JAK2 V617F Mutation in Adult T Cell Leukemia-Lymphoma

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Abstract Adult T cell Leukemia/lymphoma (ATL) is a mature T-cell neoplasm that has strong association with the human T-lymphotropic virus type 1 (HTLV-I) infection. This infection is endemic in our region (north eastern Iran). It has been highlighted that Janus Kinase family proteins and specially JAK2 mutations have a pivotal role in the development of many types of hematological malignancies and in particular myeloproliferative neoplasms. So far, the underlying molecular mechanisms leading to the ATL are not well understood. Therefore, in this study it was hypothesized that JAK2 (V617F) mutation may be present in samples from patients with ATL. This case control study was performed in north-eastern Iran. Using polymerase chain reaction, JAK2 (V617F) mutation was performed in 20 DNA samples from ATL patients and 20 HTLV-1 asymptomatic carriers (control group). The results of ATL subjects and the control group were compared by using SPSS software. In the case group 13 (65 %) and 7 patients (35 %) were male and female respectively, with the age range between 40 and 80 years. Only one patients has JAK2 mutation and this mutation was absent in 95 % of ATL patients as well as the HTLV-1 asymptomatic carriers. The results of our study demonstrated that JAK2 V617F mutation is not a common phenomenon in ATL. However, further studies are required to investigate the possible dysregulation of JAK signaling in ATL.

Keywords JAK2 (V617F) · Adult T-cell leukemia/lymphoma (ATL) · PCR · HTLV-1 · Janus Kinases (JAKs)

Introduction

In the modern pathology, detection of molecular biomarkers in different hematological malignancies including adult T-cell leukemia/lymphoma (ATL) has an important role in the disease classification as well as the treatment strategies [1]. Human T-cell Leukemia Virus Type-1 (HTLV-1) is a member of human retrovirus and about 10–20 million people are infected worldwide. It has been strongly evidenced that HTLV1 is a contributing agent of ATL, a violent T cell lymphoproliferative disorder [2]. HTLV-1 infection remains clinically latent for several years, and it has been reported that up to 10 % of infected patients will develop ATL, which usually occurs after a 40–60 years period of latency [3]. Moreover, very few studies have investigated chromosomal aberrations in ATL [4–6].

The epidemic regions of HTLV-1 infection are in SouthWest, north east of Iran, the Caribbean basin, southern Japan, some in South America, sub-Saharan Africa and Middle East. This infection is majorly widespread in our region (Khorasan province) and mainly in the cities of Mashhad and Neishaboor in north eastern Iran [2, 7]. The prevalence of HTLV-I infection is reported to be 2.12 % in the city of Mashhad (the capital of Khorasan) [8].

It is known that HTLV-1 infection of T lymphocytes is a key for leukemic transformation in ATL, but the molecular mechanisms of this leukemogenesis are not well understood. Although in ATL viral integration shows a clonal

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pattern, HTLV-1 does not contain an oncogene, and also no a proto-oncogene has been found [9]. It is implicated that Jak-Stat pathway is constitutively activated in HTLV-1-transformed cells [10]. The Janus kinase (JAK) family proteins contain four members: JAK1, JAK2, JAK3, and TYK2. These proteins link the cytokine receptors with and activate cytoplasmic transcription factors called STATs (signal transducers and activation of transcription), which shuttle into the nucleus and activate gene transcription. JAK2, couples with the cytoplasmic regions of various cytokine receptors, including those for interleukins, erythropoietin, leptin, interferon- γ and growth hormone. Acquired mutation in JAK2 (V617F) associates with a change of valine to phenylalanine at the position of 617 appears to render hematopoietic precursor cells more sensitive to the growth factors [9, 11, 12]. The JAK2 (V617F) mutation has been identified in different types of myeloproliferative neoplasms (MPNs) such as polycythemia vera (up to 95 %) [13], essential thrombocytosis (68.8 %) [14], primary myelofibrosis (57 %) [15], chronic myelomonocytic leukemia (2–10 %) [16] and in some other subtypes of myelodysplastic syndrome, in addition to few cases of acute leukemia and lymphoma [1].

