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Investigating the circadian rhythm signaling pathway in HTLV-1 pathogenesis using Boolean analysis

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ABSTRACT

The Human T-cell lymphotropic virus type 1 (HTLV-1), an oncogenic virus belonging to the Deltaretrovirus genus, expresses various proteins, including Tax and HBZ, which can affect many cellular pathways. In this study, we have investigated the role of the circadian rhythm signaling pathway, a key regulator of human health, in the pathogenesis of HTLV-1 using Boolean Network analysis and laboratory methods. After an extensive search of the circadian rhythm pathway, we analyzed the relationships between the genes of this pathway using the R programming language and the BoolNet package. Subsequently, we examined the impact of viral proteins on the cellular clock rhythm genes. Finally, we identified three genes, PER2, CRY1, and DEC1, as the main checkpoints from the attractors obtained. These three genes and two viral genes, Tax and HBZ, were quantitatively assessed on two groups of individuals, including ten asymptomatic carriers infected with HTLV-1 and ten healthy individuals using the qRT-PCR method. Our results showed that the expression level of PER2 and DEC1 genes was significantly higher in the asymptomatic carriers compared to the healthy control group. Also, we recorded positive correlations between PER2 and DEC1, CRY1 and DEC1, and negative correlations between HBZ and CRY1 and DEC1. In this study, we suggested that in asymptomatic carriers, the virus might try to induce a chronic infection by escaping from the immune system due to an alteration in circadian rhythm pathways. We also detected three promising genes in this pathway that could have therapeutic or diagnostic value in these individuals. However, this possibility requires further research in different periods, different groups (e.g., ATLL and HAM/TSP), and examining a more significant number of circadian rhythm genes.

1. Introduction

HTLV-1 (Human lymphotropic virus type 1), the first human oncogenic retrovirus, has infected about ten to twenty million people worldwide (Willems et al., 2017). Regarding geographical distribution, HTLV-1 has a heterogeneous distribution with high prevalence in some regions, including southwestern Japan, Central and South America, Caribbean islands, and northeastern Iran (Mahdifar et al., 2023). This virus can infect different types of cells, such as dendritic cells, macrophages, monocytes, and CD8 T cells, but mainly CD4 + lymphocytes act as virus reservoirs (Brites et al., 2021). Over 90 % of infected people remain asymptomatic, while the rest may progress to Adult T-Cell Leukemia/Lymphoma (ATLL) and HTLV-1-associated myelopathy/-tropical spastic paraparesis (HAM/TSP) (Martin et al., 2014).

HTLV-1, as an enveloped virus, contains a linear single-strand RNA genome with positive polarity, which produces different proteins, including Tax and HBZ, to affect various cellular pathways. Recent studies have demonstrated different paths in this virus pathophysiology, including suppression of immune system responses, activating cellular oncogenes, continuous antigenic stimulation, production of some matrix metalloproteinases, and circadian rhythm alteration (Boxus et al., 2008; Keikha et al., 2020). Circadian rhythm is an internal timing mechanism

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coordinating various other pathways in human physiology and homeostasis during 24 h. Fluctuations of this rhythm affect physiological processes such as sleep, nutrition, metabolism, and immunity against both viral and bacterial infections (Huang et al., 2023). Various researchers have examined the effects of viral infections on circadian rhythm pathways. For instance, Yang et al. reported that the overexpression of the HBZ protein from the Hepatitis B virus could alter the expression of several key circadian genes, including CLOCK, BMAL1, PER1–3, and CRY1–2.

Similarly, studies have indicated that Hepatitis C virus (HCV) infection upregulates miR-10, downregulating BMAL1 by suppressing RORα. Additionally, HCV patients often exhibit lower expression levels of PER2 and CRY2.

Human Immunodeficiency Virus (HIV) also affects the expression of CLOCK, BMAL1, REV-ERB, and PER2 genes. Research has shown that CLOCK/BMAL1 promotes HIV transcription via the E-box in the long terminal repeat (LTR), while REV-ERB reduces HIV promoter activity.

Moreover, disruptions in this circadian rhythm pathway have been reported in infections caused by Dengue virus, Herpesvirus, Influenza virus, Parainfluenza virus type 3, and Zika virus. However, data regarding HTLV-1′s impact on this pathway remains limited. Despite extensive research, the exact mechanism facilitating this virus's effect on circadian rhythm and checkpoints is still unknown and has not been thoroughly investigated, and many ambiguous aspects remain in this field (Ekhtiari et al., 2023; Gudo et al., 2015).

