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Determination of human T-Cell leukemia Virus-I among pregnant women

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ABSTRACT

Human T-cell leukemia Virus (HTLV) is an important cause of mortality and morbidity rate in adults. In India the incidence of HTLV infection are 100 in 100,000. The transmission of HTLV is through unprotected sex, HTLV donor and from infected mother to baby by means of breast feeding. Transcription of HTLV provirus leads to T-cell multiplication. A study was done to determine the presence of HTLV-I infections in pregnant women during third trimester. 57 maternal samples of three trimesters were collected. The serum was separated by centrifugation and aliquot. The presence of HTLV infection was assayed by Enzyme Linked Immunosorbent Assay (ELISA). Seropositivity for HTLV -1 infection among the third trimester of pregnant women was found to be 2.44 %.

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Introduction

HTLV (human T-cell Leukemia) is a lymph proliferative disease seen in adults. It is an etiological agent in ATL (Adult T-Cell Leukemia). HTLV is a major cause of disease in pregnant women and transmitted vertically from the mother to child, usually via breast milk. HTLV-I (Human T-cell leukemia virus type 1), also called the human T-cell lymphotrophic virus type 1, has been seriously implicated in several kinds of diseases including HTLV-I associated myelopathy.

The outbreaks of HTLV are mainly through unprotected sex between injecting drug users and their non-injecting sexual partners. No vaccine is available to prevent HTLV-1 and HTLV-2 transmission. These illnesses impose enormous social and financial costs on infected individuals, their families, and health care systems. For this reason, public health interventions aimed at counseling and educating high-risk individuals and populations are of vital importance.

A virus which is transmitted from mother to child and named as HTLV. It was the first identified as human retrovirus [9]. HTLV was divided in to four types HTLV- I, HTLV- II, HTLV- III, HTLV- IV. The five major molecular and geographic subtypes of HTLV-I are HTLV-Ia or Cosmopolitan, distributed throughout the world, HTLV-I b or Central African, HTLV-I c or Melanesian, HTLV-I d from Central African Republic pygmies and HTLV-I e, identified in a sample from an Efe pygmy from the Democratic Republic of Congo and f, from one individual from Gabon HTLV-1 subtype g was recently described, isolated from populations in southern Cameroon. There are three main well-established subtypes of HTLV-2: a, b, and d. A molecular variant of subtype a was characterized and named as subtype c, based on the properties of its Tax protein

The results of seroepidemiologic studies of human T-lymphotropic virus type I (HTLV-I) and type II (HTLV-II) infections in different population groups of Argentina have been compiled [1]. The studies have shown a high prevalence of HTLV-I/II infection in blood donors in the provinces of north Argentina (1.0% in Jujuy, 0.7% in Salta, and 0.6% in Formosa) and a low prevalence in the provinces in the central region of the country (<=0.1%). High rates of HTLV-I (0.45%-2.78%) and HTLV-II (2.78%-21.9%) infections have been documented in Native Indian groups and have highlighted the importance of sexual and mother-to-child transmission of the viruses.

In India, HTLV-I infection has been reported in individuals with ATLL, TSP, and sexually transmitted infections (STI). This infection has also been proved by molecular techniques. Most of the cases of ATLL published from India are from the state of Kerala, south India. Despite this fact, there are no systematic studies among the other risk groups in Kerala.

HTLV- I is an endemic in southwestern Japan, the Caribbean basin, southeastern United States, southern Italy, and sub-Saharan Africa. Up to 15 percent of normal blood donors in endemic areas of Japan and the Caribbean basin are positive for antibodies to HTLV- I, in nonendemic areas, less than 1 % are positive.

HTLV-II immunodominant proteins.A virus closely related to HTLV-I, HTLV-II shares approximately 70% genomic homology (structural similarity) with HTLV-I. It is found predominantly in intravenous drug users and Native Americans, as well as Caribbean and South American Indian groups. HTLV-II has not been clearly linked to any disease, but has been associated with several cases of myelopathy/tropical spastic paraparesis (HAM/TSP) like neurological disease. HTLV-II can cause another rare cancer called hairy-cell leukemia. HTLV-II infection has also been endemic for the past 10-20 years among intravenous drug users.

HTLV- II is endemic in certain Native American tribes and in Africa. It is generally considered to be a New World Virus that was brought from Asia to the Americas 10,000 to 40,000 years ago during the migration of infected populations across the Bering land Bridge. HTLV- II may be less readily transmitted sexually than HTLV –I. The seroprevalance of HTLV- II was higher in Southwest and the Midwest than in Northeast. In contrast, the seroprevalence of HTLV- II was higher in Northeast than Southwest or Midwest.

