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Frequency of Mitochondrial DNA D-Loop Somatic Mutations in Patients with HTLV-I

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ABSTRACT

Human T-cell Lymphotropic virus type-1 (HTLV-1) is endemic in Northeast of Iran. Still, it is unclear that genetic background has role in infection by HTLV-1. ***Methods:*** *We ascertained the frequency of mitochondrial DNA (mtDNA) D-loop region nucleotide changes in 45 HTLV-1 infected individuals and 463 healthy control subjects using Sanger sequencing method.* ***Results:*** *Out of totally 164 identified single nucleotide polymorphisms (SNPs) among HTLV-1 patients, 89 SNPs found statistically significant in comparison to the control group ($P<0.05$). In this study, no deletion was identified in mtDNA D-loop region. But, for the first time a high frequency of point mutations was observed in HTLV-1 patients.* ***Conclusion:*** *Such nucleotide changes in HTLV-1 patients propose that these mutations may result in impaired mitochondria function directly and/or indirectly. Moreover, these variations may act as a predisposing factor along with the environmental factors, and might play an important role in pathogenesis of HTLV-1.*

Keywords: Human T-cell lymphotropic virus, Mitochondrial DNA, Displacement loop, Polymorphism

INTRODUCTION

Human T-cell lymphotropic virus type-1 (HTLV-1) belongs to retroviridae family in the genus deltaretrovirus; it causes the myelopathy or spastic paraparesis as well as inflammatory diseases [1,2]. HTLV-1 is the first virus certainly linked to human cancer, Adult T-cell Lymphoma Leukaemia (ATLL). Distribution of HTLV-1 infection varies geographically through the world. North-east of Iran is an endemic area for HTLV-1 infection [3,4]. Many studies conducted to identify the genetic disorders, mainly genomic mutations involved in pathogenesis of oncogenic virus like HTLV-1.

An emerging body of evidence has established that HTLV-1 targets mitochondria as a mechanism to change cell physiology. Mitochondria are as an important regulator of apoptosis involving in oxidative phosphorylation. In addition, mitochondrial DNA (mtDNA) is a main target of oxidative damage. Reactive oxygen species (ROS) are small molecules that the deleterious effect of intracellular ROS produced in mitochondria is associated with a variety of human pathologies like neurodegenerative diseases and cancer [5]. HTLV-1 can generate ROS via its oncogenic products p13 and Tax proteins. It has been supposed that mtDNA sequence changes may alter the encoded protein subunits of the respiratory chain complexes, which can lead to changed ROS production and finally cancer initiation and progression [6].

The two complementary strands of mtDNA are characterized by base compositions; a heavy chain (H) is G rich, whereas the other G-poor that called light strand (L) [7]. Non-coding control region (CR) of mtDNA, D-loop that containing 1122 bp (refers to mitochondria database http://www.mitomap.org) regulates mtDNA replication and transcription [8]. This region has a high rate of mutation, about 10-fold higher than nuclear DNA which is due to

absence of an effective DNA repair machinery and protecting proteins, histones, in mitochondria [9]. D-loop consists of two hyper variable regions (HV1 with 342 bp at nucleotides 16024- 16383 and HV2 with 286 bp at nucleotides 57-372) [10]. Transcription of the whole mtDNA genome might be affected by mutations occurring in the D-loop region. Many studies have been extensively examined sequence changes that particularly accumulate in the regulatory region or D-loop in cancers [11] and metabolic diseases [12], but their predictive value still is unclear. Recently, somatic mtDNA alterations have been reported in cancers such as pancreatic [13], gastric [14,15], and hepatocellular carcinoma [16].

the fact that north-east of Iran has been identified as endemic region for HTLV-1 infection [17,18], so far, no study has been conducted on possible association of mtDNA D-loop polymorphisms and HTLV-1 infection. This study aims to evaluate the frequency of nucleic acid changes in the regulatory region of mtDNA in HTLV-1 infected individuals who were resident in this part of Iran, and compared it to control group to verify whether SNPs of D-loop region of mtDNA increase risk of HTLV-1 infection or not.