The enormous complexity of this pathway and the numerous involved genes have made examining affected proteins daunting. To solve this problem, it seemed necessary to use bioinformatics methods, including Boolean analysis. To the best of our knowledge, this research is the first study to investigate the circadian rhythm signaling pathway in the presence of HTLV-1, confirmed through Boolean analysis in asymptomatic carriers and healthy individuals.

2. Materials and methods

This research was conducted in two stages. The first stage involved a thorough analysis of the circadian rhythm pathway and the potential sites of impact from HTLV-1 on this pathway. In this step, we utilized a Boolean analysis using the BoolNet package of the R programming language to investigate the exact relation between different genes and estimate each one's effect on the outcome.

Next, to confirm the proposed results, the expression level of suggested genes from the first step was measured using qRT-PCR among HTLV-1 asymptomatic carriers and normal subjects.

2.1. Determination of circadian rhythm pathway

A systematic search was performed in the Scopus, Web of Science, and PubMed databases to determine the circadian rhythm pathway and the relationships of the involved genes. Then, using the KEGG database information, the preliminary path was drawn. In the next step, the possible action sites of HTLV-1 on the circadian rhythm pathway were determined according to the obtained data.

2.2. Boolean analysis

The variety of genes involved in this pathway and their complicated connections make it difficult to analyze it using old methods. To conquer this issue, we use the Boolean model, which has been approved for its accuracy in previous research (Mardi et al., 2024; Wang et al., 2012)In this method, each gene's activity is reduced to "on" and "off" states, and their influence on each other is limited to activation and inhibition. Based on these two assumptions, we are able to detect all possible states of pathways and predict genes' activity in different situations.

To do so, we use the BoolNet package of R programming language. First, to provide this information to the computer, each gene was translated as a node, and the relation between them was presented as a code. At this stage, a node was defined for each gene, and their relations were coded to the R programming language. After the processing, the software suggested all possible states as multiple attractors. These attractors represent a stable state, which could be regarded as different clinical manifestations. Obtained attractors were reviewed several times, and the most appropriate map and coding that justifies former research and the behavior of the pathway in the normal state were considered. Finally, according to activation or inactivation of light-related (night) and viral-related nodes (Tax and HBZ), each attractor was linked to a clinical manifestation.

2.3. Robustness analysis

Biological networks should have more structural strength against disturbances than random networks. To investigate our pathway's robustness against mutation, we performed a robustness analysis using the BoolNet package. This function generates a set of initial states and then creates disordered copies of these states by randomly sending bits. It randomly generates 100 states and 100 copies with one IP bit and performs a single-state transition for each state. It then measures the normalized Hamming distance (fraction of different bits) between each state and the corresponding perturbed copy. The primary assumption is that a strong network creates a low Hamming distance.

2.4. Laboratory analysis

In the second step of this project, the expression level of these genes among healthy people and asymptomatic carriers was measured to evaluate and confirm the role of the proposed genes.

This study was approved by the ethics committee of Alborz University of Medical Sciences (IR.ABZUMS.REC.1402.181) and conducted ethically according to Alborz University of Medical Sciences ethical standards. All study methods complied with relevant guidelines and regulations, and each participant was given signed informed consent.

2.5. Sample collection

In this study, with a standard deviation of 6, a confidence interval of 95 %, and a test power of 80 %, the relevant formula estimated the number of participants in each group to be ten. Accordingly, ten patients recently diagnosed with HTLV-1 infection were enrolled in the asymptomatic carrier group. On the other hand, ten healthy participants from the blood transfusion organization of Alborz province were selected for the control group after matching their age and sex. All participants met specific criteria, including the absence of immunodeficiency, genetic, or inflammatory disorders and no usage of any particular medications. Additionally, the samples tested negative for blood-borne diseases such as HBV, HCV, and HIV.

After providing complete explanations regarding the process of this research study and ensuring the confidentiality of the obtained information, written consent was obtained from them to participate in this study. Then, six to ten milliliters of blood were taken in an EDTA-containing tube.

2.6. RNA extraction and cDNA synthesis

The RNA was extracted using the RNjia kit manufactured by ROJE following the manufacturer's instructions. Subsequently, cDNA was synthesized using 5 μl of the extracted RNA and 1 μl of random hexamer primers with the RT-ROSET Kit (ROJE, Iran) as per the manufacturer's guidelines. RNA concentrations were measured spectrophotometrically at 260/280 nm using a NanoDrop spectrophotometer after eluting the NanoDrop column with 100 μl of H2O, and cDNA production was verified using the RPLP0 gene.

