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ORIGINAL INVESTIGATION

Different roles of CXCR1 and CXCR2 in HTLV-1 carriers and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) patients

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Abstract

One of the prominent features of HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is the excessive recruitment of leukocytes to the central nervous system (CNS), which leads to an inflammatory response—with chemokines and their receptors playing the main role in this recruitment. The aim of the study was to examine the relation of CXCR1 and CXCR2, both of which are involved in the trafficking of lymphocytes into the CNS, with the outcome of HTLV-1 infection. The mRNA levels of CXCR1 and CXCR2 were examined in peripheral blood mononuclear cells (PBMCs) of HAM/TSP patients, HTLV-1 asymptomatic carriers (ACs), and healthy controls (HCs). Furthermore, the frequency of $CD4^+$ and $CD8^+$ T cells expressing CXCR1 and CXCR2 was evaluated in the studied groups. The results of the present study showed a substantial increase in the mean mRNA expression of CXCR2 in the HAM/TSP patients compared to the HCs and ACs ($p < 0.001$). A positive correlation was also found between PVL and CXCR2 mRNA expression in the total population of HTLV-1-infected subjects ($R = 0.526$, $p < 0.001$). Moreover, the percentage of $CD8^+$ CXCR2-expressing cells was higher in HAM/TSP patients compared to ACs and HCs ($p < 0.05$, $p < 0.01$, respectively). Although the percentage of $CD4^+$ CXCR2-expressing cells was higher in HAM/TSP patients than in ACs and HCs, a significant difference was only found between HAM/TSP patients and HCs ($p < 0.05$). No significant difference in the CXCR1 mRNA expression was observed in the studied groups. The frequency of the $CD8^+$ CXCR1- and $CD4^+$ CXCR1-expressing cells was significantly lower in HAM/TSP patients than in ACs and HCs ($p < 0.001$ and $p < 0.01$, respectively). In conclusion, the high frequency of CXCR2 $CD8^+$ T cells and the high levels of CXCR2 mRNA expression in HAM/TSP patients are associated with disease pathogenesis, while the high frequencies of CXCR1 T cells in ACs might suggest that these cells act as effector CD8 T cells and are involved in controlling the viral spread and modulation of the immune response.

Keywords HTLV-1 · HAM/TSP · CXCR1 · CXCR2

Introduction

Human T-lymphotropic virus type 1 (HTLV-1) is an oncogenic human retrovirus that infects human $CD4^+$ T lymphocytes. HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and adult T-cell leukemia (ATL) are the two major diseases associated with HTLV-1 [1].

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Taraneh Rajaei and Hamid Farajifard have the same contribution in the work.

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Approximately 15–20 million people worldwide are infected with HTLV-1 [2], and it is particularly prevalent in Japan, Africa, the Caribbean Islands, Central and South America, and Northeast Iran [3]. It is not fully understood why only a small percentage of HTLV-1-infected individuals go on to develop HAM/TSP while the majority remain lifelong asymptomatic carriers (AC) of the virus [4].

HAM/TSP is a chronic progressive inflammatory disease of the nervous system [5]. Similar to other inflammatory responses, the products of HTLV-1 genomes, particularly Tax, trigger the inflammatory responses, in which immune cells infiltrate the central nervous system (CNS) [6, 7]. Migration of the immune cells to the CNS may lead to neuronal damage and demyelination [8], with these cells mainly consisting of $CD8^+$cytotoxic T lymphocytes (CTL) and monocytes [9].

One of the prominent features of HAM/TSP is the excessive recruitment of leukocytes to the site of inflammation [10]. CXCR1 and CXCR2 are expressed on different cell types, including neutrophils, monocytes, mast cells, and natural killer cells (NKCs) [11]. Furthermore, both $CD8^+$ and $CD4^+$ T cells express CXCR1 and CXCR2, although the frequency of these expressing cells is lower than that of neutrophils [12]. It has been shown that CXCL8 and its receptors (CXCR1 and CXCR2) have pivotal roles in the trafficking of leukocytes into the CNS, neuronal survival, CNS development, and neuro-immune responses [13].

High expressions of CXCR1 and CXCR2 in peripheral blood mononuclear cells (PBMCs) have been found in cases of untreated multiple sclerosis (MS) patients [14, 15]. In addition, it has been reported that the ligands of CXCR1, CXCR2; CXCL8/ IL-8, and CXCL1/GRO-a are absent in the CNS tissue of normal subjects, while they are expressed at high levels on hypertrophic astrocytes at the edge of the active MS lesions [15].

The widespread expression of neuronal CXCR2 has been shown in several regions of the brain and spinal cord [16]. CXCR2 plays a role in inflammation, oligodendroglial biology, and myelin disorders [17] as well as controlling the positioning and timely proliferation of oligodendrocyte precursors in the developing spinal cord [16]. Moreover, it has been argued that CXCR2 signaling is pivotal for neutrophil chemotaxis and oligodendrocyte development [13, 16].