MATERIAL AND METHODS

Thirty-six patients who had referred to the clinical laboratory of Razavi Hospital, Mashhad, Iran during the years between 2013 and 2015 and were diagnosed with HTLV-I infection using ELISA test, enrolled in this study. Qualitative PCR assay was used to confirm positive ELISA results. The mean age of the patients was 52.5 years (range, 20-65 years). Twenty-eight patients (77.7%) were female and eight patients (22.2%) were men. All the cases were born in Northeast of Iran and had Fars ethnicity; also, there was no any family relationship between them. Furthermore, the two generations before them were living in this province. Four hundred and sixty-three blood samples of healthy subjects were received from the National Institute of Genetic Engineering and Biotechnology, Tehran, Iran. The controls were randomly selected from people who had negative HTLV-I-Ab result or family history of HTLV-1 infection. All the patients and controls gave written informed consent to participate in the study, and signed the consent approved by the ethical committee.

Genomic DNA was extracted from peripheral blood samples collected in EDTA tube using a Genet bio blood DNA Mini-Kit (Genetbio, Korea), According to the manufacturer's unstructured and stored at -20°C.

To amplify the D-loop region of mtDNA, following PCR primers were designed; ONP98F:(5'-ATCATTGGACAAGTAGCATG-3') andONP77R:(5'-GCTCCGGCTCCAGC-GTCTCG-3') to amplify the first part of the D-loop region corresponding to 850bp, and also the second part of the D-loop genome was amplified using primers (740bp) ONP38F:(5'-GATCACAGGTCTATCACCCT-3') and ONP79R:(5'-GAGCTGCATTGCT-GCGTGCT-3'). This 1590bp PCR product covered the complete mtDNA D-loop region which was amplified in a total volume of 25 µL including 0.5 µL of each primer (10 pmol/µL), 2.5 µL of 10x buffer, 0.5 µL of dNTPs (10 mM), 1.5 µl of $MgCl_2$, 0.3 µL of Taq DNA polymerase (5 U/µL), and 60ng of the genomic DNA sample. The PCR condition was as following: an initial denaturation at 94°C for 5 minutes, 35 cycles of denaturation at 94°C for 60 s, annealing at 55°C for 60 s, an extension at 72°C for 60 s, and the final extension was at 72°C for 10 minutes. The PCR products were analyzed on 2% agarose gel electrophoresis containing 0.1% ethidium bromide. PCR sequencing was performed using BigDye® Terminator v3.1 kit ABI (Applied Biosystems, USA). After PCR product purification, products were sequenced bi-directionally on ABI 3500 genetic analyzer. DNA sequences were analysed by Sequencher, DNA sequence analysis software and compared to the complete Mitochondrial DNA sequences (Gene bank accession number: NC_012920.1).

Statistical and DNA analysis

All statistical analyses were calculated using R software. The prevalence of nucleotide variation between the case and healthy group was compared by using Fisher's exact test. $P<0.05$ was considered statistically significant.

RESULTS

In the present case-control study, polymorphisms of non-coding CR of mtDNA were analysed between nucleotide position 16024 and 576, in HTLV-I infected individuals and a healthy control group. The control group consists of 463 persons with the negative HTLV1-Ab result and also without any sign of mitochondrial disorders.

Nucleotide variations of mtDNA D-loop region were analysed by direct sequencing method (Figure 1), which showed

164 different variations in our studied population. Among these, 89 variations were only seen in HTLV-I infected cases (Table 1), 24 only in control group (Table 2). The results indicated that 51 polymorphisms did not show any statistically difference between two studied groups.

