Serum Human T-Lymphotropic Virus 1 Proviral Load in Patients on Hemodialysis

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Original Paper

Introduction. Patients on hemodialysis are a high-risk group for human T-lymphotropic virus 1 (HTLV1) infection and other viruses transmitted by blood or blood products. The Razavi and South Khorasan provinces in Iran are the endemic areas for this virus. This study compares proviral load of HTLV1 in patients on hemodialysis with otherwise healthy carriers of HTLV1.
Materials and Methods. In this case-control study the proviral load of the HTLV1 virus was compared between 25 patients on long-term hemodialysis who were positive for HTLV1 and 25 healthy carriers of HTLV1, to determine the effect of uremia and chronic hemodialysis on the proviral load. Virus proviral load was determined using a real-time polymerase chain reaction method.
Results. There was a significant difference in the proviral load between the hemodialysis patients and the control group (903 ± 182 copies per mL versus 117 ± 186 copies per mL, respectively; $P = .008$). No significant correlation was found between the proviral load and haematocrit or serum levels of urea, creatinine, parathyroid hormone, calcium, and phosphorus level in hemodialysis patients, but proviral load of HTLV1 was significantly correlated with leukocyte count ($r = -0.46$, $P = .02$), hemodialysis duration ($r = 0.48$, $P = .02$), and the numbers of blood transfusions ($r = 0.71$, $P < .01$).
Conclusions. The immune deficiency related to end-stage renal disease and uremia is the probable cause of significantly higher HTLV1 proviral load in hemodialysis patients compared to healthy HTLV1 carriers. This high HTLV1 proviral load might be due to immune dysfunction in chronic hemodialysis patients.

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INTRODUCTION

Human T-lymphotropic virus 1 (HTLV1) is a retrovirus that is transmitted by blood transfusion, sexual contact, intravenous drug abuse, and breastfeeding. Human T-lymphotropic virus from the family of retroviruses has been divided into 3 major types of I, II, and III.[1] The structure, as well as epidemiology of the virus and related diseases, have been investigated broadly in the last two decades, in the world and also in Iran. Human T-lymphotropic virus 1 is a 9-kb deltaretrovirus that could not be found in the serum of animals or human beings. It has affinity to T lymphocyte, monocytes, fibroblasts, and synovial cells. Thus, the transmission of the virus occurs by an infected cell.[2,3] Induction of lymphoma and leukemia by HTLV1 seems to happen by a tax protein.[4] The proviral load is an useful biological marker for understanding HTLV1 pathogenesis and elucidating whether or not the virus is related to the clinical

manifestation of the disease.[5] Proviral stage is the stage before virus DNA incorporation into host lymphocyte DNA, and proviral load is the quantity of virus in this stage.

Some articles have discussed the prevalence of the HTLV1 antibody in chronic hemodialysis patients in Japan and Iran,[6-9] and also some other studies have evaluated the prevalence and occurrence of adult T-cell leukemia in hemodialysis patients from two districts of Japan where the human T-cell leukemia virus type I is highly endemic.[6,7] However, no study has been reported the evaluation of the HTLV1 proviral load in hemodialysis patients. The province of Khrosan in Iran, like the Kagoshima district in Japan,[6] is endemic for the HTLV1 virus. Chronic hemodialysis patients should be considered high risk groups for HTLV1 infection, due to repeated blood transfusions and the sharing of hemodialysis equipment and units. This study compares proviral load of HTLV1 in patients on hemodialysis with HTLV1 carriers not on dialysis.

MATERIALS AND METHODS

Thirty patients on long-term hemodialysis who were positive for HTLV1 and 30 HTLV1-positive carriers not on dialysis were included in this study. The primary selection of both cases and controls was according to the HTLV enzyme-linked immunosorbent assay antibody test positivity. The exclusion criteria were the presence of other viral infections, such as hepatitis B virus antigen positivity, hepatitis C infection, human immunodeficiency virus infection, malignancy, administration of immunosuppressive or cytotoxic medications, age of less than 18 years, and a duration of hemodialysis of less than 6 months (in the case group). The research protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences (No 89066). Written consent was obtained from all case and control participants.