CXCR2 and/or its ligands are upregulated in various types of CNS inflammation, infection, and injuries, such as MS, experimental autoimmune encephalomyelitis (EAE), and Theiler's murine encephalomyelitis virus (TMEV) [18]. CXCL1 and CXCL2, the CXCR2 ligands, are significantly upregulated during the JHM strain of mouse hepatitis virus (JHMV) infection and remain expressed within the spinal cord throughout the chronic infection, even in the absence of the infectious virus. With respect to the presence of CXCR2 and its ligands during numerous CNS inflammatory conditions, a possible role of CXCR2 signaling in protection or disease progression is considered to be feasible. Currently, the functional role of CXCR2 in chronic viral infections and neuroinflammatory disease remains unknown [18]. However, it has been demonstrated that HTLV-1 Tax-specific $CD8^+$ T cells express CXCR1 and CXCR2, suggesting that these cells might be involved in the pathogenesis of HTLV-1-associated diseases [19].

The aim of the present study was to examine the contribution of CXCR1 and CXCR2 in the outcome of HTLV-1 infection. Thus, the mRNA expression and protein levels of CXCR1 and CXCR2 were investigated in peripheral blood mononuclear cells (PBMCs), and the frequencies of expressing CXCR1 and CXCR2 were evaluated in the T cells of HAM/TSP patients, asymptomatic HTLV-1 carriers, as well as healthy controls. Furthermore, the correlation of PVL with the gene and protein expressions of CXCR1 and CXCR2 was evaluated.

Materials and methods

Study population

The study population included 22 patients with HAM/TSP and 22 ACs, who were referred to the Department of Neurology, Ghaem Hospital, Mashhad University of Medical Sciences, and Mashhad, Iran. HTLV-1 infection was screened using an enzyme-linked immunosorbent assay (ELISA) test and confirmed by Western blot. Twenty HCs were recruited from Mashhad Blood Transfusion Center. Viral infections including human immunodeficiency virus (HIV), hepatitis B (HBV), and hepatitis C (HCV) were evaluated in all studied groups. The study was approved by the ethics committee of the Mashhad University of Medical Sciences, Mashhad (Grant no. 89,659), and informed consent was obtained from all participants. All HAM/TSP patients met the diagnostic criteria for the disease, as previously described [20]. The mean age of the HAM/TSP patients was 45 ± 27 (range 22–64 years), of which 63% were women. The mean ages of the ACs and HCs were 41.13 ± 13 (range 25–53 years) and 41.8 ± 12.4 (range 20–60 years), respectively. The majority of the ACs was women (53%), with an even higher proportion in the HCs (80%). No significant differences were found in terms of gender ($p = 0.801$) or mean age ($p = 0.218$) between the three analyzed groups.

Flow cytometric analysis of CXCR1 and CXCR2 surface molecule expression

The expression of surface CXCR1 and CXCR2 in $CD4^+$ and $CD8^+$ T cells was measured using flow cytometry, and immunofluorescence staining was performed according

to the manufacturer's instructions (Biolegend, San Diego, California). Briefly, optimal concentrations of desired fluorochrome-conjugated monoclonal antibodies (FITC anti-CXCR1 and anti-CXCR2, PE anti $CD4^+$ and anti-$CD8^+$, PerCP anti-CD3) (Biolegend, San Diego, California) were added to 100 µL of anti-coagulated whole blood and incubated for 20 min at room temperature, in the dark. The cells were washed with cell-staining buffer, and data were collected on a FACSCalibur (BD Biosciences, San Jose, CA, USA) and analyzed using FlowJo software. Data were analyzed in lymphocyte gate.

Real-time quantitative RT-PCR

Total RNA was extracted from the PBMCs using the Tripure isolation method (Roch Diagnostics GmbH Roche Applied Science, Mannheim Germany), and cDNA was synthesized using the RevertAid™ First-Strand cDNA Synthesis kit (Fermentas, Germany) with random hexamer primers. Relative CXCR1 and CXCR2 mRNA expression levels were quantified by real-time quantitative RT-PCR. Two different primer/probe sets were designed for CXCR1 and CXCR2 based on sequenced data available from the NCBI databases using Beacon Designer software (PREMIER Biosoft International, Palo Alto, CA; version 7) (Table 1). The specificity of oligonucleotides was checked by BLAST analysis (NCBI). Amplification of a single product for each primer set was confirmed by electrophoresis analysis on 2% agarose gel followed by DNA sequencing performed by Applied Biosystems (SEQLAB, Germany).

Quantitative real-time RT-PCR was performed on the cDNA samples using two standard curve techniques with a Rotor-Gene Q Machine (Qiagen, Hilden, Germany). The test was carried out with the Universal Master Mix (Takara, Otsu Shiga, Japan) utilizing the TaqMan method. The PCR conditions were as follows: 95 °C for 10 min, 40 cycles of denaturation at 95 °C for 15 s, and annealing and extension at 60 °C for 45 s. The relative quantity of the gene of interest was normalized to the relative quantity of β2M as a reference gene, and it was reported as the relative gene expression.

