

Determination and Analysis of HTLV-1 virus load by Real-time PCR among HTLV-1 Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP) and asymptomatic carriers

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ABSTRACT

Introduction: HAM/TSP (HTLV-1-associated myelopathy/tropical spastic paraparesis) is a set of gradual symptoms and progressive paralysis in the neurons of the lower motor organs and sensory disorders as well as sphincter disorders and infects the spinal cord and white tissue of the brain.

Methods: In this study, blood samples were taken from 57 people in three groups of HAM/TSP patients (20 people), asymptomatic carriers (17 people) and healthy control people (20 people). Demographic information of the patients was recorded in the questionnaire and informed consent was obtained from them. Initial identification of the virus was done using the ELISA method. Then peripheral blood mononuclear cells were isolated using Faycol gradient and their DNA was isolated using the kit. Finally, Real Time PCR technique

Results: There was a significant difference ($p=0.037$) in demographic characteristics, i.e. gender, among asymptomatic carriers infected with HTLV-1 (7 men and 13 women), HAM/TSP patients (6 men and 11 women) and control group (13 men and 7 women). In the (HAM/TSP) group, $P=0.015$, in the (asymptomatic carriers) group, $P=0.042$, and in the (control) group, $P=0.049$. The mean and standard deviation of the age in the healthy control group were $78/15 \square 44/36$, $76/15 \square 47/44$ in the asymptomatic carriers of HTLV-1 virus, and $10/15 \square 45/46$ in the HAM/TSP patients. Statistical analysis of the samples using ANOVA test shows that there is no significant difference in terms of age between the groups ($P > 0.05$). As expected, the average provirus load in the HAM/TSP group was 07/1 times its value among asymptomatic

Key words: HTLV-1 virus, HAM/TSP, proviral load, clinical symptoms.

INTRODUCTION

Human T-lymphotropic virus type 1 is a retroviral Delta that infects about 20-10 million people worldwide. HTLV-1 infection is mostly asymptomatic, but for unknown reasons, it leads to the development of aggressive malignant diseases such as T-cell leukemia/acute lymphoma (ATL) in adults in 5-3% of cases. In addition, HTLV-1 infection leads to HTLV-1-dependent myelopathic inflammatory disorder / tropical spastic paraparesis (HAM/TSP). The virus is endemic in Khorasan Province and Nishapur city. About 5-2% of people infected with the virus have HAM/TSP, and the rest of those infected remain as asymptomatic vectors. [1] Among the numerous factors associated with the virus and host, the CTL cells produced in the host body seem to play a decisive role in controlling viral load and determining the risk of inflammatory disease HAM/TSP. Differences in viral load in different patients appear to be caused by differences in the effective responses of these cells in the host body. Studies show that CTL cells in asymptomatic vectors are more efficient than in people with HAM/TSP [29]. Iran is one of the countries that is considered part of the endemic areas in terms of HTLV-1 prevalence, and contamination with this virus causes diseases such as ATLL and HAM/TSP, for which there is unfortunately no cure.[30]. Despite the high importance of contamination with this virus, no specific measures have been taken yet regarding easy and low-cost diagnostic methods for identifying this virus in our country. Because the province of Khorasan, especially Nishapur, has the highest prevalence of the HTLV-1 virus. In this study, we considered the facilities and equipment to identify the virus and confirm its identification in three groups of healthy people, carriers and patients with HAM/TSP, as well as to examine clinical symptoms.[31]

Objectives of the research**General objective:**

Detection of HTLV-1 virus and determination of viral load by real-time PCR among patients with HAM/TSP and asymptomatic carriers

Partial objectives:

- 1 -Detection of HTLV-1 infection using real-time PCR
- 2 -Investigation of viral load in HAM/TSP patients
- 3 -Investigation of virus load in asymptomatic carriers

Research questions:

Is there a relationship between HAM/TSP and viral load

Research hypotheses:

Virus load in patients HAM/TSP is more common.

Statement of the problem:

Human T-lymphotropic virus type 1 is classified as a complex retrovirus in the genus Deltaretrovirus of the subfamily Orthoretrovirinae [2]. HTLV - 1 can be sexually transmitted [3], taking an injectable drug [4] and breastfeeding passed from mother to child [2]. For more than two decades, a study of HTLV-1-mediated pathogenesis has been conducted focusing on Tax, one of the HTLV-1-encoded viral oncoproteins. Tax has since been considered vital for leukemogenesis due to its polytropic effects both on viruses and on many cell genes responsible for cell proliferation, genetic instability, disruption of cell cycle regulation and apoptosis. However, tax expression is not detected in about 60% of fresh cases of isolated samples from ATL cases. In 2002, another regulatory protein encoded in the negative string or antisense of the virus genome called the HTLV-1 leucine-based zipper, the HBZ factor, was identified. The form of hbz connection is expressed in all cases of ATL and HAM/TSP, and its expression depends heavily on the proviral load (PVL) in people infected with HTLV-1 and with the severity of the disease in HAM/TSP patients [2]. Hbz protein also causes ATL cell proliferation and induction of Tcell lymphoma in transgenic expression CD4 + T cells, indicating possible interference of hbz expression in the development of ATL disease [3]. Moreover, among the encrypted viral HTLV-gene, only the hbz gene sequence remains intact under the influence of nonsensical mutations and deletions [5]. These findings suggest that hbz expression was necessary for the reproduction and survival of ATL and HTLV-1 cells of infected cells, and tax expression is not always necessary for ATL preservation [6].