HTLV-I and -II are viruses that are not related to HIV (10). The viruses can cause blood or nervous system diseases in a small number of infected people HTLV-II is endemic in the

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Americas. Both of these viruses, although rare, were found in the US blood donor population in the 1980s. Few people got HTLV as a result of transfusion, but because of the small transfusion risk that existed in the 1980s, tests to detect HTLV-I antibodies were developed and quickly implemented. These tests also detected many, but not all, HTLV-II infections.

HTLV can transmit from an infected mother to her baby. Up to 1 in 4 children born to mothers with HTLV-I infection become infected, with most infections occurring through prolonged breast feeding, between sexual partners through unprotected intercourse (no condom used) and through transfused blood from an HTLV infected donor. The risk of transmission from an infected man is greater than from an infected woman. Seropositive pregnant women were infected with HTLV- I. By testing the HTLV- I antigen and HTLV- I proviral genome in cultured placental villous cells. The incidence of HTLV- I infection rates between placentas and cord blood samples suggests that there is a placental barrier system against mother to fetus HTLV- I transmission [7].

The peak prevalence of HTLV-I is among 39 to 49 years of age but the mean age was 56 years and the age range was 9–89 year and lower prevalence in younger and older age groups and for HTLV- II is 30-49 years of age according to the study done in America. HTLV-II–infected participants had a higher incidence of acute bronchitis, bladder or kidney infection, arthritis and asthma, and a higher incidence of pneumonia than did HTLV-seronegative participants followed concurrently [11]. The finding of a higher rate of these infectious diseases among HTLV-II participants was supported both by survival analysis, which considered only the first diagnosis, and by negative binomial modeling, which considered both first and recurrent infections.

In pregnant women HTLV-I became severe in third trimester. It has related to the development of inflammatory diseases in various organs such as the eyes, lungs and joints. In particular, a higher seroprevalence of HTLV-I in patients with inflammatory ocular disease, such as endogenous uveitis, episcleritis, retinitis pigmentosa, dermatitis of children and degenerative choroiditis. Most infectious agents to whom pregnant women are exposed are without long-term consequence for mother or child. However, an important subgroup of viruses can be transmitted vertically, causing intrauterine infection of the developing fetus or in the fully developed child around the time of birth. Clinical manifestations are varied; they can be severe, even fatal.

The most frequent pathway of vertical transmission of HTLV-I is breast-feeding, however bottle fed children may also become infected in a frequency varying from 4 to 14% [12]. In these children the most probable routes of infection are transplacental or contamination in the birth canal. Forty-one bottle-fed children of HTLV-I seropositive mothers were in ages varying from three to 39 months (average age of 11 months). No case of infection was detected. The absence of HTLV-I infection in these cases indicates that transmission by transplacental route may be very infrequent.

More clarification is needed in the possible role of HTLV in rheumatologic, psychiatric, and infectious diseases. Since cures for ATL and HAM/TSP are lacking and no vaccine is available to prevent HTLV-1 and HTLV-2 transmission, these illnesses impose enormous social and financial costs on infected individuals, their families, and health care systems.

The investigation was done for the association between human T-lymphotropic virus type-1 (HTLV-1) infection and cancer risk in a longitudinal study [4]. The baseline survey, including analysis of antibody to HTLV-1, took place during 1985–1987 and follow-up was performed until the end of 2001. These findings support the idea that HTLV-1 infection is not associated with an increased general cancer risk. Confounding by hepatitis C virus (HCV) and the interaction between HTLV-1 and HCV may explain the increased risk of liver cancer among HTLV-1 carriers.

The complications that have been associated from various sources of HTLV T-cell leukemia / lymphoma during pregnancy are Myelopathy, HTLV-I Associted Myelopathy, B-cell chronic lymphocytic leukemia, Syphilis.

The basic features of HTLV-I infection causes a chronic infection of $CD4^+$ T cells, and is associated with various disease outcomes, with the development of adult T-cell leukemia (ATL) [8]. The T-cell dynamics after HTLV-I infection can be described in a mathematical model coupled with differential equations. The infection process is modeled assuming cell-to-cell infection of $CD4^+$ T cells. The model allows for $CD4^+$ T cell subsets of susceptible, latently infected and actively infected cells as well as for leukemia cells.