2.7. Primer design and qRT-PCR

The following primers were designed and utilized to measure the expression levels of PER2, DEC1, CRY1, RPLP0, HBZ, and TAX. PER2 (forward primer: 5'-CAGCCTCAGTTTCCGAGCCA-3', reverse primer: 5'-GACTGAAAGAGCGGTGGGGA-3'), DEC1 (forward primer: 5'-CACGGACGCAGGTTCACGAT-3', reverse primer: 5'-GGCAGAAAGGAGGCTGGTGT-3'), CRY1 (forward primer: 5'-TGGGCAACTGTTATGGCGTGA-3', reverse primer: 5'-CCCTCCTGACGAAGCTGTGT-3'), RPLP0 (forward primer: 5'-GACAAAGTGGGAGCCAGCGA-3', reverse primer: 5'-ACACCCTCCAGGAAGCGAGA-3'), HBZ (forward primer: 5'-ACGTCGCCCGGAGAAAACA-3', reverse primer: 5'-ACGTCGCCCGGAGAAAACA-3'), TAX (forward primer: 5'-AGCACCTCCAACCCTGTCT-3', reverse primer: 5'-CAGGTGATGGGGGGGGAAAG-3'). The qRT-PCR was performed on the cDNA samples using a SYBR Green master mix (Takara, Otsu, Japan) and a Q-6000 machine (Qiagen, Germany). The RPLP0 gene was used as a housekeeping gene to normalize the mRNA expression levels and to control for errors between samples.

2.8. Statistical analysis of data

Statistical analysis was conducted using GraphPad Prism Software Version 7 (GraphPad Software, Inc.). Quantitative data were expressed as mean ± SD and percentages. Comparisons between different groups were performed using Mann–Whitney U test. Pearson's or Spearman's tests were utilized to analyze the correlation between variables. Results were considered significant if $P \leq 0.05$.

3. Results

3.1. Circadian rhythm signaling pathway

Various studies on the circadian rhythm pathway and conducted studies on HTLV-1 stated that Tax protein could form hetero- and homodimer complexes with CREB and increase the expression of some genes. Using CREB, Tax attaches to 21-bp enhancer regions and increases the efficiency of that region in HTLV-1 (Adya and Giam, 1995; Zhao and Giam, 1992; Yoshida, 1995). CREB is a general transcription-activating factor that controls and regulates more than 4000 genes (Steven et al., 2020) and, through the MAPK/ERK signaling pathway, regulates and controls the PER 1–2 (Travnickova-Bendova et al., 2002; Motzkus et al., 2000).

The circadian rhythm pathway includes a transcription-translation feedback loop that converges on BMAL1 and CLOCK. Whose transcription activators include BMAL1 and CLOCK, and its repressors include PER 1, 2, 3, and CRY 1 and 2. BMAL1/CLOCK heterodimers also induce the transcription of REVERBα/β and RORα nuclear receptors, which form lateral repressor and activator loops, respectively (Huang et al., 2023; Hand et al., 2020; Nobis et al., 2019; Fortier et al., 2011). The combination of information obtained from this pathway is summarized in Fig. 1. Also, the translation of these relations to R programming language codes is provided in Table 1

3.2. Robustness against disruption

We measured the distance between the two successor states using normalized Hamming distance. This test was repeated for 100 randomly selected states of the Boolean network model, and the mean normalized Hamming distance was determined. Additionally, we conducted this test on 1000 randomly generated networks. As shown in Fig. 2, the