Detection of JAK2 mutation is helpful in the diagnosis of MPNs, prognosis as well as the prediction of therapeutic response [17]. Currently there are several therapeutic reagents for inhibition of JAK2 in MPNs [18]. Hence, the occurrence of such mutation in ATL may lead to the target therapy in ATL patient in the future. In light of the limited efficacy of standard therapies for ATL and HAM/STP, the current preclinical results with JAK3-targeted therapy offers a potential new therapeutic regimen for such disorders [19, 20]. Therefore, the inhibition of JAK-STAT pathway may provide a new approach for the treatment of ATL. In this study, the presence of JAK2 (V617F) mutation was therefore investigated in ATL patients in an endemic region of Mashhad, Iran.

Materials and Methods

A prospective case-control study was conducted on 20 DNA samples of ATL patients and 20 HTLV-1 asymptomatic carriers (control group) from Ghaem Hospital and Imam Reza Hospital of our university medical center. The study was approved by local ethic committee and also financially supported by the research vice chancellor of the University. Following the diagnosis, blood or bone marrow of ATL patients was taken and sent to the laboratory of molecular hematopathology at Ghaem Hospital. The control samples were obtained from HTLV-1 positive blood donors in Khorasan-Razavi Blood Transfusion Center. In

this center, all donors are routinely screened for HTLV-I antibodies by using enzyme-linked immunosorbent assay (ELISA) and then positive samples were confirmed by using polymerase chain reaction (PCR) or Western blotting.

Medical history and laboratory reports of two groups were perused and two pathologists reviewed the archived slides. All data related to the patients such as lymphadenopathy, splenomegaly, hepatomegaly, complete blood counts (CBC) and hypercalcemia were recorded from their background information files.

DNA Extraction; after obtaining a medical history, 2 milliliters of blood was taken, and was kept in tubes containing Ethylenediaminetetraacetic acid (EDTA-K2) as anticoagulant then Ficoll gradient centrifugation was used to isolate mononuclear blood cells and the DNA was extracted from these cells. DNA concentration was determined applying the Thermo Scientific NanoDrop 2000 Spectrophotometer and samples with low DNA concentration (<20 ng/ μ L) were excluded from the study.

Amplification Refractory Mutation-polymerase chain reaction (ARMS-PCR) for JAK2 mutation was performed according to the previous published methodologies [21]. Mismatches are included to maximize discrimination of the wild-type and mutant alleles. Amplifications were done in 20 μ L reaction volumes containing approximately 100 ng of genomic DNA, 10 mM dNTPs, 50 mM Magnesium chloride (MgCl₂), 1 unit HotStart Taq polymerase (Takara), 2.5 μ L 10X buffer (Cina Gene), 10 pmol for each forward and reverse primers. PCR process was performed using specific primers for JAK2: Forward Outer: 5'-TCC TCA GAA CGT TGA TGG CAG-3', Reverse Outer 5'-ATT GCT TTC CTT TTT CAC AAG AT-3', Forward wild-type specific 5'-GCA TTT GGT TTT AAA TTA TGG AGT ATA TG-3' and Reverse mutant-specific 5'-GTT TTA CTT ACT CTC GTC TCC ACA AAA-3' in Veriti 96 well thermocyclers (Applied Biosystems, USA). Thirty-four cycles of PCR were carried out with denaturing at 94 °C for 30 s, annealing at 60 °C for 45 s and elongation at 72 °C for 45 s. At the end PCR products were electrophoresed on 2 % agarose gel for 45 min (Fig. 1).