Classification of the HTLV-1

The genus Deltaretrovirus includes types of HTLV viruses 1, 2, 3 and 4 (HTLV-1/2/3/4), T-cell lymphotropic virus, Simian virus types 1, 2, 3 and 4 (STLV-1/2/3/4), and cow leukemia virus (BLV) [4]. Among human deltaretroviruses, only HTLV-1 is causally associated with human diseases such as ATL/lymphoma (ATLL) and tropical spastic paraparesis (TSP), also known in Japan as HTLV-1-related myelopathy (HAM), in addition to other pathologies associated with abnormal lymphocytic proliferation caused by the interaction of viral proteins with the host cell biochemical apparatus. However other members of the HTLV group were not causally related to any human disease and their role in humans is uncertain. Although some researchers claim that HTLV-2 is one of the triggers of neurological disorders in humans(4).. HTLV 3 and 4 variants were also not associated with any human disease and are found only among Indigenous people of Central Africa([4].

Human T-lymphotropic virus type 1 is a complex retrovirus in the genus deltaretrovirus of the subfamily Orthoretrovirinae and of the family retroviridae viruses. HTLV-I 1 is a type of human oncogenic retrovirus that causes a stable infection in the host body, which is associated with ATL (adult T cell Leukemia/Lymphoma), HAM/ TSP (HTLV-I-dependent myelopathy/tropical spastic paraparesis) and other HTLV-I-dependent diseases such as uveitis, arthritis, myositis, alveolitis and sugarcane syndrome in 5-5/ 0% of the time it has (YAKOVA et al., 2005; Higuchi

et al., 2003; Espíndola et al., 2015). It was first identified in 1980 as a Type C retrovirus in a lymphoblastoid T cell class[5].

Genetic structure of the HTLV-1 virus

Human T-lymphotropic virus type 1 belongs to the retroviridae family and has a BP 9032 long RNA genome containing the genes gag, pol and env. These genes encode capsid and nucleocapsid proteins, envelop protein including surface glycoprotein and trans-membrane protein. The PX region at the 3' end of the genome contains virus regulatory genes. This region encodes the regulatory proteins p27rex, P40Tax, as well as P21rex. Ltr regions in the virus genome have 755 nucleotides that contain areas necessary to initiate the translation of viral genes such as TATA patterns, responsive components (TRE) tax, game sequences necessary for transcription termination, and mRNA polyadenylation (made of the HTLV-I Gene). The ltr region is methylated and removed on the 5-CE side in adult T-cell leukemia during translation, while the existing part remains on the 3-ce side [6]

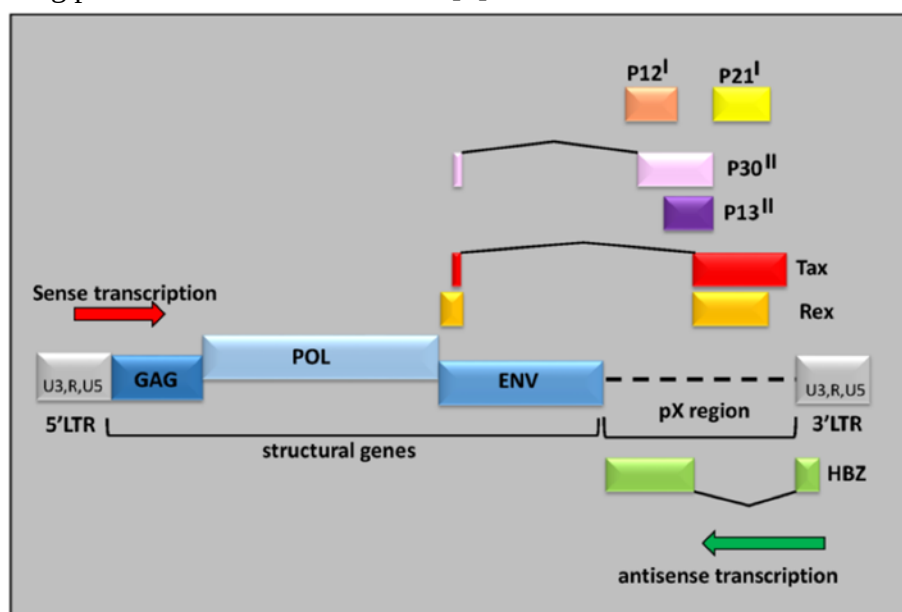


Figure 1. Genomic structure, sequencing of HTLV-1 virus genes and proteins [7]

The HTLV-1 genome in the virion is of the RNA molecule type, and after entering the host cell, it is first transcribed by the reverse transcriptase enzyme of a DNA copy of the RNA. This DNA is made to bind the host cell chromosome and multiply with the help of the host. In other words, the genetic material of the HTLV-1 virus is in the RNA vein and inside the host cell is DNA.