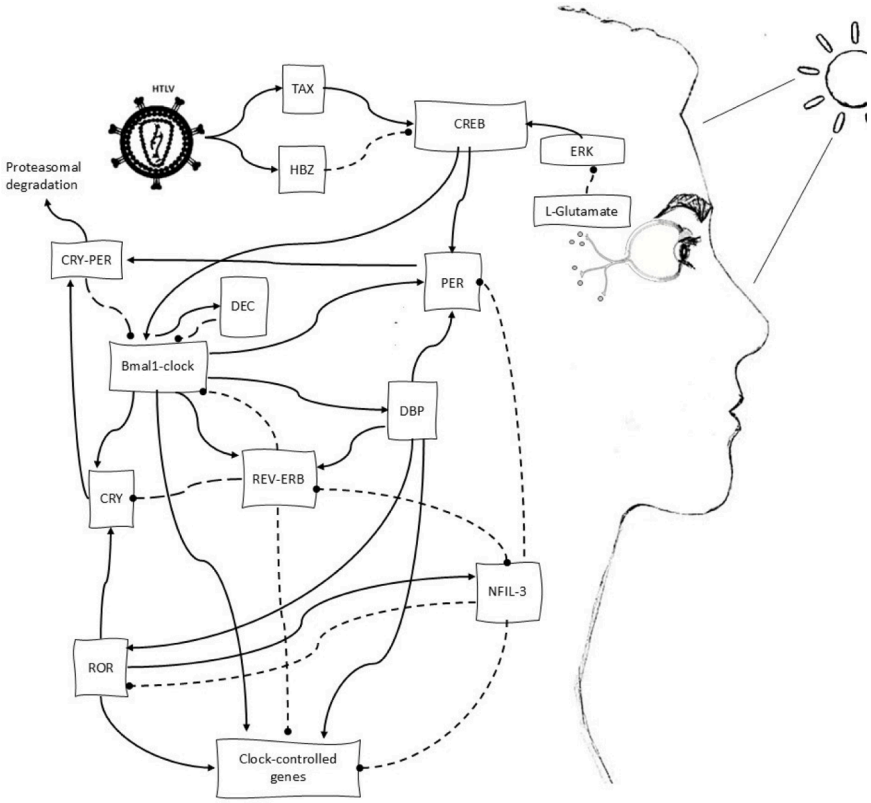

Fig. 1. HTLV-1 effect on circadian rhythm pathway.
Circadian rhythm signaling is simplified and reduced to its most essential nodes. Black lines represent the interaction between two molecules. Arrowheads (>) represent activation, and circle-headed (—•) arrows represent inhibition.

Table 1
The circadian pathways involved Genes and their relations.

Targets	Boolean Function	Reference
CREB	(Night | Tax) & ! (HBZ)	(Arnulf et al., 2002; Lemasson et al., 2007)
BMAL1_CLOCK	(CREB) & ! (PER_CRY & REV_ERB & DEC)	(Busino et al., 2007; Hergenhan et al., 2020; Shearman et al., 2000; Finger and Kramer, 2021)
PER	(BMAL1_CLOCK | DBP | CREB) & ! (PER_CRY & NAFIL3)	(Busino et al., 2007; Hergenhan et al., 2020; Finger and Kramer, 2021)
CRY	(BMAL1_CLOCK | ROR) & ! (PER_CRY & REV_ERB)	(Busino et al., 2007; Hergenhan et al., 2020)
DEC	BMAL1_CLOCK	(Kato et al., 2014)
DBP	BMAL1_CLOCK	(Ripperger and Schibler, 2006; Takahashi, 2017)
NFIL3	ROR & !REV_ERB	(Finger and Kramer, 2021; Kato et al., 2014; Patke and Young, 2020; Chen et al., 2018; Gachon et al., 2004)
REV_ERB	(BMAL1_CLOCK | DBP) & ! (NFIL3)	(Hergenhan et al., 2020; Finger and Kramer, 2021; Cho et al., 2012)
ROR	(DBP) & ! (NAFIL3)	(Finger and Kramer, 2021; Patke and Young, 2020; Chen et al., 2018)
CCG	(BMAL1_CLOCK | DBP | ROR) & ! (NFIL3 | REV_ERB)	(Finger and Kramer, 2021; Patke and Young, 2020)

Abbreviations used: CREB: cAMP response element binding protein, BMAL1: Brain and muscle Arnt-like protein-1, PER: Period Circadian Regulator 2, CRY: Cryptochrome 1, DEC: Human differentiated embryo chondrocyte 1, DBP: Albumin d-site-Binding Protein, NFIL3: Nuclear Factor, Interleukin 3 Regulated, REV-ERB: nuclear receptor subfamily 1 group D member 1, ROR: RAR-related orphan nuclear receptor, CCG: Circadian clock genes.

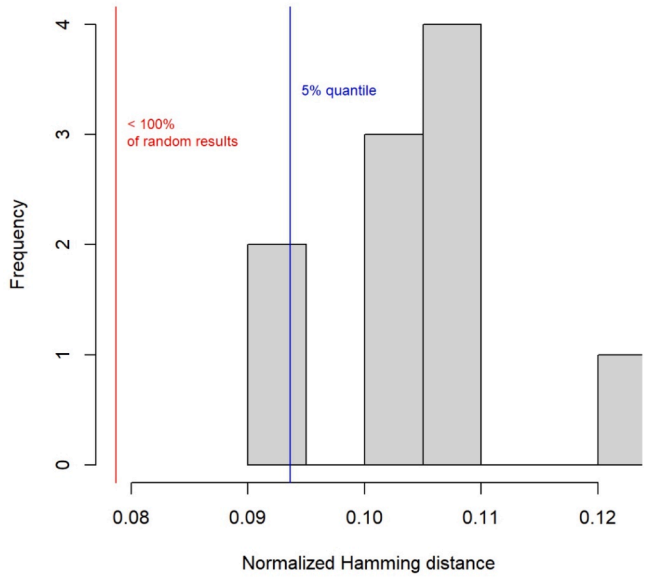

Fig. 2. Transition robustness.
One hundred randomly drawn states of the circadian rhythm pathway were muted by bit flip, and their successor states were computed. The successor states of the mutated and original states were then compared using the normalized Hamming distance (red line). The same was done for 100 randomly generated networks of the same size (histogram).

suggested pathway for the impact of HTLV-1 on Circadian Rhythm displayed a statistically significant Hamming distance of 0.075 (P-value >0.001), indicating its robustness to perturbation (Fig. 2).