HTLV-I proviral load assay

DNA was extracted from PBMCs using a commercially available kit according to the manufacturer's instruction (Blood Mini Kit, Qiagen, Germany). The PVL of HTLV-1 in PBMCs was measured using a real-time PCR utilizing a commercial absolute quantification kit (HTLV-1 RQ, Novin Gene, Iran), and the number of HTLV-I proviruses per 10^4 was calculated [3].

Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 13 (IBM SPSS Inc., Baltimore, MD) was used for statistical analysis of the gathered data. Normality of the data was checked prior to the data analysis.

For the non-parametric analyses, we used the Mann–Whitney *U* test and Kruskal–Wallis test. For the parametric analyses, one-way ANOVA was used. Pearson and Spearman correlation coefficients were used to show statistical dependence or correlation between two variables. Pearson's correlation coefficient was used for parametric analysis when both variables displayed normal distributions, whereas Spearman's correlation coefficient was used for the non-parametric measurement of statistical dependence. *P* values of < 0.05 were considered to indicate statistical significance.

Results

Clinical findings in HAM/TSP patients

The main clinical findings and demographic data of 13 available HAM/TSP patients including clonus (38%), sensory complaints (69%), type of sensory complaints (mostly, tingling, numbness and burning pain in feet and legs), lower extremities' weakness (85%), lumbar pain (23%), lower extremities pain (23%), urinary disturbance (61%), type of urinary disturbance (61%), paraparesis (38%) and tetraparesis (7%) are shown in Table 2.

Table 1 CXCR1, CXCR2 and β2M primers and probe sequences

Gene	Forward	Reverse	Probe
CXCR1	5′-GCTGTTAAGTCACTCTGATCTCTG-3′	5′-GTCCTCTTCAGTTTCAGCAATGG-3	5′AGCTCCTACTGTTGGACACACCTGGC-3′
CXCR2	5′-TCTTCTGGAGGTGTCCTACAGG-3	5′-GAAATCTTCAAAGCTGTCACTCTCC-3′	5′AAAGCCCAGCGACCCAGTCAGGAT-3′
β2M	5′-TTGTCTTTCAGCAAGGACTGG-3′	5′-CCACTTAACTATCTTGGGCTGTG-3′	5′-TCACATGGTTCACACGGCAGGCAT-3′

Table 2 The demographic and the main clinical manifestations of HAM/TSP patients

No.	Gender	Age	Clonus	Sensory complaints	Type of sensory complaint	Lower extremity weakness	Lumbar pain	Lower extremity pain	Urinary disturbance	Tape of urinary disturbance	Paraparesis	Tetraparesis
1	Female	42	Pos	Pos	Numbness	Pos	No	No	Pos	No	Pos	No
2	Female	49	No	Pos	Burning pain in feet and legs	Pos	No	Pos		Overactive bladder, nocturia	No	Pos
3	Female	30	No	No	Burning pain in feet and legs	Pos	Pos	Pos	Pos	Nocturia	Pos	
4	Female	49	No	No		Pos	No	No	No	No	No	No
5	Female	54	Pos	No	Burning pain in feet and legs	Pos	No	Pos	No	No	No	No
6	Female	63	Pos	No	No	Pos	No	No	Pos	Nocturia Urinary incontinence Urinary frequency	No	No
7	Female	56	Pos	Pos	Numbness Burning pain in feet and legs	Pos	Pos	No	No	Nocturia Urinary incontinence Urinary frequency	Pos	No
8	Female	45	No	Pos	Tingling Numbness Burning pain in feet and legs	Pos	No	No	Pos	Nocturia Urinary incontinence Urinary frequency	Pos	No
9	Female	37	No	Pos	Tingling Numbness Burning pain in feet and legs	Pos	Pos	No	Pos	Nocturia Urinary incontinence Urinary frequency	No	No
10	Female	47	No	Pos	Tingling Numbness Burning pain in feet and legs	No	No	No	No	No	No	No
11	Male	57	Pos	Pos	Numbness	No	No	No	Pos	No	No	No
12	Male	35	No	Pos	No	No	No	No		Urinary frequency	No	No
13	Male	55	No	Pos	Tremor numbness	Pos	No	No	Pos	Urinary incontinence Urinary frequency	Pos	No

No negative, *Pos* positive

Increased CXCR2 mRNA expression in HAM/TSP patients

The mean mRNA expressions of CXCR2 in the HAM/TSP patients, ACs, and HCs were 7.11 ± 1.30, 0.57 ± 0.10, and 0.53 ± 0.09, respectively. The CXCR2 mRNA expression in the HAM/TSP patients was significantly higher than that in the ACs ($p < 0.001$) and HCs ($p < 0.001$). However, no significant difference was found in CXCR2 mRNA levels between the ACs and HCs ($p > 0.05$) (Fig. 1).