MATERIALS AND METHODS

The materials, equipment and devices used

The materials, equipment and devices used in this study are listed in tables 1.

Table 1. The used material for the Eliza test.

Country of manufacture	Sher Kat maker	Name of kit and equipment
Australian	E-Bioscience	Human IL-6 Platinum
Germany	Apendrov	Sample (100-1000microl
		microliter sample (1-100)
Italy	LP (ITALIANAS PA)	Yellow and blue sample head
Germany	IKA KS4000	Shaker
Iranian		Iranian glass pipe
USA	STAFAX Awareness	Eliza reader device

Study population

The study is descriptive-cross-sectional. The population studied includes three groups of HAM/TSP patients, asymptomatic vectors, and healthy individuals. Patients who have visited the neurology clinic in Nishapur. Patients entered the study after being diagnosed with clinical symptoms by a neurologist, obtaining a description. All people involved in the study are residents of Nishapur and surrounding villages. A questionnaire was prepared from the information, description and tests of the patients, which is attached. The diagnosis of neurological and motor disabilities of HAM/TSP disease was based on the International Guide. Patients infected with HTLV - 1 who did not have clinical symptoms were considered vectors .

the Inclusion criteria:**A. patient group HAM/TSP:**

1. -confirmation of the diagnosis of symptoms by a neurologist with a clinical examination
2. confirmation of infection with the virus by serological method (anti-HTLV-I Positive)
3. -confirmation of infection with the virus by Real timePCR

B-group of unmarked carriers:

1. confirmation of infection with the virus by serological method (anti-HTLV-I Positive)
2. confirmation of infection with the virus by Real time PCR
3. lack of clinical neurological symptoms in the clinical examination

c - control group:

1. no infection with the HTLV-1 virus
2. -no inflammatory diseases

Exclusion criteria

- 1-history of interferon treatment in the HAM/TSP group
- 2-no confirmation of virus contamination in both groups
- 3-no history of inflammatory diseases such as Chagas, HBV, HCV , HIV 1/2
- 4-people with other neurological symptoms unrelated to HTLV ,
- 5-immunosuppressants
- 6-pregnant women

Sample size:

The sample size studies were selected according to the limitation of HAM/TSP patients. In the present study, about 57 people (20 HAM/TSP, 17 HTLV-1 unmarked carriers and 20 healthy control subjects) were examined. The number of samples was calculated from the following formula:

Sampling:

Each patient was examined by a neurologist and given a full description of his justification and consent for blood transfusion. Then 12 CC of complete blood samples were taken from the patient. 6 CC of Whole Blood was poured into the gel tubes to isolate the plasma and to check for anti-HTLV-1,2 antibodies, and 6 CC of it was transferred to the tube containing EDTA-K2. Then the tube was gently shaken so that all the blood was mixed with the anticoagulant in the tube and no clot formed.

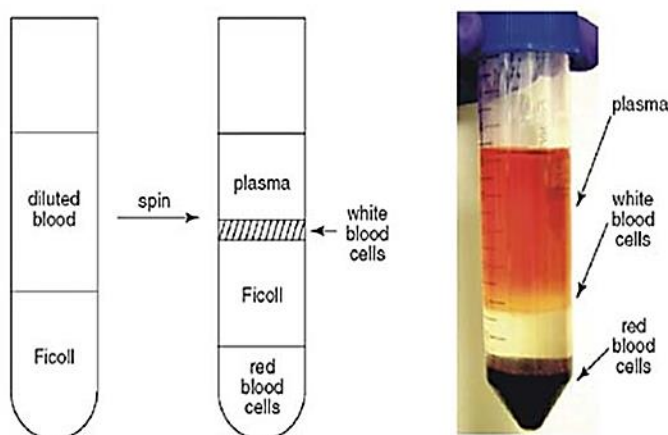


Figure 2. Isolation of White Blood Cells using Density Gradient Centrifugation.

Extraction of DNA from PBMCs

After extracting the lymphocytes, using GeNet Bio's DNA extraction kit(Iran), the DNA was extracted from the cells and used to determine the viral load. The kit DNA extraction protocol is as follows:

- First, in a 1.5 mL microtube, 20 microliters of K proteinase were poured, and the amount of 200 microliters of isolated lymphocyte was poured on it, and then vortexed.
- Two hundred microliters of GB buffer was poured onto the mixture inside the microtube and vortexed for 15 seconds.
- Then put it at 56 °C for 10 minutes, and then it was vortexed.

About 200 microliters of absolute ethanol were poured on it.

- Then transferred the mixture inside the microtube on the cartridge.

It is then centrifuged at RPM 10000 for 1 minute. after that discarded the microtube under the cartridge and put the cartridge on another microtube, and added 500 microliters of GW1 washing solution to it, and it was 10,000 centrifuges around the rpm for a minute. then discarded the underground microtube and added 500 microliters of GW2 washing solution to the amount, and it was centrifuged at rpm 14800 for two minutes.