3.3. Attractors of the circadian rhythm pathway

The presented path analysis (Fig. 3) showed five single-state attractors and three attractors with four states. According to the activity or inactivity of Tax, HBZ, and light nodes, the attractors have been assigned to different clinical states. Attractors A, C, E, and G represent pathway activity at night, and attractors B, D, F, and H show circadian rhythm state at the time. Also, studies claimed that the expression of Tax and HBZ follows a periodic pattern, and HTLV-1 infected patients have factual expression levels of these two proteins over time. Considering this, we assumed that attractors C, E, and G represent the circadian rhythm pathway in HTLV-1 infected patients at night, and attractors D, F and H show the circadian rhythm pathway state in HTLV-1 infected patients in the daytime.

Attractors A and B show the activity of this pathway in the absence of viral genes (Tax and HBZ). Accordingly, we assumed these two attractors to be the pathway states in healthy cells. Attractor A demonstrates the pathway state in healthy cells at night. As expected, the entire pathway remains inactive without light or intervening factors.

On the other hand, attractor B shows the pathway state during the day. At the start of the night, the MAPK signaling pathway activates, which leads to the activation of CREB. This leads to the attachment of CLOCK-BMAL1 to the E-box and the transcription of CCGs, PER2, and CRY1. These two molecules form a heterodimer molecule that inhibits BMAL1-CLOCK at the dawn. The activation of CCGs at night enables further relevant pathways to circadian rhythm.

Comparing attractors C and D suggests that the sole activation of Tax inhibits the effect of light on this pathway, and its presence leads to the transcription of CCGs in the daytime. On the other hand, the sole activation of HBZ inhibits the whole path, even at night. Therefore, no clock-control genes are transcripted in these patients. Finally, the co-activation of both viral genes, as presented in attractors G and H, can have a similar effect and block all nodes.

3.4. Laboratory findings

In the last step, we measured the PER2, DEC1, and CRY1 expression levels in the healthy group and asymptomatic carriers to approve the suggested pathway and attractors. The mean age of participants in the healthy control group was 53.2 ± 7.32; in the asymptomatic carriers, it was 59.9 ± 3.1. Also, there were five males in each group.

The expression of PER2 was 0.004378 ± 0.003020 in the control group, which was significantly lower than its expression in asymptomatic carriers (0.1681 ± 0.2303, P-value =0.0014). Similar results were recorded for the expression of DEC1. The expression level of this gene in asymptomatic carriers was 0.3153 ± 0.5453, significantly higher (P-value =0.0044) than control group 0.05132 ± 0.04315. in contrast, the expression of CRY1 had no significant variation in asymptomatic carriers and control group (P-value =0.1457, 0.1701 ± 0.1945 vs 0.07134 ± 0.05875) (Fig. 4).

Conducting the Spearman correlation test reveals that HBZ expression has a negative correlation with CRY1 (Spearman: −1.00, P-value< 0.0001) and DEC1 (Spearman: −0.73809, P-value= 0.045). On the other hand, the expression level of DEC1 had a positive correlation with CRY1 (Spearman: +0.73909, P-value= 0.046) and PER2 (Spearman: +0.7619, P-value= 0.036) (Fig. 5).

4. Discussion

Life on Earth has evolved in a very rhythmic environment. To adapt to this matter, living organisms have circadian rhythms that consist of cycles with fluctuations of almost 24 h; this system coordinates immunity, nervous, endocrine, cardiovascular, metabolic, and other biological systems (Ortega-Campos et al., 2023; Du et al., 2023).Recent studies have suggested that the disruption of this system has a crucial role in the physiopathology of various diseases, including viral infections like