Statistical Analysis

Data management and analysis was performed using SPSS V.18 (Statistical software for social analysis-version 11.5). The Kolmogorov-Smirnov test applied to determine of data distribution (the equality of continuous) and then Chi Square and independent sample *t* test were used to compare the categorical variables and mean of continuous variables, respectively. Differences were statistically significant at *P* < 0.05.

Fig. 1 Electrophoresis of amplified PCR products for JAK2 V617F mutation. *Left to right: Lane 1* positive control, *Lane 2 and 4:* Negative controls, *Lane 3* positive results of JAK2 V617F mutation, *Lane 5* size marker, *Lane 6–12* patients with negative results of JAK2 mutation

Results

A total of 20 patients were included comprising 20 ATL patients and the same number of control group. For patients, the age range was 34–78 years and the mean plus standard deviation (SD) of the age in the case and control group was 54.80 ± 12.46 and 55.25 ± 3.65 respectively. There were no significant difference between two groups found by an independent *t* test ($P = 0.87$). In case group 13 patients (65 %) and 7 patients (35 %) were male and female respectively and in control group 12 individuals (60.0 %) and 8 individuals (40.0 %) were male and female respectively. Pearson Chi square test showed no significant differences between case and control group ($P = 0.74$).

No JAK2 mutation band was detected in control samples, while only one of the patients showed DNA band of the corrected molecular weight. By using Fisher's exact test no statistical differences were found between two groups ($P = 1.000$). The JAK positive patient was a 52 years old woman with chronic form of ATLL, symptoms of anemia (fatigue, palpitation and headache), supraclavicular lymphadenopathy, without hepatomegaly and splenomegaly, laboratory results: RBC: $0.3.3 \times 10^{12}/L$, Hb:84 g/L, Plt: $238 \times 10^9/L$, WBC: $7900 \times 10^9/L$, lymph:77.6 % (33 % ATL cells in peripheral blood smear), Serum creatinine:0.7 mg/dL, Urea:27 mg/dL, AST:18 U/L, ALT:23 U/L, Calcium: 10.5 mg/dL U/L, LDH:425 U/L, ALP:107 U/L.

Lymphadenopathy was present in 12 patients (60.0 %), splenomegaly in 6 (30.0 %), hepatomegaly in 5 (25.0 %), skin lesion in 9 (45.0 %), bone marrow involvement in 9 (45.0 %) and hypercalcemia in 7 (35.0 %). In patients group minimum, maximum and mean plus SD of serum calcium level were 7.9, 15.8 and 10.56 ± 2.2 respectively. Table 1 shows the CBC findings of samples from ATL

patients and Table 2 compares the case and control group for laboratory finding.

Discussion

Relationship between viruses and pathogenesis of various benign and malignant tumors remain a concern for physicians [22–25]. JAK/STAT signaling pathway is one of the most important cytokine-signaling pathways regulating T-lymphocyte function. JAK1, JAK2 and JAK3 are expressed in hematopoietic cells and play an important role in the differentiation of these cells [10]. Activation of JAK1 and JAK3 is also observed in HTLV-1 infected T cell lines or in uncultured leukemic cells from ATL patients [26]. The HTLV 1 is relatively common in the general population of our region (northeast Iran) [27] and it is very important that we find the contributing pathway in the pathogenesis of this virus. In the present study 20 ATL patients were investigated in addition to the 20 HTLV-1 carriers to analyze whether JAK2 is present; by using ARMS-PCR method. In the current research, we have demonstrated that there is no association with JAK2 V617F mutation and ATL.