Table 1 List of variations in both HTLV-1 patients and healthy control samples

Row	SNPs	Patients with HTLV-I Case (n=45)		Controls Case (n=463)		Odd Ratio	95% CI		P -Value
		N	%	N	%		Lower	Upper	
1	A73G*	36	80	266	57.5	2.956679	1.357857	7.146468	0.003777
2	C78A	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
3	A93G	1	2.2	8	1.7	1.29189	0.0284913	10.018371	0.569
4	C96T*	2	4.5	1	0.2	21.18087	1.082367	1258.2746	0.02176
5	G100A	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
6	G103A*	2	4.5	1	0.2	21.18087	1.082367	1258.2746	0.02176
7	G109T	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
8	C110A	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
9	A111G	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
10	C112A	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
11	C113A	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
12	C114A	1	2.2	2	0.4	5.207116	0.0868392	101.91749	0.2433
13	C122A	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
14	T146C	8	17.8	52	11.2	1.706817	0.6508815	3.990882	0.2225
15	C147T	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
16	C150T	4	8.9	41	8.9	1.004156	0.2488729	2.9839313	1
17	C151T	3	6.6	27	5.9	1.153082	0.2149593	3.9949278	0.7412
18	T152C	13	28.9	109	23.5	1.318619	0.6125025	2.693347	0.4646
19	T152G	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
20	A153G	1	2.2	10	2.1	1.029491	0.0232028	7.5449166	1
21	A156G	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
22	A189G	5	11.1	19	4.1	2.911887	0.8067379	8.6461698	0.05143
23	C194T	3	6.6	12	2.6	2.676968	0.4666275	10.47108	0.1392
24	T195C	8	17.8	66	14.2	1.299847	0.50044	3.000628	0.5085
25	T195A	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
26	T204C*	7	15.6	28	5.6	2.853322	0.9862343	7.2777439	0.02653
27	G207A*	6	13.3	21	4.5	3.226819	1.005598	8.916003	0.02446
28	A210G	2	4.5	8	1.7	2.637994	0.2649054	13.806441	0.2193
29	T217C*	3	6.6	4	0.9	8.126393	1.152279	49.804791	0.01769
30	T233G	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
31	A234G	1	2.2	1	0.2	10.39399	0.1308108	819.81957	0.1695
32	T236A	1	2.2	2	0.4	5.207116	0.0868392	101.91749	0.2433
33	C271T	1	2.2	10	2.1	1.029491	0.0232028	7.5449166	1
34	C285T	3	6.6	10	2.1	3.224132	0.5492715	13.183282	0.09905
35	C295T	2	4.5	45	9.7	0.4325747	0.0491517	1.7604879	0.4147
36	A297G	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
37	C321T*	5	11.1	0	0	Inf	10.07345	Inf	4.42E-06
38	T322G*	2	4.5	1	0.2	21.18087	1.082367	1258.2746	0.02176
39	C325G*	3	6.6	6	1.3	5.406765	0.8449849	26.429254	0.0374
40	C328A	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
41	C333A	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
42	A336T	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
43	C339A*	2	4.5	0	0	Inf	1.955899	Inf	0.007688
44	C341T*	2	4.5	0	0	Inf	1.955899	Inf	0.007688
45	C344T*	3	6.6	0	0	Inf	4.371381	Inf	0.0006533