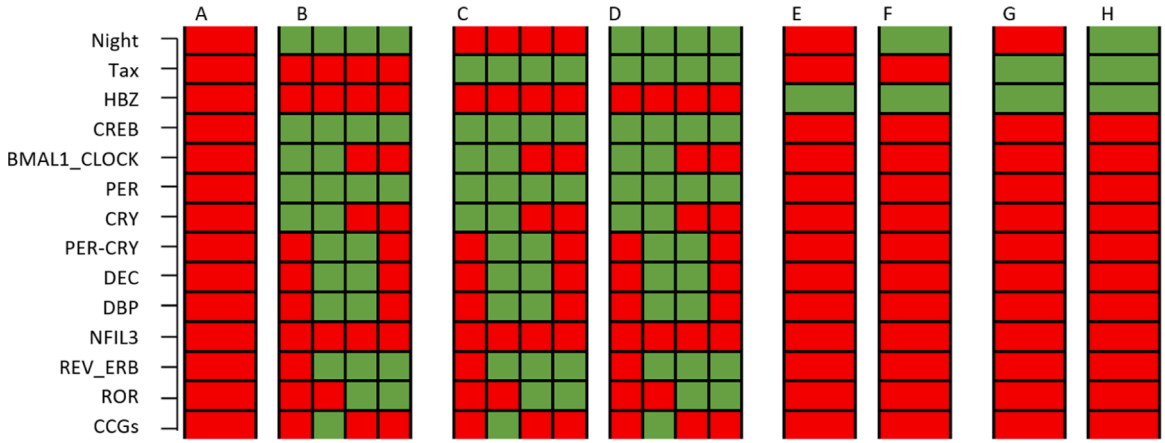

Fig. 3. Attractors of the circadian rhythm pathway with HTLV-1 infection.
An exhaustive attractor search of the circadian rhythm pathway yielded five single-state attractors and three four-state pathways. The frequency of occurrence of each attractor is 12.5 %. Each block represents an attractor. The nodes are listed on the y-axis. Each rectangle symbolizes the state of a node: red indicates inactivity, and green indicates activity.

HTLV-1. In this study, by both bioinformatic and laboratory methods, we proposed a novel pathway in which HTLV-1 can alter the circadian rhythm. Our extensive literature search proposed a novel pathway in which HTLV-1 viral genes (Tax and HBZ) can affect circadian rhythm (Fig. 1). Next, our Boolean analysis demonstrated that the viral genes could disrupt the normal functions of this path in both day and night; it also suggested that PER2, CRY1, and DEC1 are acting as this pathway's main checkpoints, and their activation or inactivation can alter the overall results of the path. Finally, to confirm these results, we measure the expression level of these three genes in healthy and asymptomatic carriers of HTLV-1. Our laboratory analysis demonstrates a significantly higher expression of DEC1 and PER2 in asymptomatic carriers. Also, HBZ expression had a negative correlation with CRY1 and DEC1.

As discussed earlier, in the first step, we extensively search HTLV-1 and circadian rhythm pathways and merge them (Fig. 1). As demonstrated in Fig. 1, in addition to all tissues and cells, a central clock is located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus (Persson, 2019). It consists of transcriptional/ translational feedback loops regulated by genes. After transcription of CRY1 and PER2 genes, PER2 and CRY1 form a complex in the cytoplasm, re-enter the cell nucleus, and suppress their transcription by suppressing the BMAL1/-CLOCK heterodimer complex as negative feedback. With the destruction and consumption of PER2 and CRY1, the BMAL1/CLOCK complex is activated again, which starts a new cycle. Also, there are additional loops, including stabilizing loops (ROR, REV-ERBs) and auxiliary loops (DEC1, NFIL3, DBP), which are necessary for the precise and rhythmic regulation of the cell clock (Dintwa et al., 2023). According to this biological logic, we ran a Boolean analysis that proposed Fig. 3-A and B attractors, showing the circadian rhythm's day and night time activity in healthy people. Confirming this, in 2021, Lee revealed that the expression of PER2 and CRY1 increases at night, inhibiting the BMAL1/CLOCK heterodimer complex (Lee, 2021).

In the next step, we added HTLV-1 viral genes to predict pathway activity in infected patients. Tax and HBZ bind to the transcription factor CREB and change the transcription of the CRE sequences. This process alters the expression of several genes and cellular pathways, including migration, phagocytosis, metabolism of immune cells, activation of signaling pathways, inflammatory responses, recognition of innate immunity, and immune adaptation processes (Ding et al., 2024). This, along with our results, suggests that these two viral genes dysregulate the circadian rhythm, which can make infected patients susceptible to HTLV-1-related diseases like ATLL and HAM/TSP.

To test our hypothesis, we compare the activity rate of each node between healthy and asymptomatic patients. This comparison suggested three genes, PER2, DEC1, and CRY1, as this pathway's main checkpoints, which was later confirmed by laboratory methods. Our results demonstrate higher expression of DEC1 and PER2 in infected patients and a negative correlation between HBZ and CRY1 expression. Further research should investigate These three genes as diagnostic or therapeutic targets in HTLV-1 infection.