The JAK2V617F point mutation that first described in 2005 is now widely used as a diagnostic marker and major criteria for Philadelphia chromosome negative MPNs [1]. In this study there was no relationship between JAK2V617F and ATL, however; several other studies have shown that the dysregulation of JAK/STAT signaling is strongly associated with different other types of hematopoietic malignancies and solid tumors [18]. Mullighan et al. [28] have demonstrated the activating mutations in the JAKs in 10.7 % of acute lymphoblastic leukemia (ALL) cases, and Kameda and colleagues [29]

Table 1 Show the CBC finding in our patients

CBC index	Minimum	Maximum	Mean	SD
WBC $\times 10^3/L$	1.2	97.7	29.5	27.3
RBC $\times 10^6/L$	3.0	5.0	4.05	0.56
Hemoglobin g/dL	8.7	14.4	11.79	1.77
PLT $\times 10^3/L$	23	442	180.40	103.07
Lymphocyte (%)	7	85	35.38	22.93
Absolute lymphocyte count $\times 10^3/L$	0.72	48.35	10.77	12.84

Table 2 Compared case and control groups for laboratory finding

Variables	Group	Mean	SD $\pm$	P value
RBC $\times 10^6$	Patient	4.05	0.57	0.72
	Control	4.12	0.71	
Hemoglobin mg/dL	Patient	11.79	1.77	0.65
	Control	11.49	2.31	
WBC $\times 10^3$	Patient	29.53	27.31	0.002
	Control	7.26	2.81	
PLT $\times 10^3$	Patient	180.400	103.77	0.287
	Control	212.800	84.86	
Lymphocyte (%)	Patient	59.35	22.00	0.001
	Control	24.68	10.53	
Absolute lymphocyte count $\times 10^3$	Patient	10.82	12.80	0.04
	Control	4.70	2.41	

showed the JAK1 mutation in 18 % of adult precursor T-ALL. It was shown that the JAK3 mutation is present in only one case of ATL and there were no JAK1 mutations in 20 ATL patients. In another study Tomita et al. [30] observed constitutive Jak-Stat pathway activation in HTLV-1 infected T-cell lines and it was concluded that this activation is a reason for the proliferation and survival of leukemic cells in ATL and therefore, inhibition of this pathway may be a new strategy for treating of ATL. Moreover, JAK2 mutations as well as several translocations of the JAK2 gene (e.g. PCM1-JAK2 and ETV6-JAK2) have been reported in T cell lymphoproliferative disorders [31]. Nevertheless, amplification of the JAK2 locus (9p.24) has been shown in Hodgkin's and non-Hodgkin's lymphoma [32].

JAK2 exon 12 mutations, contributes to constitutive activation of JAK2 and its downstream proteins. JAK2 possesses a pseudokinase domain which promotes growth-factor independence in transfected cell lines [33]. Valine to phenylalanine substitution destabilizes the JAK homology 2 (JH2) domain of JAK2 causes loss of the auto-inhibitory activity of this pseudokinase domain and explains some of the clinical finding observed in patients with MPNs [34]. Although there may be a relationship between leukemogenesis and activation of the Jak-Stat pathway in ATL, there is an activation of the IL-2R signaling pathway mediated by Jak-Stat in ATL cells [30]. In addition, it is

well documented that the TAX protein induced by the genome of HTLV-1 (ATL cells), may activates the transcription of several host cell genes, including genes encoding the cytokine IL-2 and its receptor [9]. Therefore, this pathway may play a key role in transformation by HTLV-1.

Currently there is no uniformly effective therapy for patients with HTLV-1 associated ATL [19]. It has been suggested that JAK3 inhibitor might serve as an effective therapeutic agent in patients with chronic and smoldering ATL [15]. Yang et al. [35] have shown that AZ960, a novel inhibitor of JAK2 kinase, effectively induced cell growth arrest and apoptosis of HTLV-1-infected T cells and also downregulation of the phosphorylated forms of JAK2 family proteins. However, it is not clear whether disruption of Jak-Stat pathway could completely reverse the phenotypic condition of HTLV-1-infected T-cells.

ATL is an uncommon disease and the current study was carried only on 20 samples of ATL patients and only one sample was positive for JAK2 V617F mutation. An important limitation of our study was the small population size. Further studies are recommended to investigate JAK2 signaling pathway in larger sample sizes of ATL patients.

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