The mean CXCR1 mRNA expression in the HAM/TSP patients (5.75 ± 0.76) was higher when compared with the ACs (2.71 ± 0.66) and HCs (1.47 ± 0.30); however, no significant differences were observed between the groups ($p > 0.05$) (Fig. 2). Furthermore, the mean ratio of CXCR1/CXCR2 mRNA expression was evaluated (Table 3). This ratio was found to be significantly higher in the ACs than in the HAM/TSP patients ($p < 0.001$).

Increased frequency of CXCR2 CD8$^+$ T cells in HAM/TSP patients

The mean percentages of CD4$^+$ CXCR2 T cells (CXCR2-positive T-helper cells) in the HAM/TSP patients, ACs, and HCs were 5.62 ± 1.80, 3.88 ± 2.11, and 1.16 ± 0.89, respectively (Fig. 3). The mean percentage of CD4$^+$ CXCR2 T cells was higher in HAM/TSP patients than in ACs and HCs; however, no significant differences were observed between the HAM/TSP patients and ACs or between the HCs and ACs ($p > 0.05$ and $p > 0.05$, respectively) (Fig. 3d).

The mean percentages of CD8$^+$ CXCR2 T cells (CXCR2 positive CTLs) in the HAM/TSP patients, ACs, and HCs were 10.18 ± 6.89, 5.87 ± 3.09, and 1.14 ± 0.95, respectively (Fig. 3). The mean percentage of CD8$^+$ CXCR2 T cells was higher in the HAM/TSP patients than in the ACs and HCs ($p < 0.05$ and $p < 0.01$, respectively). Furthermore, the mean percentage of CD8$^+$ CXCR2 T cells was significantly higher in HTLV-1 ACs than in HCs ($p < 0.05$) (Fig. 3d).

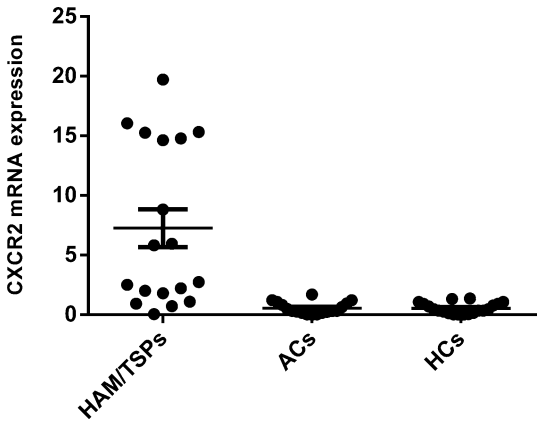

Fig. 1 The CXCR2 mRNA expression in healthy controls (HCs), HTLV-1 asymptomatic carriers (ACs) and HAM/TSP patients. CXCR2 mRNA expression was higher in the HAM/TSP patients than in the ACs ($p < 0.001$) and HCs ($p < 0.001$). No significant difference in the CXCR2 mRNA levels was observed between ACs and HCs. The results are expressed as the mean ± SEM values

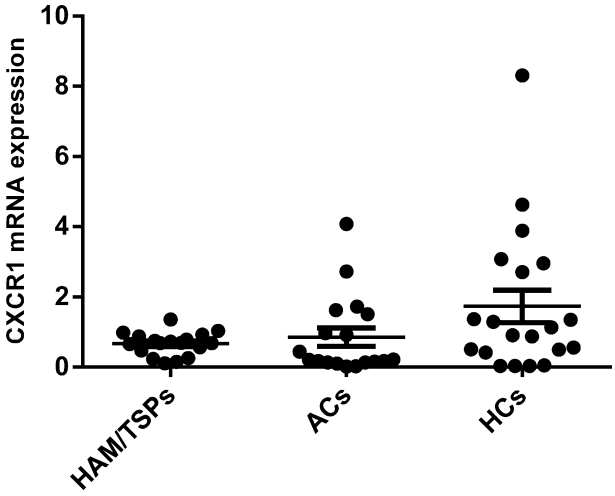

Fig. 2 The CXCR1 mRNA expression in healthy controls (HCs), HTLV-1 asymptomatic carriers (ACs) and HAM/TSP patients; no significant differences in CXCR1 mRNA expression were observed between groups ($p > 0.05$). The results are expressed as the mean ± SEM values

Increased frequencies of CXCR1 CD8$^+$ T cells and CXCR1 CD4$^+$ T cells in asymptomatic carriers

The mean percentages of CD4$^+$CXCR1 T cells (CXCR1-positive T-helper cells) in the HAM/TSP patients, ACs, and HCs were 1.30 ± 0.44, 3.94 ± 2.60, and 1.60 ± 1.32, respectively (Fig. 3). The mean percentage of CD4$^+$CXCR1 T cells was higher in the ACs than in the HAM/TSP patients and HCs ($p < 0.01$ and $p < 0.01$, respectively). However, no significant differences were found in the mean percentages of CD4$^+$CXCR1 T cells between HAM/TSP and HCs (Fig. 3d).