RESULTS

The results of the diagnostic Eliza test for samples of HAM/TSP patients and asymptomatic vectors are shown in the table below. As shown in Table 2, in 2 of the subjects entered into the study, the serum sample was diagnosed negative, and the rest were positive.

Table 2. The results of Eliza test for HTLV-1 infection with the virus

Result<0.9 Negative 0.9-1.1 Equivocal >1.1 Positive	S/Co	Cut-Off (Co)=NC+0.2	Sample (S)	Row in ELISA
Positive	7.63	0.13+0.2=0.33	2.524	1
Positive	7.27	0.33	2.401	2
Positive	7.68	0.33	2.536	3
Positive	7.66	0.33	2.530	4
Positive	7.59	0.33	2.506	5
Positive	7.58	0.33	2.501	6
Positive	7.59	0.33	2.505	7
Positive	7.59	0.33	2.506	8

Positive	7.57	0.33	2.500	9
Positive	7.62	0.33	2.517	10
Positive	7.62	0.33	2.516	11
Positive	7.95	$0.12+0.2=0.32$	2.545	12
Positive	7.97	0.32	2.551	13
Positive	7.97	0.32	2.553	14
Positive	7.97	0.32	2.550	15
Positive	5.95	0.32	1.905	16
Positive	7.93	0.32	2.539	17
Positive	7.53	$0.14+0.2=0.34$	2.561	18
Positive	7.53	0.34	2.562	19
Positive	7.50	0.34	2.551	20
Positive	2.98	0.34	1.013	21
Positive	7.49	0.34	2.548	22
Positive	7.50	0.34	2.549	23
Positive	12.12	$0.01+0.2=0.21$	2.546	24
Positive	8.89	0.21	1.868	25
Positive	11.99	0.21	2.517	26
Positive	12.1	0.21	2.541	27
Positive	11.93	0.21	2.506	28
Negative	0.32	0.21	0.068	29
Negative	0.228	$0.05+0.2=0.25$	0.057	30
Positive	10.10	0.25	2.525	31
Positive	10.264	0.25	2.566	32
Positive	12.09	$0.009+0.2=0.209$	2.527	33
Positive	12.16	0.209	2.541	34
Positive	11.92	0.21	2.504	35
Positive	12.11	0.209	2.531	36

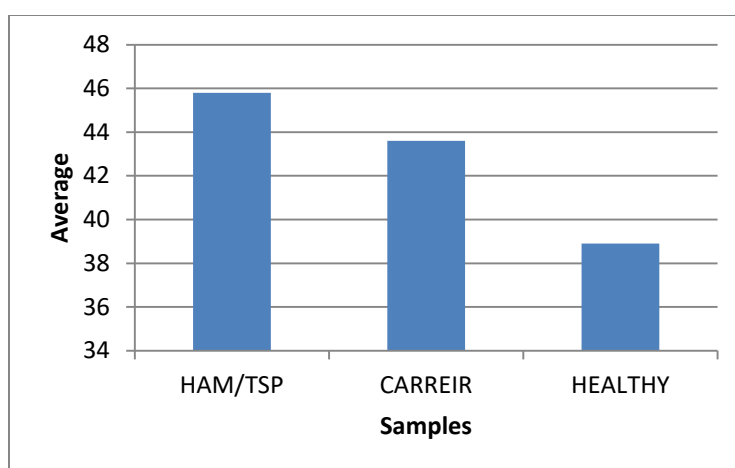
Examination of groups by age

In these 20 HAM / TSP, 20 HTLV-1 asymptomatic vectors, 20 were examined as healthy control subjects. The study reported an average age of 45-8 patients, an asymptomatic vector of 43-6 and healthy people of 38-9 years. The mean and standard age deviation in the total subjects

studied in this thesis was 12.5 ± 43.30 . Statistical analysis of samples using the ANOVA test showed that there was no significant age difference between the study groups ($P = 0.254$).

Table 3. The average age of groups

		Number	Average	Standard deviation	P-value
Age	HAM/TSP	20	46.4500	15.10481	$P > 0.05$
	Carrier	17	44.4706	15.76435	
	Control	20	36.4444	15.78786	
	All	57	41.7031	15.99571	

**Figure 3. The average age of the subjects.**

Examination of groups by gender

Using statistical analysis, it was found that groups differ significantly in gender. In the HAM/TSP group, $P = 0.015$ and in the CARRIER group, $p = 0.042$ and in the CONTROL group, $p = 0.049$.

Table 4. The distribution of the sex of the subjects by male and female separation

Gender * Group Crosstabulation					
		Group			Total
		HAM/TSP	Vector	Control	
Gender	Number of men	7	6	11	24
	Number of women	13	11	9	33
	Total sum	20	17	20	57