46	C363A*	4	8.9	0	0	Inf	7.111883	Inf	5.43E-05
47	G367A*	3	6.6	0	0	Inf	4.371381	Inf	0.0006533
48	C370A	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
49	C376A	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
50	G390A*	3	6.6	0	0	Inf	4.371381	Inf	0.0006533
51	T392A	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
52	C395T	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
53	A403T*	2	4.5	0	0	Inf	1.955899	Inf	0.007688
54	C405T*	3	6.6	0	0	Inf	4.371381	Inf	0.0006533
55	C412T*	3	6.6	4	0.9	8.126393	1.152279	49.804791	0.01769
56	A426T*	2	4.5	0	0	Inf	1.955899	Inf	0.007688
57	T453A	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
58	C457T	1	2.2	1	0.2	10.39399	0.1308108	819.81957	0.1695
59	C463T	2	4.5	22	4.7	0.9324651	0.1028842	4.0118489	1
60	C470T	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
61	T483C*	2	4.5	1	0.2	21.18087	1.082367	1258.2746	0.02176
62	C487T	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
63	T490C	6	13.3	39	8.4	1.670574	0.5440463	4.3225366	0.2701
64	C492A*	3	6.6	0	0	Inf	4.371381	Inf	0.0006533
65	C498T	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
66	A509G*	2	4.5	0	0	Inf	1.955899	Inf	0.007688
67	T594C	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
68	A16051G*	3	6.6	0	0	Inf	4.371381	Inf	0.0006533
69	C16069T*	2	4.5	83	17.9	0.2133516	0.0245529	0.8479209	0.01973
70	C16071T	1	2.2	12	2.6	0.8543977	0.0195431	6.0334048	1
71	T16086C	3	6.6	8	1.7	4.044068	0.666273	17.679768	0.06476
72	T16093C	3	6.6	17	3.7	1.871117	0.3377355	6.8620539	0.4076
73	C16111T*	2	4.5	0	0	Inf	1.955899	Inf	0.007688
74	C16114T	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
75	T16124C	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
76	T16126C	13	28.9	113	24.4	1.257721	0.5847065	2.5663066	0.4759
77	G16129C*	2	4.5	0	0	Inf	1.955899	Inf	0.007688
78	G16145A*	3	6.6	0	0	Inf	4.371381	Inf	0.0006533
79	C16148T	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
80	A16163G	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
81	C16179T	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
82	A16182C	1	2.2	22	4.7	0.4561034	0.0107999	2.9639846	0.7104
83	A16183C	3	6.6	43	9	0.6981131	0.1329019	2.3338424	0.786
84	C16184T*	2	4.5	0	0	Inf	1.955899	Inf	0.007688
85	C16186T	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
86	T16189C	7	15.6	98	21.1	0.6865731	0.250887	1.619904	0.4448
87	C16192T	1	2.2	27	5.8	0.3675046	0.0087706	2.3453086	0.4977
88	T16195G	1	2.2	3	0.6	3.470996	0.0649212	44.272022	0.3108
89	C16197G	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
90	C16197T*	2	4.5	0	0	Inf	1.955899	Inf	0.007688
91	C16201A*	4	8.9	0	0	Inf	7.111883	Inf	5.43E-05
92	C16201T	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
93	T16209A*	3	6.6	0	0	Inf	4.371381	Inf	0.0006533
94	C16211A*	4	8.9	0	0	Inf	7.111883	Inf	5.43E-05
95	C16214A	2	4.5	7	1.5	3.020031	0.2972741	16.531683	0.1855
96	T16217C	3	6.6	19	4.1	1.667133	0.3036808	6.0156596	0.4319
97	T16217A	1	2.2	0	0	Inf	0.2638176	Inf	0.08858