HTLV-I associated myelopathy causes prolonged morbidity and was not recognised as a clinical entity until 1985. The rate of morbidity rate and mortality rate increases from 1986-1992 and in 2006 the mortality rate became 86% studied by CHEST 2006-case reports. According to the study done by The Tsushima ATL Study Group between 1985 to 1996, 530 pregnant women were found to be positive for anti-HTLV-I antibodies. HTLV- II has not been associated with a particular disease and infact has been thought of as "a virus sharing for a disease". HTLV- II may play a role in certain neurologic, hematologic and dermatologic diseases. The mortality rate of HTLV decreases from 25% to 5% when the child is given bottle feed. The mortality rate of HTLV-I is 5.8% HTLV- II is 5.3%

At present there is no treatment to eradicate HTLV infection from infected individual. Infection with HTLV is lifelong. As 95% of people infected with HTLV never develop any HTLV related symptoms, any treatment to eradicate infection would have to be very safe for its use to be justified. ATLL is usually treated with anti-cancer drugs, but recently transferring to anti-viral treatment after starting with anti-cancer treatment has been shown to improve the outcome.

Blood donations are screened routinely for HTLV but distinguishable results between HTLV-I and HTLV-II cannot be detected by lab tests. The prevention of HTLV-I infection includes avoidance of breast-feeding, if possible; screening of blood donors for HTLV antibody; safe sexual practice; and the avoidance of sharing or reusing of needles.

Evaluation of the human T-cell lymphotropic virus type I (HTLV-1) infection among 6754 pregnant women in Salvador, Bahia, Brazil using enzyme-linked immunosorbent assay, Western blot analysis, and polymerase chain reaction assay concluding the rate of infection to 0.84% (57 of 6754 women) [2]. The adherence of recommended algorithm for HTLV antibody testing will dramatically improve HTLV WB testing efficiency [3]. They encourage the development of a systematic plan to educate health care professionals regarding the recommended guidelines for HTLV antibody testing.

HTLV-I has a very low level of disease penetrance. The transformation of an infected cell is a rare event, and the cumulative lifetime risk of developing ATL is 1% - 5% in persons infected with HTLV-I. The latent period from infection

to clinical disease is estimated to be 30 to 50 years most persons with ATL appear to have acquired the infection in childhood.

Materials and methods

Sample collection and sample processing

Maternal blood samples were collected from the donor mothers during trimester as per the standard method of collection by Becton Dickinson Vacutainer Systems Europe, France using the strict aseptic conditions the site was cleaned with alcohol swap. The venipuncture was done and the peripheral blood was collected in the standard BD blood collection tubes. The tubes were transported immediately from the hospital in a thermocol box with frozen gel pack without delay within 24 hours at 18-24°C.The tubes were allowed to stand for an hour to clot. Then the tubes were centrifuged at 1500rpm for 10 minutes at room temperature. The serum was separated and aliquot as 1mL to 2mL Cryogenic vials. Each tube was then labeled with appropriate sample identification number (RS1 to RS57) respectively. The samples were stored at -20°C and documented.

Detection Htlv – I Test

80µL of sample diluent was added into each microtiter plate well. 20µL of negative control was added into A_1 well. 20µL of cut-off control was added into B_1, C1 and D_1 wells. 20µL of positive control was added into wells E_1. 20µL of samples was added into individual wells from F_1 using a separate pipette for each sample respectively. The plate was sealed with plate sealer. Microtitre plate was incubated at 40°C for 30 minutes. Plate sealer was removed and washed. 100 µL of conjugate solution was added into each well of microtitre plate. The plate was covered with plate sealer incubated at 40°C for 30 minutes. Plate sealer was removed and washed. The substrate solution was prepared by dissolving two chromogen tablets in 20 mL of substrate buffer. 100 µL of freshly prepared substrate solution was added to each microplate well in dark. Microplate was incubated in dark for 30 minutes at room temperature. 50 µL of stop solution was added to each well to stop the reaction. The bottom of the plates was wiped and the microtitre plate was read at 490/630nm with a ELISA reader within 30 minutes after the addition of the stop solution. Absorbance results were recorded on the data sheet with the kit lot number and expiry date and the assay as repeated for three times, the results were interpreted.

Calculations

The presence or absence of anti-HTLV-I antibodies is determined for each sample by comparing the measured absorbance with the calculated cut-off value.

CUT-OFF SERUM (OD)

$$\overline{OD\ (CS)} = \frac{OD\ (B_1) + OD\ (C_1) + OD\ (D_1)}{3}$$

CUT-OFF VALUE (C.O.V)

The cutoff value is determined by the ratio

$$\text{Cutoff value} = \frac{\overline{OD\ (C\ S)}}{5} = C.O.V$$

Assay Validation

- The absorbance of the negative control serum should be less than 70% of the cut-off value OD (NC) $< 0.7 \times C.O.V$
- The mean absorbance of cut-off serum should be greater than 0.8 $\overline{OD\ (C\ S)} > 0.8$
- The ratio OD (PC) /OD (CS) should be greater than or equal to 1.3

$$\frac{OD\ (PC)}{OD\ (CS)} \geq 1.3$$

Results

Generation of epidemiological data on perinatally transmitted infections is the fundamental tool for the formulation of health policies. Perinatally transmitted pathogen such as HTLV-I constitutes serious public health problems because they frequently cause clinical disease in the infected mothers and their infants. Once acquired during the pregnancy, some of these pathogens can induce devastating effects on the fetus, causing an array of pathological alterations including abortions, still births, early infant deaths and congenital abnormalities.