DEC1 's role in this pathway was similar to its vital functions in various cellular pathways, including immunity against viral infections and chronic inflammatory diseases (Chakrabarti et al., 2004). For instance, Li et al., in 2022, conducted a study on 118 participants with metabolic syndrome and observed that these patients have higher expression levels of DEC1 and BMAL1 (Li et al., 2022). Also, many investigations have revealed the link between this gene and some malignancies, such as oral squamous cell carcinoma. For instance, in 2020, Mao et al. reported significantly higher gene expression in oral leukoplakia and oral squamous cell carcinoma (Mao et al., 2020). Likewise, a survey in 2019 stated that patients with non-small-cell lung carcinoma are more likely to have a circadian rhythm and DEC1 disruption. They also showed that a higher level of DEC1 is associated with poor overall survival of these patients (Qiu et al., 2019). In line with these results, some studies suggest its role in breast cancer metastasis, thyroid cancer invasion, and colon cancer progression (Gallo et al., 2018; Ehata et al., 2007; Li et al., 2002). These results are more notable considering the carcinogenic nature of HTLV-1 and its progression to ATLL. This gene overexpression may be one of HTLV-1′s pathways for progression to ATLL, as in an in vitro study, Sato et al. demonstrated the critical role of this gene in the pathogenesis of HPV as an oncogenic virus. They also showed that combination therapy with cisplatin inhibits apoptosis and expression of DEC1, SOX2, and c-MYC in cells (Sato et al., 2020).

Our result also enlightens the vital role of PER2 in this infection. PER2, discovered by Konopka and Benzer in 1971, plays a crucial role in circadian rhythms in such a way that studies have shown that its mutation in mice leads to a shorter circadian period than in wild-type mice (Kim et al., 2018). Also, its disruption has been linked to various types of cancer. As reported in a review study by lymphoma, teratoma, liver, lung, and ovarian cancers (Lee, 2021). Also, Guo et al. conducted an in vivo study to investigate the role of circadian rhythm in pituitary tumorigenesis. Similar to our results, they showed that the expression level of PER2 was significantly higher in those with malignancies than in the control group. Their study also found that loss of PER2 protects mice against developing estrogen-induced pituitary adenoma. This raises the hypothesis that this gene can be a promising therapeutic target in preventing HTLV-1 progression to ATLL (Guo et al., 2023). In line with this theory, Ayan et al., by utilizing bioinformatics and laboratory methods

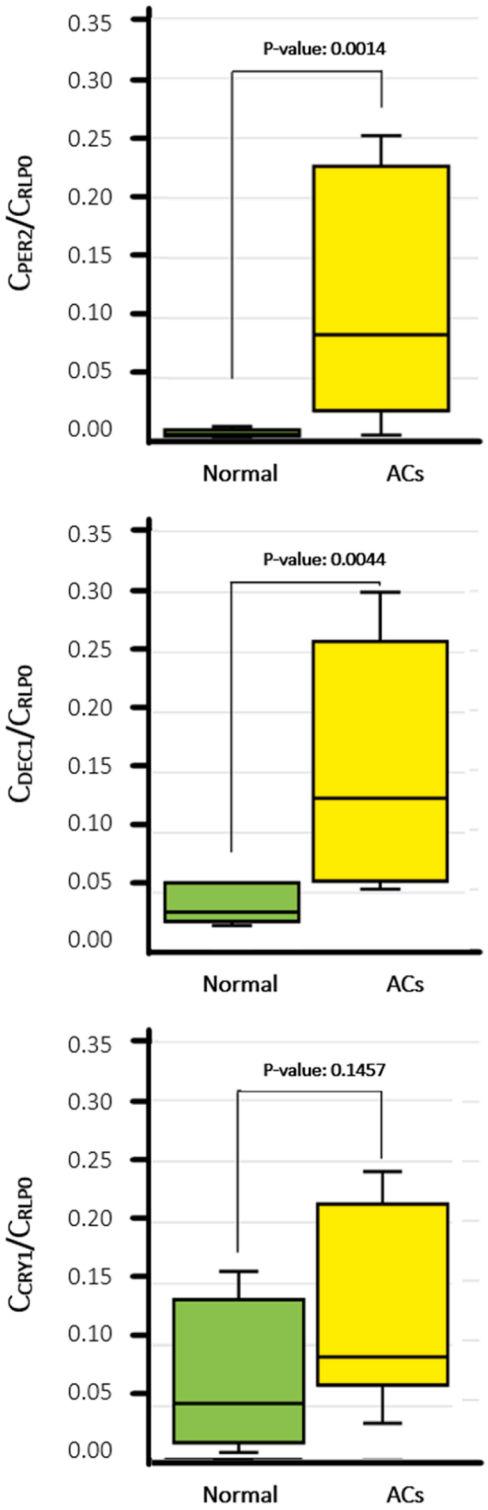

Fig. 4. Expression levels of PER2 (A), DEC1 (B), and CRY1 (C) in the asymptomatic carriers and control groups. The figure demonstrate box plots comparing the gene expression levels of PER2, DEC1, and CRY1 between asymptomatic carriers (ACs) and a control group. Each box represents the distribution of expression levels for a specific gene, with the line inside the box indicating the median value. The provided p-values indicate the statistical significance of the differences in PER2 and DEC1 gene expression between the two groups.