98	C16223T	14	31.1	98	21.2	1.680102	0.7934017	3.4045279	0.1333
99	C16234T	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
100	C16239T*	3	6.6	0	0	Inf	4.371381	Inf	0.0006533
101	T16243C	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
102	C16245G*	3	6.6	0	0	Inf	4.371381	Inf	0.0006533
103	C16248T	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
104	T16249C*	4	8.9	0	0	Inf	7.111883	Inf	5.43E-05
105	C16256T	1	2.2	26	5.6	0.3825005	0.0091163	2.4484903	0.4973
106	A16258C*	4	8.9	0	0	Inf	7.111883	Inf	5.43E-05
107	C16261T*	2	4.5	0	0	Inf	1.955899	Inf	0.007688
108	A16265C*	5	11.1	0	0	Inf	10.07345	Inf	4.42E-06
109	C16266T	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
110	A16269C*	3	6.6	0	0	Inf	4.371381	Inf	0.0006533
111	C16270T*	2	4.5	0	0	Inf	1.955899	Inf	0.007688
112	A16272T	1	2.2	8	1.7	1.29189	0.0284913	10.018371	0.569
113	G16273A	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
114	G16274A	1	2.2	13	2.8	0.7870775	0.0181057	5.4784495	1
115	A16275G	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
116	T16276A*	4	8.9	0	0	Inf	7.111883	Inf	5.43E-05
117	T16288C*	2	4.5	0	0	Inf	1.955899	Inf	0.007688
118	C16292T	3	6.6	22	4.7	1.430658	0.2633648	5.0663097	0.4773
119	A16293C*	3	6.6	0	0	Inf	4.371381	Inf	0.0006533
120	C16294T	4	8.9	44	9.5	0.9291607	0.2309585	2.7488107	1
121	C16296T*	6	13.4	24	5.2	2.805854	0.8846993	7.616455	0.03992
122	T16298C	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
123	C16301A*	2	4.5	0	0	Inf	1.955899	Inf	0.007688
124	T16304C	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
125	A16309G*	2	4.5	0	0	Inf	1.955899	Inf	0.007688
126	T16311C	6	13.3	71	15.3	0.8496655	0.2834192	2.1246509	0.8306
127	A16316G	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
128	A16318T*	3	6.6	0	0	Inf	4.371381	Inf	0.0006533
129	G16319A	3	6.6	18	3.9	1.76343	0.3198391	6.4127745	0.419
130	T16325C*	2	4.5	0	0	Inf	1.955899	Inf	0.007688
131	C16327T*	3	6.6	0	0	Inf	4.371381	Inf	0.0006533
132	C16327A	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
133	C16355T	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
134	T16356C*	2	4.5	0	0	Inf	1.955899	Inf	0.007688
135	T16357C*	2	4.5	0	0	Inf	1.955899	Inf	0.007688
136	T16359C	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
137	T16362C*	9	20	42	9	2.499923	0.9902388	5.7616523	0.03303
138	G16390A*	2	4.5	0	0	Inf	1.955899	Inf	0.007688
139	A16399G	1	2.2	0	0	Inf	0.2638176	Inf	0.08858
140	C16519T*	12	26.7	283	61	0.2319627	0.1061968	0.4753477	1.12E-05
*Show statistically significant (P<0.05)									

To compare polymorphism frequencies between age groups of HTLV-1 infected patients, Fisher's exact test was performed. Only two, C321T (P<0.05) and the minor allele of G16145A showed significantly difference (P<0.05) in age group classifications (Tables 3 and 4). Among 164 control region polymorphisms, 89 were more frequent in patients with HTLV-I infection compared to controls cohort (P<0.05) (Table 1), which indicated that an individual carrying these alleles may be susceptible to an increased risk of HTLV-I infection. Of these, the CR polymorphism A73G found to have a significantly higher frequency in HTLV-I patients compared to controls.