There are about 20 millions infected people worldwide. The incidence of late disease is 3% to 4% in British Columbia, in India it is about 73%. Hence it is of paramount importance the establishment of a sentinel surveillance program for pregnant women in order to monitor the trends of these infections.

Assay Of Htlv – I Test

HTLV – 1 test was performed using an ELISA kit. The cut off serum value was found to be 1.785 and the cut off value was interpreted as 0.358. The highest optical density was observed in the 52nd sample with the OD of 0.516, being positive for HTLV -1 infection in Fig 1.

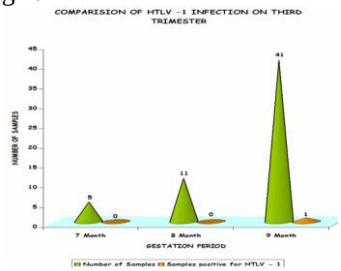

Figure1: Comparison of HTLV-1 Infection on third trimester

Serrano, V.; Gutieerrez, M.; Gonzalez-Lahoz. (1998). Detection of HTLV-II proviral sequences in HIV immunosuppresed patients with HTLV indeterminate serological patterns. *F Infect*.36, 243-244.

A total of 57 samples have been collected. One sample out of the 41 samples collected from the ninth month of the gestation period has been found to be positive for HTLV -1 infection accounting for 2.44% positivity. Samples collected at the 7th and 8th month have been shown to be negative for HTLV – 1 infection. Table 1 shows the total number of samples collected during the various gestation period like 7th, 8th and 9th month of the pregnancy.

Discussion

HTLV-I is a retrovirus endemic in Japan, West Africa, the Caribbean, South America and Melanesia. It has also been reported in other areas such as North America and Europe, where about 1% of the population are carriers, in Japan between 6% and 37% of the population are infected.

The two most common ways of getting the virus are through blood contact (e.g., blob components, intravenous drug use) and mother-to-child transmission through breast milk. After a long asymptomatic phase, 2% - 5% of people infected with HTLV-I go on to develop adult T-cell leukemia, a smaller proportion of infections (0.1%-2%) result in HTLV-I associated myelopathy, a progressive neurological disease.

A prospective serosurvey of 57 pregnant women delivered during January 2007 to March 2007 was done for HTLV – 1 infection using the ELISA assay. Among the 57 samples collected during the third trimester of pregnancy, one out of 57of the population in the 9th month of the gestation was found to be seropositive for HTLV -1. This was comparable with the study population done on the Spanish pregnant women where 0.064 % was seropositive for HTLV – 1 infections (5).

HTLV -1 was present in 1/57 (2.44%) from a women of the southern part of TamilNadu with poor socio economic status and had the Intra uterine death delivery. Selective antennal screening should be considered in former IDU'S, there by posing a risk in the delivery. The results were correlated with the previous studies (6).

The low prevalence of HTLV - 1 infections and the lack of the HTLV - 1 antenatal screening would pose a greater risk of transmission to the infants by breast feeding

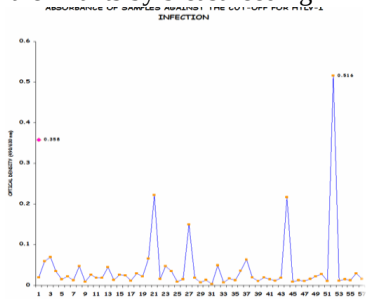

Figure 2: Absorbance of samples against the cut- off for HTLV-1 infection.

Conclusion

India has no organized screening program and many Indian women particularly the 75% who lived in rural areas lack both awareness of the disease and access to prevention and treatment facilities. Prevention of sexual transmission of HTLV-I by educational programs emphasizing the importance of using condoms to prevent all sexually transmitted disease, including HIV infection, which is spreading rapidly in some of these populations.

In areas with higher prevalence for these infections mandatory screening should be considered for pregnant women. Implementation of these recommendations will certainly prevent the transmission of several perinatally transmitted pathogens like HTLV -1 in lower economic conditions. Refraining from breast feeding of limiting the duration of breast-feeding can reduce the risk of mother-to-child transmission.

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Table 1: Results of testing sera from women attending antenatal clinics for Ab to HTLV-I

S.No.	Gestation Details	No. of Samples Collected	No. of Positive Samples for HTLV-I	% Positivity For HTLV-I
1.	7th month	5	0	0
2.	8th month	11	0	0
3.	9th month	41	1	2.44%