Peripheral blood mononuclear cells (PBMCs) were isolated using the Ficoll gradient method. For DNA extraction, the DNA Blood Mini Kit (QIAgen, Hildesheim, Germany) was used. The virus proviral load was determined by the Taqman real time polymerase chain reaction method (Mastermix, Takara Bio Inc, Shiga, Japan). In both cases and controls, when the HTLV1 proviral load was undetermined, the HTLV1 antibody was rechecked with the radio-immunoassay method. In all those patients or controls with undetermined HTLV1 proviral load, the result of the radio-immunoassay test was negative, and therefore the individual was considered HTLV1 negative and excluded from the study.

Proviral load of HTLV1 and other characteristics were compared between the two groups using the t teat and the chi-square test, where appropriate. The correlations between proviral load and continuous variables within the hemodialysis group were tested using the Pearson correlation coefficient test. The SPSS software (Statistical Package for the Social Sciences, version 16.0, SPSS Inc, Chicago, Ill, USA) was used for data analyses. A *P* value less than .05 was considered significant.

RESULTS

Overall, the prevalence of the HTLV1 infection was 0.6% in the studied dialysis units. Twenty-five patients in the case group and 25 healthy carriers of HTLV1 were included in the study analyses. There were no significant differences regarding age and gender distribution between the two groups (Table 1). Moreover, the number of blood transfusions was significantly larger in the hemodialysis group than in the health controls (Table 1).

The proviral HTLV1 load was significantly higher in the hemodialysis patients as compared to the healthy carriers (903 ± 182 copies per mL versus 117 ± 186 copies per mL, respectively; *P* = .008). A significant correlation was observed between the proviral load of the HTLV1 virus and duration of hemodialysis in the hemodialysis group. In addition, proviral load was significantly correlated with the number of blood transfusions and reversely correlated with leukocyte count (Table 2). However, no correlation was found between proviral load and age, hematocrit level, or a positive familial history of HTLV1 infection.

Table 1. Characteristics of Hemodialysis Patients and Healthy Controls With Human T-Lymphotropic Virus 1 Infection

Characteristic	Hemodialysis Patients	Control Group	*P*
Mean age, y	61.12 ± 10.63	57.83 ± 9.23	.25
Mean number of blood transfusions	0.79 ± 0.93	1.91 ± 0.40	< .001
Sex (%)			
Male	14 (56)	10 (40)	
Female	11 (44)	15 (60)	.15

Table 2. Correlations Between Human T-Lymphotropic Virus 1 Proviral Load and Characteristics of Hemodialysis Patients

Characteristic	Human T-Lymphotropic Virus 1 Proviral Load	
	r	*P*
Hemodialysis duration	0.48	.02
Leukocyte count	-0.46	.02
Numbers of blood transfusions	0.71	< .01
Hemoglobin	-0.10	.62
Serum calcium	-0.17	.41
Serum phosphorus	-0.20	.34
Serum parathyroid hormone	-0.03	.91
Serum creatinine	0.08	.68
Blood urea nitrogen	0.32	.11

The correlation between proviral load and the number of weekly recombinant erythropoietin administrations was marginally significant.

DISCUSSION

The significantly higher HTLV1 proviral load in chronic hemodialysis patients compared with healthy carriers can be ascribed to the mutual effect of the virus and the uremic milieu on the immune system. The higher proviral load in hemodialysis patients may have exasperated the immunosuppressive effects of the HTLV1 virus, providing it with the opportunity to amplify its own replication in a vicious cycle. Significantly higher numbers of blood transfusion in HTLV1 infected chronic hemodialysis patients compared with healthy carriers may be due to the routine higher frequency of blood transfusion in these patients. Although currently all blood products are monitored for HTLV1 infection, this may still contribute to the HTLV1 infection in these patients.