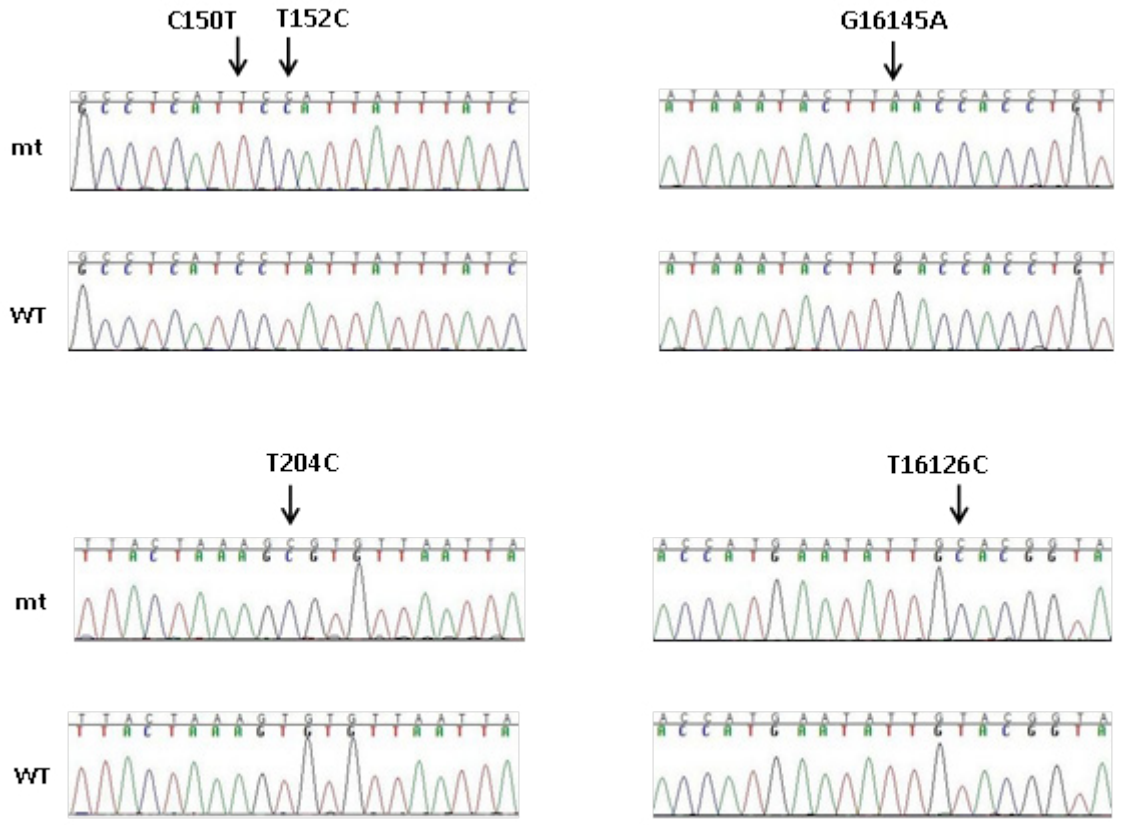

Figure 1 Detection and identification of somatic mtDNA mutation in patients with HTLV-1 infection by DNA sequencing method. Sequencing analysis showed T152C, C150T, T204C, T16126C, and G16145A. mt, Mutant; WT, wild-type

We observed significant fluctuation within SNP frequencies, the presence of T16519C polymorphism in HTLV-I patients was 26.7% (12/45) versus 61% (283/463) in control group (P<0.05).

Further analysis among statistically significant CR polymorphisms performed based on gender in HTLV-I infected group, and only 7 polymorphisms were detected with substantial nucleotide variation between males and females in HTLV1 infected group, particularly T152C, T195C, T490C, T16126C, and T16189C found to have an important higher frequency in females compared to the males (P<0.05) (Table 2).

Table 2 Comparison of SNPs in the population infected with HTLV-I based on the gender

SNPs	Patients with HTLV-I Case (n=45)		Sex		Odd Ratio	95% CI		P -Value
	N	%	Men Case (n:12)	Female Case (n:33)		Lower	Upper	
A73G	36	80	83.3	78.8	0	0	1.24188	0.08627
C78A	1	2.2	0	3.03	0	0	14.18184	0.2667
A93G	1	2.2	0	3.03	0	0	14.18184	0.2667
C96T	2	4.5	8.3	3.03	0.3535534	0.0042581	29.415806	0.4667
G100A	1	2.2	0	3.03	0	0	14.18184	0.2667
G103A	2	4.5	8.3	3.03	0.3535534	0.0042581	29.415806	0.4667
G109T	1	2.2	0	3.03	0	0	14.18184	0.2667
C110A	1	2.2	0	3.03	0	0	14.18184	0.2667
A111G	1	2.2	0	3.03	0	0	14.18184	0.2667
C112A	1	2.2	0	3.03	0	0	14.18184	0.2667