The mean percentages of CD8$^+$CXCR1 T cells (CXCR1 positive CTLs) in the HAM/TSP patients, ACs, and HCs were 1.81 ± 0.50, 5.16 ± 2.09, and 1.43 ± 1.07, respectively (Fig. 4). The mean percentage of CD8$^+$CXCR1 T cells was higher in the ACs than in the HAM/TSP patients and HCs ($p < 0.001$ and $p < 0.001$, respectively). There were no significant differences in the mean percentage of CD8$^+$CXCR1 T cells between the HAM/TSP patients and HCs (Fig. 3d). Furthermore, the ratios of CD4$^+$CXCR1/CD4$^+$CXCR2 T cells and CD8$^+$CXCR1/CD8$^+$CXCR2 T cells were evaluated. The mean ratio data are presented in Table 3. The mean ratio of CD4$^+$CXCR1/ CD4$^+$CXCR2 T cells was significantly higher in the ACs than in the HAM/TSP patients and HCs ($p < 0.01$ and $p < 0.01$, respectively). In addition, the

Table 3 The mean ratio of CXCR1/CXCR2 mRNA expression, $CD4^+CXCR1/CD4^+CXCR2$ T cells and $CD8^+CXCR1/CD8^+CXCR2$ T cells

	CXCR1/CXCR2 mRNA expression ratio	$CD4^+CXCR1/CD4^+CXCR2$ T-cell ratio	$CD8^+CXCR1/CD8^+CXCR2$ T-cell ratio
HAM/TSP	1.27 ± 0.99	1.22 ± 0.40	0.48 ± 0.10
ACs[a]	4.85 ± 1.83	3.09 ± 0.56	2.56 ± 0.47
HCs[b]	10.10 ± 6.85	1.43 ± 0.56	0.88 ± 0.29