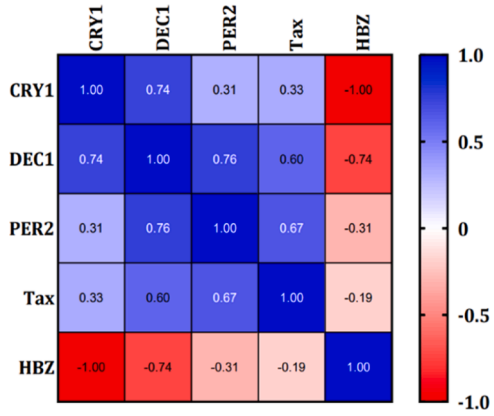

Fig. 5. The associations of viral and circadian rhythm gene expression using Spearman correlation analysis.

on patients with hepatocellular carcinoma, demonstrate that expression levels of PER2 are associated with more prolonged overall survival of these patients (Ayan and Cagatay, 2023).

In contrast to our data, some studies have reported that the expression level of PER2 decreases in some types of cancers. For example, Ma et al. 2020 showed that the expression level of PER2 in glioma stem cells is lower than in non-stem glioma cells (Ma et al., 2020), and Wang et al. showed that isolated neutrophils from CML, ALL, and CLL patients had lower PER2 levels than the control group (Wang et al., 2020). Besides methodological discrepancies (e.g., sampling time), this raises a hypothesis about HTLV-1′s physiopathology. As shown in Fig. 3, the expression of HTLV-1′s viral genes can disrupt the circadian rhythm and normal fluctuations in PER2. Our results suggest that the sole presence of Tax elevates the expression of DEC1 and PER2, and the presence of HBZ, or their co-existence, lowers its transcription. Considering the pulsatile expression of HBZ and Tax in asymptomatic carriers and a higher level of Tax than HBZ in ATLL patients, it can be hypothesized that similar to chronic viral infections like ALL, CML, and CLL in HTLV-1 asymptomatic carriers, the HBZ protein suppresses the DEC1, PER2, and other circadian rhythm proteins to suppress inflammatory responses which can lead to chronic infection and escaping from the immune system. In contrast, a higher level of Tax in some HTLV-1 asymptomatic carriers can lower the expression of DEC1 and PER2, which leads to its progress to ATLL.

Along with notable results, our study had some limitations. Although according to the low prevalence of HTLV-1 and related formula, we enrolled ten patients in each group, by increasing the sample size, the statistical power is increased, the risk of bias is reduced, and it enables a more accurate estimation of the observed effects. Further studies are needed to examine and integrate the upstream and downstream pathways with the circadian rhythm pathway, collect samples in different periods and groups of ATLL and HAM/TSP patients, and examine more genes.

5. Conclusion

Due to the numerous involved genes and their complex relationships, disruption and inconsistency in one of the genes cause disruption and dysregulation of the entire circadian rhythm, which can lead to the development of malignancies. In this study, by utilizing approved bioinformatic and laboratory methods, we demonstrated that HTLV-1 can progress to chronic infections and ATLL by altering this pathway. We also suggest two genes, PER2 and DEC1, that can act as main checkpoints of this path, which can have diagnostic, prognostic, or therapeutic implications.

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Ethical approval

The Ethics Committees of Medical Sciences Research at Alborz University of Medical Sciences, Iran (IR.ABZUMS.REC.1402.181) approved this study.

CRediT authorship contribution statement

Abdollah Amiri: Writing – original draft, Supervision, Investigation, Conceptualization. **Shayan Mardi:** Writing – original draft, Software, Methodology, Formal analysis, Conceptualization. **Atefeh Bahavar:** Investigation, Data curation. **Mohsen Sheikhi:** Investigation, Data curation. **Somayeh Yaslianifard:** Investigation, Data curation. **Sayed-Hamidreza Mozhgani:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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None

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.virusres.2025.199539.

Data availability

The data supporting this study's findings are available upon request from the corresponding author. However, the data are not publicly available due to privacy or ethical restrictions.

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