C113A	1	2.2	0	3.03	0	0	14.18184	0.2667
C114A	1	2.2	0	3.03	0	0	14.18184	0.2667
C122A	1	2.2	0	3.03	0	0	14.18184	0.2667
T146C	8	17.8	33.3	12.1	0.2854923	0.0421847	1.8911816	0.1808
C147T	1	2.2	0	3.03	0	0	14.18184	0.2667
C150T	4	8.9	8.3	9.09	0.1002823	0.0017407	1.415948	0.05205
C151T*	3	6.6	0	9.09	0	0	0.7995383	0.0155
T152C*	13	28.9	16.6	33.3	0.0078923	0.0001419	0.0880971	8.72E-08
T152G	1	2.2	0	3.03	0	0	14.18184	0.2667
A153G	1	2.2	8.3	0	Inf	0.0093395	Inf	1
A156G	1	2.2	0	3.03	0	0	14.18184	0.2667
A189G	5	11.1	16.6	9.09	0.202618	0.0147413	2.0503421	0.1091
C194T*	3	6.6	0	9.09	0	0	0.7995383	0.0155
T195C*	8	17.8	16.6	18.1	0.0705568	0.0056744	0.5079979	0.002387
T195A	1	2.2	8.3	0	Inf	0.0093395	Inf	1
T204C*	7	15.6	16.6	15.1	0.0972492	0.0077377	0.7366808	0.009904
G207A*	6	13.3	16.6	12.1	0.136957	0.0105812	1.1456384	0.03541
A210G	2	4.5	16.6	0	Inf	0.067035	Inf	1
T217C	3	6.6	8.3	6.06	0.1644904	0.002578	3.4640711	0.169
T233G	1	2.2	0	3.03	0	0	14.18184	0.2667
A234G	1	2.2	0	3.03	0	0	14.18184	0.2667
T236A	1	2.2	0	3.03	0	0	14.18184	0.2667
C271T	1	2.2	0	3.03	0	0	14.18184	0.2667
C285T	3	6.6	16.6	3.03	0.715478	0.0339994	45.63246	1
C295T	2	4.5	0	6.06	0	0	1.864564	0.06667
A297G	1	2.2	8.3	0	Inf	0.0093395	Inf	1
C321T*	5	11.1	8.3	12.1	0.0678609	0.0012391	0.8016635	0.01402
T322G	2	4.5	0	6.06	0	0	1.864564	0.06667
C325G	3	6.6	8.3	6.06	0.1644904	0.002578	3.4640711	0.169
C328A	1	2.2	0	3.03	0	0	14.18184	0.2667
C333A	1	2.2	0	3.03	0	0	14.18184	0.2667
A336T	1	2.2	0	3.03	0	0	14.18184	0.2667
C339A	2	4.5	8.3	3.03	0.3535534	0.0042581	29.415806	0.4667
C341T	2	4.5	8.3	3.03	0.3535534	0.0042581	29.415806	0.4667
C344T	3	6.6	8.3	6.06	0.1644904	0.002578	3.4640711	0.169
C363A	4	8.9	8.3	9.09	0.1644904	0.002578	3.4640711	0.169
G367A	3	6.6	8.3	6.06	0.1644904	0.002578	3.4640711	0.169
C370A	1	2.2	0	3.03	0	0	14.18184	0.2667
C376A	1	2.2	0	3.03	0	0	14.18184	0.2667
G390A	3	6.6	8.3	6.06	0.1644904	0.002578	3.4640711	0.169
T392A	1	2.2	0	3.03	0	0	14.18184	0.2667
C395T	1	2.2	0	3.03	0	0	14.18184	0.2667
A403T	2	4.5	0	6.06	0	0	1.864564	0.06667
C405T	3	6.6	8.3	6.06	0.1644904	0.002578	3.4640711	0.169
C412T	3	6.6	8.3	6.06	0.1644904	0.002578	3.4640711	0.169
A426T	2	4.5	8.3	3.03	0.3535534	0.0042581	29.415806	0.4667
T453A	1	2.2	0	3.03	0	0	14.18184	0.2667
C457T	1	2.2	0	3.03	0	0	14.18184	0.2667
C463T	2	4.5	0	6.06	0	0	1.864564	0.06667
C470T	1	2.2	8.3	0	Inf	0.0093395	Inf	1
T483C	2	4.5	0	6.06	0	0	1.864564	0.06667
C487T	1	2.2	8.3	0	Inf	0.0093395	Inf	1
T490C*	6	13.3	0	18.1	0	0	0.3050306	0.0006482
C492A	3	6.6	8.3	6.06	0.1644904	0.002578	3.4640711	0.169