[a]Asymptomatic carriers

[b]Healthy controls

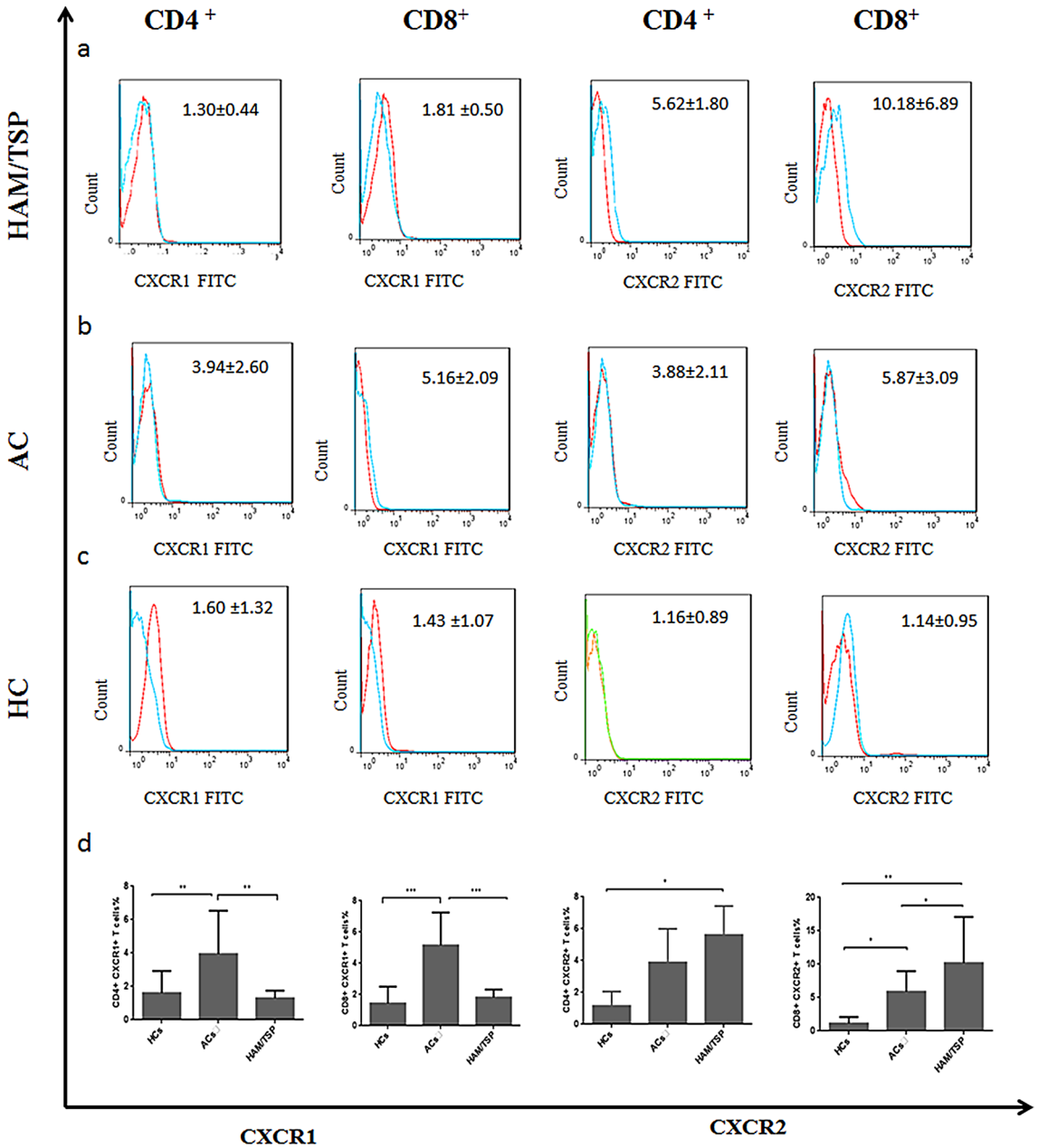

Fig. 3 The CXCR1 and CXCR2 expressions were measured by flow cytometry from 10,000 events gated on live CD3 + CD8+ (CTLs) and CD3 + CD4+ (T helper) cells. The CXCR1 and CXCR2 expression in Th cells and CTLs in HAM/TSP patients (**a**), HTLV-1 asymptomatic carriers (ACs) (**b**), and healthy controls (HCs) (**c**) in comparison with isotype-matched control. The mean percentage of CD4 + CXCR1, CD8 + CXCR1, CD4 + CXCR2 and CD8 + CXCR2 in cells in HAM/TSP patients, ACs and HCs (**d**)

mean ratio of $CD8^+CXCR1/CD8^+CXCR2$ was higher in the ACs than in the HAM/TSP patients and HCs ($p<0.001$, $p<0.01$ respectively).

PVL assessment

The HTLV-1 PVL in the HAM/TSP patients was significantly higher than that in the ACs (469.38 ± 87.36 vs. 53.38 ± 9.5; $p<0.001$) (Fig. 4).

Relationship between CXCR1, CXCR2 expression, and PVL

In the present study, a positive correlation was found between PVL and CXCR2 mRNA expression in the total population of HTLV-1-infected subjects ($R=0.526$, $p<0.001$). Furthermore, PVL was negatively correlated with the mean percentage of $CD8^+CXCR1$ T cells ($R=-0.487$, $p<0.\ 01$) and the mean percentage of $CD4^+CXCR1$ T cells ($R=-0.433$, $p<0.\ 01$) in the total population of HTLV-1-infected subjects.

Association of clinical data and laboratory findings

The study findings showed that there was a positive association between CD4CXCR1 with tremor ($p=0.04$) and sensory complaints ($p=0.03$). CD8CXCR1 had strong association with sensory complaints and lower extremity weakness ($p=0.01$ and $p=0.04$), respectively. Furthermore, viral load had relation with sensory complaints ($p=0.029$) and feet and leg pain ($p=0.023$) and HBZ expression was associated with clonus ($p=0.03$) and tetraparesis ($p=0.02$). The inflammatory cells, neutrophil, also had relationship with urinary disturbance ($p=0.04$).

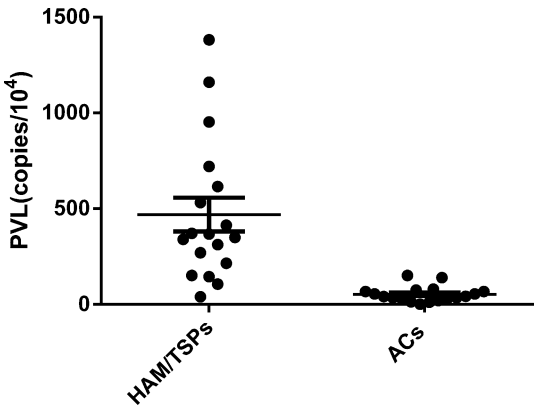

Fig. 4 HTLV-1 DNA PVL expressed as the number of copies/10^4 PBMCs was assessed in the HAM/TSP patients and ACs. HAM/TSP patients have a higher PVL ($p<0.001$) than ACs. The results are expressed as the mean ± SEM values

Discussion

The highly enhanced numbers of HTLV-1-specific CTLs in PBMCs and CSF is considered to be one of the most significant features of the cellular immune responses in patients with HAM/TSP [21, 22]. Furthermore, it has been reported that the frequency of HTLV-1 Tax-specific CTLs is notably higher in CSF than in PBMCs in HAM/TSP patients [21]. Many studies have strongly suggested that the infiltrating CTLs in the CNS produce several inflammatory markers, such as TNF-α, IFN-γ, monocyte inflammatory protein (MIP)-1α, MIP-1β, IL-16, and MMP-9 [9]. Moreover, HTLV-1-specific CTLs target the HTLV-1-infected $CD4^+$ T cells, leading to bystander damage in the CNS [23, 24].

