (P < 0.05). Among group II, the two seropositive subjects were one male (1/74) and one female (1/74). There was a noticeable increase in seroprevalence with advancing age up to the 50 year old group (Table 1). Prevalence ratios did not depend on family size. In group I, the seroprevalence was 50.0% among the 34 spouses (11 husbands and 23 wives) and 17.5% among children (Table 3). Among the 17 infected spouses, 13 were female and four were male. Among the 11 husbands, there were four seropositive subjects; yielding a seroprevalence of 36.4%. This seroprevalence was 56.5% (13/23) among wives.

In 13 out of the 32 families (40.6%) investigated in group I, there was no seropositive relative. The mean age of the subjects of these 13 families was 17.4 ± 16.4 and the mean family size was 4.2 ± 1.8. At the same time there was a clustering of infected subjects in 19 families (59.4%) in group I. The mean age of the subjects of these 19 families was 18.8 ± 16.7 and the mean family size was 4.4 ± 1.9.

Discussion

Longitudinal survey

In 4 years three seroconversions were recorded, two cases of seroconversion occurring in infants under 24 months old; a similar rate to that reported in two Gabonese studies.15,16 In our study, the annual incidence density of HTLV-I based on all the family members was 6%. A recent Gabonese study17 reported a rate of 48% among children only. This incidence is much higher than that reported in our study but it has been estimated in children only, making inappropriate any comparison with our study.

Concordant with the published results of a survey in Japan,18,19 our study found no significant effect of the serological status of the father on vertical transmission of the virus, thus demonstrating the predominant importance of mother-to-child transmission. In our cohort, all infants were breastfed until the age of 18 months and this practice probably constituted a major mode of transmission. The proportion of HTLV-I seropositive children born to seropositive mothers was 23.2%. This result concurs with data reported from Gabon18 and also from a study of long-term breastfed children in Jamaica.19 Breastfeeding in African countries is usually prolonged until infants reach the age of at least 12 months. According to a study of short-term breastfeeding children in Japan (fed for <3 months) HTLV-I transmission is less than 6%. The same study reported that children breastfed for >7 months were at least three times more liable to seroconversion than those who had short-term breastfeeding.19,20

Vertical contamination appeared unaffected by the sex of children or their rank in sibling order in this study. Similar results were reported in Gabon where no gender relationship was observed.15 However, a study of Japanese populations described increased risk for last-born female infants.21 The authors hypothesized that since mothers having their last born were on average older than mothers having their first children, they were more likely to have acquired seropositive status. This phenomenon was absent from our study population possibly because all mothers were relatively young and the number was small.

Out of the 13 seropositive mothers who had children during the study period, nine exhibited titres of >4096, yet only two children seroconverted. Unlike observations made in Japan22 and Jamaica,19 our study could not demonstrate a correlation between maternal HTLV-I antibody titres and transmission to children, probably because of the small size of the population studied. The Japanese study22 reported no HTLV carrier case in children born to mothers with titres <4000.

As far as sexual transmission of HTLV-I is concerned, just one seroconversion occurred in the 4-year follow-up period among 25 husband and wife pairs in which only one member was positive. The fact that the virus was transmitted from a man to a woman is probably not the result of mere chance: studies in Japan have shown that sexual transmission is more often observed from men to women than the reverse.23 We found a significant difference in HTLV-I seropositivity between males and females whereas mother-to-child transmission favoured neither boys nor girls.

Finally it should be noted that no HTLV-II could be detected in the study population. Indeterminate patterns corresponded with gag isolate reactivities and remained constant during the survey. In most African studies reporting such indeterminate WB patterns, subsequent molecular analysis revealed no evidence of HTLV-I infection,24 which corroborates our follow-up.

Any possible evolution in the profiles of this group of subjects will have to be detected during future follow-up. Preliminary PCR results have to be followed by others, in particular, sequence variation analysis among the members of a same family.

Family case-control study

The smaller number of relatives (n = 150) of the index cases included in this family case-control study, in contrast to the 232 relatives included in the cohort, is explained by the non-inclusion of the relatives of the six subjects with indeterminate WB pattern and the relatives of the live subjects not followed in the year of this study.
The seropositivity among families having at least one positive member for HTLV-I infection was 27.5% compared to 1.4% in the control families. The prevalence in family members of the control subjects was almost the same as the prevalence found in the general population of Benin. In Japan, have found a prevalence of 38.5% in families of infected subjects against 7.7% in control families. However, this report comes from one of the highest prevalence areas in the world—15.3%. The general prevalence for Benin is 10 times lower.

Despite this low general prevalence of HTLV-I positivity in Benin, the proportion of seropositive relatives of the index cases is rather important. A recent Gabonese study has compared 100 children aged 1–14 years born to HTLV-I seropositive mothers to 175 children of same group age born to seronegative mothers. The seroprevalence reported was 15% among the first group and 0.6% among the second group.

Besides these two family surveys, there was to our knowledge no other study comparing HTLV-I seropositivity in family members of asymptomatic infected subjects versus control families of negative subjects. However, intrafamilial clustering has been described in family members of symptomatic individuals suffering from ATL or TSP/HAM. In Japan, Sarin et al. studied the family members of one subject affected by ATL with a reported prevalence of 60%. Shoji et al., also in Japan, found a prevalence of 70% in relatives of subjects suffering from TSP/HAM. Mc Khann et al. reported a prevalence of 50% in family members from symptomatic infected subjects living in Tumaco, Colombia. Other Japanese authors report figures of 44.6% and 50.0%.

In our study, we have observed a significantly higher proportion of seropositivity in females (35.2%), than in males (19.5%). This was similar to the observation of Kajiyama, and other Japanese authors. This female predominance in family clusters confirms what has been reported from general population surveys and may be due to greater sexual transmission from males to females, as males more frequently have multiple sexual partners in these areas of the world. Biological factors may also facilitate transmission from infected males to partners compared to transmission from infected females.

Increased prevalence of infection with age in family clusters for HTLV-I corresponds to the same phenomenon in the general population and it can be explained by the accumulation of contacts during a lifetime, or by late seroconversion in subjects infected from childhood.

In the present study, HTLV-I seroprevalence and clustering do not vary in relation to family size. However, other authors have reported decreasing prevalence with increasing family size.

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