Correlation of the number of blood transfusions in hemodialysis patients with higher HTLV1 proviral load in these patients is also interesting, but a causal relationship between these two cannot be directly discerned from the current study. As we were not able to evaluate the immune system function by measuring CD4, FoxP3+CD4+ regulatory T cells in virus-infected individuals, ascription of these correlations and differences to immune dysfunction requires further studies; however, the results of the studies already done in this field may be helpful for explaining the obtained results. The immune dysfunction progresses the proviral growth and level of the proviral load. Some authors have recommended that periodically quantifying proviral load is necessary in asymptomatic carriers to identify those at risk for developing neurological disease, and it is necessary for HTLV1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) patients to monitor spinal injury and progression to walking disability.[11] Also, a close relationship between the proviral load in saliva and oral manifestations was observed in some studies.[5] Although one study has shown that HTLV1 proviral load remains stable in both asymptomatic individuals and HAM/TSP patients over time, HAM/TSP individuals usually have a higher proviral load when compared to asymptomatic ones.[10]

Results of other studies suggest that first, asymptomatic carriers with a proviral load higher than 10% should be observed closely and their by HTLV1 proviral load checked more frequently, and second, HTLV1 proviral load in blood and tissue is a good marker for the diagnosing and monitoring of HTLV-1 infection.[12] In one study, although the prevalence of human papilloma virus infection was more in HTLV1 carriers, no difference in HTLV1 proviral load between human papillomavirus-infected women and those uninfected was found.[13] In another study, interleukin-18 and interferon-gamma polymorphisms were correlated with proviral load and susceptibility to HTLV1 infection. In this study, no significant correlation was found between interleukin-18 promoter region polymorphisms and HAM/TSP development and proviral load.[14] Also, there are reports implying that co-infection by HTLV1 or HTLV2 and human immunodeficiency virus is associated with a higher mortality from pulmonary tuberculosis,[15] risk of strongyloidiasis,[16] and also accelerated progression to AIDS.[17] Another effect of this co-infection is the loss of correlation between the human immunodeficiency virus proviral load and CD4+ T-cell numbers[18]; those co-infected patients had higher absolute CD4 numbers and CD4/CD8 ratios. Human T-lymphotropic virus 1 also has a depressing effect on the immune system particularly in co-infection with hepatitis C virus infections.[19]

Uremia itself on the other hand has a deteriorating suppressive effect on the immune system. Indeed, uremic patients suffer from an acquired type of immune deficiency. In addition, uremia has suppressive effects on innate and adaptive immunity,[20] and regulatory T-cells from patients

with end-stage renal disease undergo apoptosis by early cell-cycle arrest.[22-24] Dialysis can induce death of regulatory T cells in an autocrine fashion. Also, there is a correlation between recombinant erythropoietin therapy and reduction in CD4+ T-cell numbers. The significant but negative correlation between leukocytes count and proviral load in our study can be ascribed to the reducing effect of both uremia and prescribed erythropoietin on the lymphocytes culminating to weaker immune response and higher proviral load in hemodialysis patients, although we were not able to measure the CD4+ T-cells and regulatory T-cell numbers in our study. The current study's finding of a positive but only near significant correlation between the HTLV1 proviral load and the number of weekly recombinant erythropoietin administrations may somehow be explained in this way, although others have reported no effect of erythropoietin administrations on the numbers of lymphocytes and CD4/CD8 ratios in the peripheral blood of uremic patients undergoing peritoneal dialysis.[25] Some studies have shown that low natural killer group 2 member D expression and upregulation of its ligand, MHC class I related chain A, are associated with reactive oxygen species production and may be involved in the immune dysfunction of end-stage renal disease patients.[26] Hemodialysis may aggravate the immunosuppressive effects of end-stage renal disease.[23,24,27] These factors may be explanations for the cellular immune dysfunction in uremia that contributes to higher HTLV1 proviral load in our hemodialysis patients. A positive significant correlation between hemodialysis duration and the HTLV1 proviral load can be explained this way. The longer duration of hemodialysis gives the virus more opportunity for replication under the immune suppressed conditions of chronic uremia.

CONCLUSIONS

Due to the probable suppressive effects of uremia, chronic hemodialysis, and also HTLV1 virus on the immune system, the HTLV1 proviral load in hemodialysis patients is significantly higher in comparison with healthy carriers. A history of a long duration of hemodialysis, more frequent numbers of blood transfusions, and probably more frequent and higher doses of recombinant erythropoietin are correlated with a greater HTLV1 proviral load in hemodialysis patients. Frequent monitoring of asymptomatic hemodialysis patients with low proviral load is recommended, and further studies should be conducted to assess the course of proviral load and the immune system function in these patients over extended periods of time.

CONFLICT OF INTEREST

None declared.

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