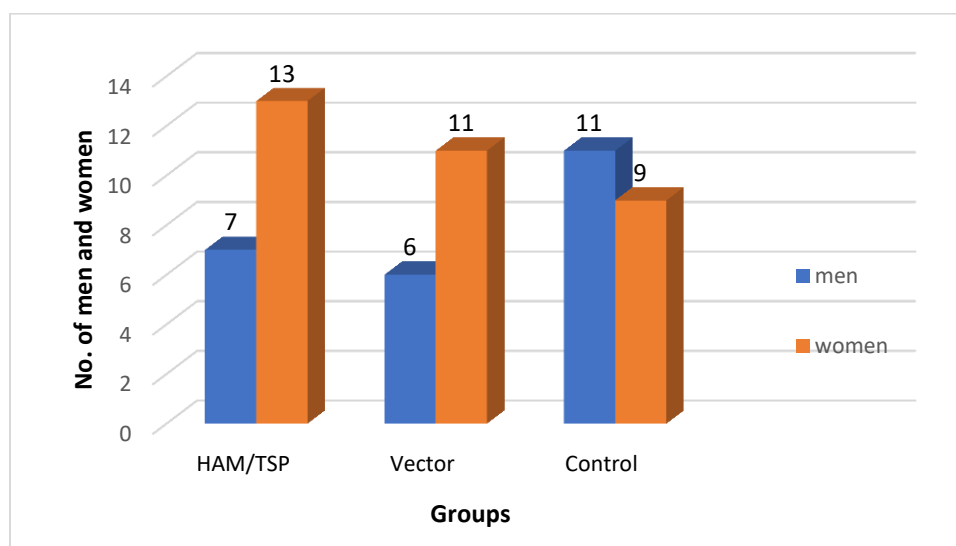


Figure 4. The chart of sexual distribution in the control group, vector and HAM / TSP Verification of serological diagnosis with Real-Time PCR.

Confirmation of infection with the virus was carried out using the Real-time PCR method. The Real-time PCR results for HTLV-1 of the 37 samples tested showed that all samples previously confirmed by the attending physician for the presence of HAM/TSP disease and asymptomatic vector were confirmed by the Real-time PCR reaction.

Curve of kit standards

In the proviral load measurement, the standard curve for the kit was drawn, as shown in Figure 5.

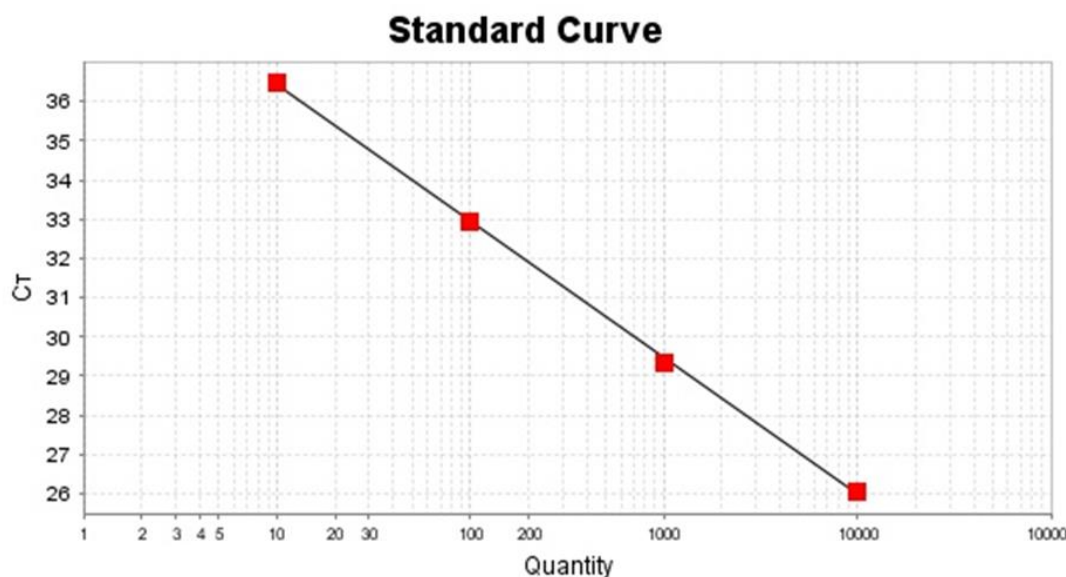


Figure 5. The curve of the standards of the viral load sensing kit.

The increase in HTLV/FAM radiation related to HTLV and the increase in Albumin / VIC radiation caused by Albumin are considered. If the sample has a sigmoidal and CT chart of less than 40 on both the FAM and VIC channels, it is usually considered positive. If a sample in the fam channel lacks a Sigmoid curve and in the VIC channel it has a Sigmoid curve and its CT is within the range of kit standards, it is usually called a negative sample. The following graphs are the results of the PCR Rail time. The following are the machine diagrams associated with the PCR Rail time.