Previous studies demonstrated that the median age of HAM/TSP patients in Iranian (43 years) compared to Japanese (57.3 years) and Brazilian (54 years) was lower in these populations [25]. The most clinical onsets of Japanese HAM/TSP patients were numbness of lower legs (13%), gait impairment (65%), urinary disturbance (33%), and back pain (9%) [25], while in Brazilian patients lower limbs (100%), motor disability (73%), low back pain (62.5%), sensory complaints (52.3%), hand numbness (35%), foot numbness (23.9%), and urinary problems (11%) [25]. In the present study, the most clinical findings in HAM/TSP patients were lower extremity weakness (85%), sensory complaints (69%), urinary disturbance (61%), clonus (38%), lumbar pain (23%), lower extremity pain (23%), paraparesis (38%) and tetraparesis (7%). Taken together it seems that spasticity is a more common clinical onset in Iranian and Brazilian patients, while in Japanese gate impairments and urinary disturbance. Differences in clinical development and progression of HAM/TSP might be due to viral virulence factors such as proviral load, HTLV-I subgroups, Tax and HNX expressions, HLA background and routes of transmission [25].

It is well known that during neuroinflammation, chemokines and their receptors play critical roles in the migration and recruitment of leukocytes from the blood stream into the CNS across the blood–brain barrier [10, 11]. Previous studies have shown the impact of CXCR1 and particularly CXCR2 and their associated ligands in protection and/or disease progression in various types of CNS inflammation and infection, including MS, EAE, JHMV, and Theiler's murine encephalitis virus (TMEV) [14, 15, 26–29]. To the best of our knowledge, no clear evidence exists regarding the contribution of CXCR1 and CXCR2 to the outcome of HTLV-1 infection. As a result, in the present study, the percentages of T cells expressing CXCR1 and CXCR2 and the mRNA expression of these chemokine receptors were evaluated in PBMCs of HAM/TSP patients, ACs, and HCs.

The results of the present study demonstrated substantial increases in CXCR2 mRNA expression in the HAM/TSP patients compared to the HCs and ACs. There was also a positive correlation between PVL and CXCR2 mRNA expression in the total population of HTLV-1-infected subjects.

Upregulation of the CXCR2 and/or its ligands has been demonstrated in different models of CNS infection and inflammation [14, 26–29]. However, the roles of CXCR2 in either repair and/or exacerbation of pathology in these diseases remain controversial. Some findings suggested that CXCL1/CXCR2 mediated a signaling pathway that plays a neuroprotective role during the course of CNS demyelination. Hosking and his colleagues demonstrated that in mice infected with JHMV, the inhibition of CXCR2 signaling by neutralizing antibody delayed clinical recovery as well as increasing the severity of demyelination in the CNS, which is associated with an increase in the number of apoptotic cells in white matter tracts, mainly of oligodendrocytes [18].

Omari et al. also reported a neuroprotective role for CXCR2 during autoimmune demyelination [30]. Their findings demonstrated that during EAE in transgenic mice, the overexpression of CXCL1 resulted in milder forms of EAE, which is associated with a decrease in demyelination and reduced clinical severity [30]. On the other hand, some reports have addressed the contribution of CXCR2 signaling to the pathogenesis of the disease. The effect of CXCR2 signaling on initiating neuroinflammatory demyelinating diseases such as EAE has been studied. Carlson et al. reported that CXCR2 plays an important role in the presentation and relapse of EAE by promoting the accumulation of polymorph nuclear cells (PMN) into the CNS during preclinical and acute stages of disease, resulting in regulation of the permeability of the blood–brain barrier(BBB) [26]. Similarly, Hosking and colleagues showed that the CXCR2 ligands are essential in host defense during acute JHMV-induced encephalomyelitis by attracting neutrophils into the CNS, helping to facilitate the subsequent loss of BBB integrity, which permits the entrance of virus-specific T cells into the CNS [31].

Moreover, other studies on bone marrow chimeric mice suggest that myelin repair was more proficient in $CXCR2^{-/-}$ versus CXCR2+/+ mice [32, 33]. These data might indicate that demyelination and OPC (oligodendrocyte precursor cells) proliferation and differentiation are improved in the absence of CXCR2 activity [34]. Additionally, it has been suggested that the administration of CXCR2 antagonist or a small molecule inhibitor of CXCR2 at onset or during EAE resulted in marked reductions in both the clinical severity and numbers of demyelinated lesions [18, 34, 35].

Based on our results, it seems that upregulation of CXCR2 on T cells, $CD4^+$, and especially $CD8^+$ cells may lead to the infiltration of these cells into the CNS. The increased number of activated T cells infiltrating the CNS serves as an important source of pro-inflammatory mediators, resulting in the dysfunction and death of oligodendrocytes (bystander damage). Although HTLV-I-specific CTL has been considered to play pivotal roles in the elimination of virus-infected cells and decreasing HTLV-I PVL, the increased number of these cells might induce an immunopathology in the CNS, ultimately leading to the HAM/TSP pathogenesis. Further studies are needed to clarify the presence of CXCR2 $CD8^+$ T cells in the CNS and the role of these cells in the outcome of infection with HTLV-1.

In the present study, we also noticed that the CXCR1 mRNA expression was higher in the HAM/TSP patients than in the ACs and HCs. However, we could not find any significant differences among the three groups. The frequency of the $CD8^+$ CXCR1- and $CD4^+$ CXCR1-expressing cells was significantly lower in the HAM/TSP patients than in the ACs. The ratios of CXCR1/CXCR2 mRNA and protein expression were significantly higher in the ACs than in the HAM/TSP patients.

Previous studies have shown that CXCR1 is involved in inflammatory demyelinating diseases such as MS [14], which is consistent with our results suggesting that CD4CXCR1 and CD8CXCR1 are associated with clinical symptoms. Further studies have reported that CXCR1 and CXCR2 are differentially regulated on the cell surface and exhibit different functions. CXCR1 and CXCR2 bind IL-8 to induce a group of equipotent responses, such as phosphor inositol hydrolysis, intracellular Ca2 + mobilization, chemotaxis, and exocytosis, while stimulation of CXCXR1 leads to a respiratory burst in neutrophils, which suggests that these receptors might play different physiological roles in inflammation [36].

It has been argued that the regulation of the surface expressions of CXCR1 and CXCR2 are controlled differently, and following stimulation with IL-8, the expressions of both of these chemokine receptors are reduced. However, removal of IL-8 could lead to the fast re-expression of CXCR1, while the re-expression of CXCR2 is slow and incomplete [37, 38]. Furthermore, it has been shown that CXCR2 is more sensitive to low ligand concentrations and might play a significant role in recruiting neutrophils to the site of infection [39]. Effector HTLV-1-specific $CD8^+$ T cells in HAM/TSP patients express both CXCR1 and CXCR2, and it seems that recruitment of $CD8^+$ CXCXR2 T cells to the CNS contributes to the demyelination process, which is a clinical manifestation of HAM/TSP [19]. Moreover, it has shown that Tax has the ability to transactivate the IL-8 gene through the transcription factors, nuclear factor kappa B (NF-kB), and AP-1 [40]. HTLV-1-infected T-cell lines and fresh ATL cells constitutively express IL-8 [41]. Different cell types secrete IL-8, and this chemokine has potent

chemotactic activity for T lymphocytes and neutrophils [42]. Chaves et al. reported that HAM/TSP patients exhibit high levels of IL-8 and CXCL9 compared to ACs, suggesting that IL-8 might be a promising marker for the clinical management of HTLV-1 infections [43, 44]. However, in Iranian HAM/TSP patients and ACs we could not find any such correlation (unpublished data).

In the present study, we expected that the CXCR1 protein would be expressed more in HAM/TSP patients compared to ACs; however, our findings demonstrated that the CXCR1 protein expression was higher in the ACs than in the HAM/TSP patients. Although we could not examine the expression of IL-8 in this study, it is likely that high levels of IL-8 in the HAM/TSP patients downregulated the protein expression of CXCR1, whereas this issue is less common in ACs. Therefore, with regard to the negative correlation of PVL with the mean percentage of $CD8^+CXCR1$ and $CD4^+CXCR1$ T cells, as well as the higher ratio of CXCR1/CXCR2 mRNA and protein expression in ACs in comparison with HAM/TSP patients, it seems that the high frequency of CXCR1 T cells, especially CD8 + T cells in ACs, could modulate the immune response or control the viral spread. Takata et al. reported that human cytomegalovirus (CMV)-specific CD8 T cells from HCs show cytolytic function and express CXCR1, suggesting that this chemokine is a marker for cytolytic effector $CD8^-$ T cells [45]. It is likely that the high frequency of CXCR1 T cells, especially HTLV-1-specific CD8 + T cells in ACs, exhibits more efficient CTL immune response and control HTLV-1 spread and replication (HA). However, the data regarding the role of this chemokine in the outcome of HTLV-1 infection should be interpreted with caution due to the controversial results obtained.

The limitations of this study were mainly related to the relatively small sample size and the inability to evaluate the CXCR1 and CXCR2 ligands CXCL8, CXCL5, and CXCL6. The assessment of these chemokines and their ligands in blood and CSF would be useful for further clarifying the precise roles CXCR1 and CXCR2 play in the pathogenesis of HTLV-1-associated diseases. Additional studies are needed to clarify the roles chemokines and their receptors play in HAM/TSP pathogenesis.

In conclusion, the high expression of CXCR2 in HAM/TSP patients is implicated in the induction of inflammatory responses into the CNS by increasing the recruitment of HTLV-1-infected $CD4^+$ lymphocytes and specific $CD8^+$ T cells to this milieu, resulting in CNS damage. Furthermore, the high levels of CXCR1 protein expression in ACs may be beneficial in controlling viral spread. These results suggest that the ratio of CXCR1/CXCR2 mRNA and protein expression may serve as a useful biomarker for monitoring the progression of HTLV-1-associated diseases.

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Compliance with ethical standards

Conflict of interest There is no conflict of interest for this study.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee. The study was approved by ethics committee of Mashhad University of Medical sciences, Mashhad (No. 89659).

Informed consent Informed consent was obtained from all individual participants included in the study.

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