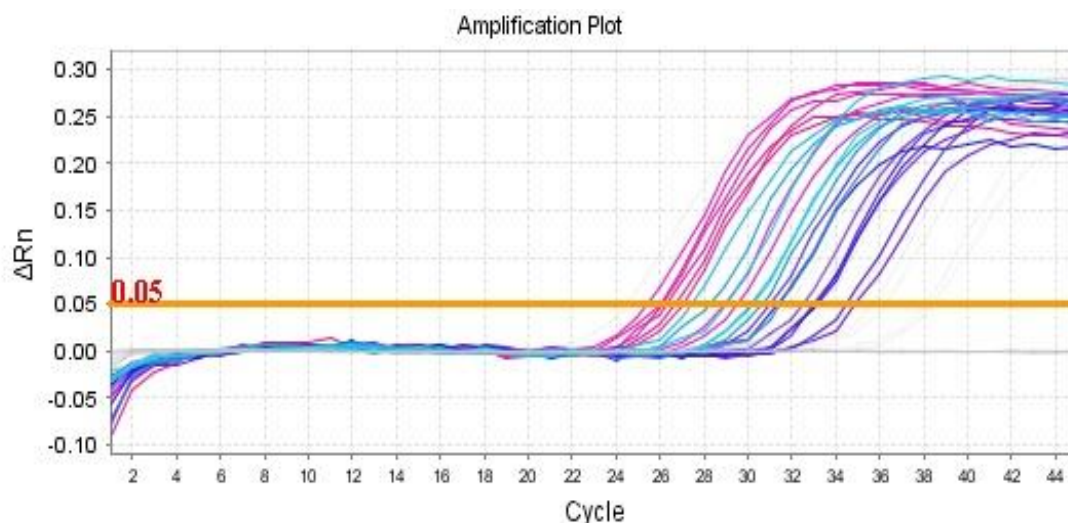


Figure 6. Sigmoid curve related to the Vic channel proviral load HAM/TSP patient samples and asymptomatic vector.

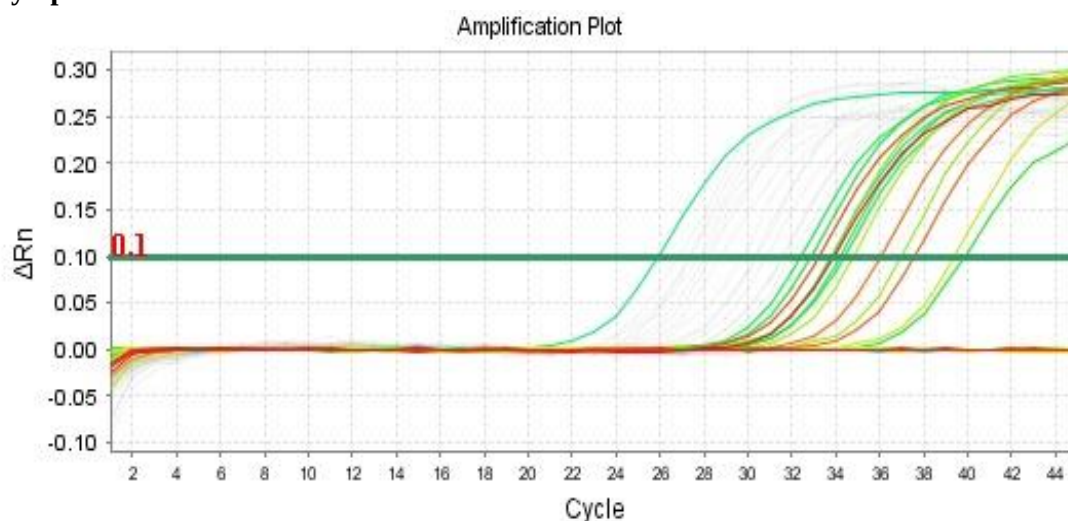


Figure 7. Sigmoid curve related to the proviral load of the fam channel patient samples HAM / TSP and asymptomatic vector.

Ct values and concentration of standard and control samples - various Ct values, given and calculated relative concentrations for standard samples and HTLV-1 virus are given in Table 5.

Table 5. CT values: kit concentration and calculated concentration for standard and virus loading samples.

Ct (copy in microlit)	Concentration given	Name	No.
26.08	10000	S1	1
29.34	1000	S2	2
32.29	100	S3	3
36.48	10	S4	4

Table 6. The kit concentration and calculated concentration for albumin control samples related to viral load virus.

Ct (copy in microliter)	Concentration given	Name	No
26.96	10000	C1	1
30.24	1000	C2	2
34.7	100	C3	3
37.59	10	C4	4

Results related to proviral load in HAM/TSP patients

Real time PCR results for the HTLV-1 virus load in the HAM/TSP patient group are given in Table 7.

Table 7. The real time PCR results for the HTLV-1 virus load in the HAM/TSP group.

Sample number	CT HTLV-1	CT Albumin	HTLV1 titre *2/ALB titre (copy/10000PBMC)
1	37.28	30.57	25212.8
2	34.26	30.24	23423.2
3	39.24	31.25	25961
4	36.79	34.04	22345.1
5	31.66	24.38	26848.2
6	39.73	35.52	23125.3

7	39.25	35.9	22604.1
8	30.97	24.51	26124
9	39.28	32.55	24949.5
10	33.91	31.6	22186.2
11	33.22	31.55	21769.2
12	39.4	35.94	226703
13	37	33.85	22597.5
14	33.87	30.28	23132.8
15	32.89	27.43	27490.2
16	34.2	28.3	24985.1
17	31.56	22.28	29286.2
18	27.26	20.51	27479.1
19	32.75	24.87	27591.7
20	29.69	23.43	26198.7

Results related to the proviral load in asymptomatic carrier

Real time PCR results for the HTLV-1 virus load in the asymptomatic carrier group are shown in Table 8.

Table 8. Real time PCR results for the study of HTLV-1 virus loading in the asymptomatic carrier group

Sample number	CT HTLV-1	CT Albumin	HTLV1 titre *2/ALB titre (copy/10000PBMC)
1	38.85	34.83	23061.1
2	36.26	32.66	22953.7
3	39.32	32.76	24814.8
4	40.13	29.17	28442.9
5	35.68	33.05	22319.9
6	36.8	34.46	22078.7
7	38.44	37.21	2135.1
8	37.59	31.96	24316.8
9	37.59	31.96	22729.7
10	34.81	32.37	22233
11	39.85	30.85	26706.4

12	32.41	27.27	24573.4
13	34.39	27.68	25686.6
14	33.8	28.03	24930.7
15	34.62	24.51	29202.9
16	29.11	25.81	23318.2
17	30.69	22.66	28001.3

Statistical analysis of the results of Real time PCR

Data OR CT (obtained from asymptomatic vector subjects and Ham/TSP patient subjects) was obtained. Comparison of the average rate of peroviral load among asymptomatic carriers and people with HAM/TSP is given in Table 9. As can be seen, the average peroviral load in sick people is higher than in asymptomatic vectors, but not statistically significant ($p = 0.40$).

Table 9. The comparison of mean and deviation from the virus load criterion in two groups of carriers and patients with the T Test

Comparison of copy number results (T Test)	Average	Deviation from the standard	Sig
Ham/TSP patient group	24799. 01	2242. 48	0.40
Healthy carrier group	19875. 56	10141. 62	

Clinical signs of HAM/TSP patients

By examining the demographic information of the sick people whose information was contained in the questionnaire, the desired analyses were carried out, the details of patients with HAM/TSP, including the degree of the disease and the type of disease, are given in Table 10. Among patients with HAM/TSP, 95% had abnormal gait, 90% had abnormal muscle reflexes, 65% complained of a decrease in the sensory system, 80% had a urinary disorder and 100% had an Autonomic Disorder. The HAM/TSP group of patients had a high degree of chronic diseases (80%) with a low degree of inflammation, and only 5% were in the acute phase of the disease. Of these, 55% showed tetraparasia.

Table 10. The characteristics of HAM/TSP patients in this study.

Percentage of disorder or disease	Parts	HAM/TSP
-	%95	Qualified
5	Acute	Degree of disease
15	Under acute	
80	Chronic	
95	-	Walking mode
55	Tetraparzi	Type of disease
20	Paraparzi (right)	
15	Paraparzi (left)stem)	
90	-	Muscle reflex
65	-	Sensory system
80	-	Urinary incontinence
100	-	Anarchy of the autonomous system

DISCUSSION

HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM / TSP) is a progressive chronic neurological disease caused by infection with the HTLV-1 virus and is similar to other inflammatory responses of leukocytic artery in brain and spinal cord ulcers. Most of these cells are CD4 T-lymphocytes, CTL cells, and monocytes that have been able to cross the blood-brain barrier and bring themselves to the parenchyma of the brain. In patients with HAM/TSP, there is a high headline of anti-HTLV-I antibodies and a large number of active CTL cells, and the immune responses in these patients appear to be the cause of inflammatory injuries and tissue injuries [8]

There are many questions about HTLV-1 infection and the quality of immune responses created in the human body, including why only a few people infected with the HTLV-1 virus develop inflammatory diseases such as ATL and HAM / TSP, while most infected people remain as asymptomatic vectors throughout their lives. Does the difference in the type of HTLV-1 virus cause these differences, or are the differences in host people, such as the strength of the immune system, the determining factor? So far, the cause of these differences is not known exactly, but in general, specific host-related factors such as genetics and immune responses and Specific factors related to the virus, such as its genotype, are involved [8].

Host immune responses played a key role in the pathogenesis and course of HAM/TSP disease, and studies show that the average percentage of proprietary + TCD4 cells against HTLV-I virus

was higher in HAM/TSP patients compared to asymptomatic vectors with the same rate of viral load. Also, the response of proprietary+TCD4 cells against HTLV-I is more associated with the production of pre-inflammatory cytokines such as interferon gamma. In addition, the percentage of HTLV-I proprietary + TCD4 cells that produce interferon gamma has been reported in HAM/TSP patients to be much higher than asymptomatic vectors with the same virus load rate[32].

The factor that leads to an increase in the high percentage of specific +CD4 cells against HTLV-I in HAM/TSP patients is unclear, but this seems to interfere with tissue damage and inflammation, especially since in active (acute) inflammatory injuries, the majority of cells are +CD4 cells (9). Studies have shown that the number of HTLV-I-specific CD8+ T cells in peripheral blood and cerebrospinal fluid that identify the tax 11-19 peptide is much higher in HAM/TSP patients than in asymptomatic vectors.

There is a positive correlation between the virus load and the percentage of HTLV-I proprietary CTL cells, but the reason for this correlation is not entirely clear. This increases the load of the provirus, which in turn causes a strong antigenic stimulation, which in turn causes the proliferation and activation of HTLV-I-specific CTL cells. Increasing the number and activity of CTL cells more effectively destroys cells that express HTLV-I, thereby inhibiting the replication of the HTLV-I virus and reducing the viral load. On the other hand, various studies have shown that increasing the number of virus-specific CTL cells is not a good indicator of their efficiency, but instead of the number of CTL cells, one should focus on the efficiency and lethal power of these cells. So the effective response of htlv1-specific CTL cells plays a very important role in controlling the viral load, and the difference in the proviral load among people infected with the virus is due to the differences in the effective response of these cells in these people [9]

The present study sampled 37 patients visiting the Nishapur Neurological Clinic. All 37 people infected with the virus were then diagnosed using the Eliza test and detection of anti-HTLV-1,2 antibodies. Out of a total of 37 people surveyed, 20 were placed in the HAM/TSP group based on clinical symptoms and according to the global standard, and 17 were clinically asymptomatic and infected with the virus, which were categorized into the asymptomatic Vector Group of the virus. In addition, 20 people who did not have serologically anti-HTLV-1,2 antibodies were included in the study as healthy controls.[27]

A survey of HAM/TSP patients in the study looked at 7 males (35%) and 13 females (65%). There were also 6 men (35.29%) and 11 women (64.71%) among the asymptomatic vectors of the virus, and 13 men (65%) and 7 women (35%) among the healthy control group. Among people who tested positive for the HTLV-1 virus (patient and Vector) and healthy control group using the Fisher test, it has been shown that there was a significant difference ($0.037/0=p$) in demographic characteristics, i.e. gender, among asymptomatic vectors infected with HTLV-1 (7 males and 13 females), HAM/TSP patients (6 males and 11 females) and witness Group (13 males and 7 females). In the group (HAM / TSP) $P=0.015$ and in the group (unmarked carriers)

$P=0.042$ and in the group (Control) $P=0.049$. Of the 37 HTLV-1 positive individuals, 70% were male.

The mean and age standard deviation were reported in healthy control group subjects 78/15 at 44/36, in HTLV-1 asymptomatic vector subjects 76/15 at 47/44, and in HAM/TSP patients 10/15 at 45/46. Statistical analysis of samples using the ANOVA test shows that there is no significant age difference between groups ($P > 0.05$).

In the other part of the article, the infection was confirmed in the patients and patients of HAM/TSP using the Real-time PCR method, all of which were confirmed to be infected by the HAM/TSP group and the HTLV-1 virus. The results of the PCR Real time test showed that there was no difference between the results of the HTLV-1,2 virus detection serological analysis and the results of the Real-time PCR proprietary molecular test.

After that, the rate of proviral load or virus load was determined in both asymptomatic and HAM/TSP carrier groups. The average Proviral load was measured in the HAM/TSP group, 24,967.3, and in the asymptomatic Vector Group, 23,189.6 in 104 PBMC cells. The statistical relationship between the rate of load or load proviral in vector and patient HAM/TSP was examined with the help of the T Test, and a significant relationship between load proviral was not found between the two groups of vector and patient ($p=0.185$). In addition, the correlation between gender and virus load was also checked and no significant correlation was found ($p=0.161$).

As expected, the average load rate of the provirus in the HAM/TSP group was 0.71 times its value among asymptomatic vectors (which was not a statistically significant difference). This indicates that the progression of the disease from asymptomatic vector state to HAM/TSP is related to an increase in the virus rate.[22]

These results are consistent with previous research in HAM/TSP patients[14]. In this study, the average viral load in patients was higher than in other studies in Iran, Japan and Brazil[14]. Virus-infected t-CD4 and T-CD8 lymphocytes can penetrate nerve tissues and cerebrospinal fluid, leading to a high load of the virus in CSF, causing the demyelination of neurons in the nervous system, resulting in HAM/TSP consequences [11].

Although some studies have conducted studies on immune system response in people with HTLV-1, few studies have focused on the Association of immune response and disease degree among HAM/TSP patients and asymptomatic vector groups[10]. Compared to healthy people or asymptomatic vectors, the progression of the disease in HAM/TSP cases is dependent on the loading of the HTLV-1 virus and the secretion of pre-inflammatory and anti-inflammatory cytokines including IFN- γ , TNF- α , IL-2, IL-4, IL-6, and IL-8 [11,17]

In a 2015 study comparing virus load, expression of viral genes and IL-10 and gamma interferon (IFN) cytokines in two groups of HTLV-1 asymptomatic carriers and HAM/TSP patients, HAM/TSP patients and asymptomatic carriers with higher provirus loads were more than IL-10, but in asymptomatic carriers with lower provirus loads the expression of IFN and IL-10 was proportional [12,21]

Conclusion

In this intermittent study, high HTLV-1 levels were identified among serum samples using the Eliza test in Nishapur. The results of the study emphasize that HTLV remains an important indigenous disease in Nishapur. Serology test results and molecular test results were consistent. Peroviral load was measured more in HAM/TSP patients than in asymptomatic vectors, and the role of peroviral load in the pathogenesis of HAM/TSP disease and the progression of the disease was diagnosed with importance.

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