C498T	1	2.2	8.3	0	Inf	0.0093395	Inf	1
A509G	2	4.5	8.3	3.03	0.3535534	0.0042581	29.415806	0.4667
T594C	1	2.2	0	3.03	0	0	14.18184	0.2667
A16051G	3	6.6	16.6	3.03	0.715478	0.0339994	45.63246	1
C16069T	2	4.5	0	6.06	0	0	1.864564	0.06667
C16071T	1	2.2	0	3.03	0	0	14.18184	0.2667
T16086C	3	6.6	8.3	6.06	0.1644904	0.002578	3.4640711	0.169
T16093C	3	6.6	8.3	6.06	0.1644904	0.002578	3.4640711	0.169
C16111T	2	4.5	16.6	0	Inf	0.067035	Inf	1
C16114T	1	2.2	0	3.03	0	0	14.18184	0.2667
T16124C	1	2.2	0	3.03	0	0	14.18184	0.2667
T16126C*	13	28.9	16.6	33.3	0.0078923	0.0001419	0.0880971	8.72E-08
G16129C	2	4.5	8.3	3.03	0.3535534	0.0042581	29.415806	0.4667
G16145A	3	6.6	8.3	6.06	0.1644904	0.002578	3.4640711	0.169
C16148T	1	2.2	0	3.03	0	0	14.18184	0.2667
A16163G	1	2.2	0	3.03	0	0	14.18184	0.2667
C16179T	1	2.2	0	3.03	0	0	14.18184	0.2667
A16182C	1	2.2	0	3.03	0	0	14.18184	0.2667
A16183C	3	6.6	8.3	6.06	0.1644904	0.002578	3.4640711	0.169
C16184T	2	4.5	8.3	3.03	0.3535534	0.0042581	29.415806	0.4667
C16186T	1	2.2	0	3.03	0	0	14.18184	0.2667
T16189C*	7	15.6	8.3	18.1	0.0350869	0.0006669	0.3601554	0.0006894
C16192T	1	2.2	0	3.03	0	0	14.18184	0.2667
T16195G	1	2.2	8.3	0	Inf	0.0093395	Inf	1
C16197G	1	2.2	0	3.03	0	0	14.18184	0.2667
C16197T	2	4.5	8.3	3.03	0.3535534	0.0042581	29.415806	0.4667
C16201A	4	8.9	8.3	9.09	0.1644904	0.002578	3.4640711	0.169
C16201T	1	2.2	8.3	0	Inf	0.0093395	Inf	1
T16209A	3	6.6	8.3	6.06	0.1644904	0.002578	3.4640711	0.169
C16211A	4	8.9	8.3	9.09	0.1002823	0.0017407	1.415948	0.05205
C16214A	2	4.5	16.6	0	Inf	0.067035	Inf	1
T16217C*	3	6.6	0	9.09	0	0	0.7995383	0.0155
T16217A	1	2.2	0	3.03	0	0	14.18184	0.2667
C16223T*	14	31.1	33.3	30.3	0.0316274	0.0025141	0.2152724	1.66E-05
C16234T	1	2.2	0	3.03	0	0	14.18184	0.2667
C16239T	3	6.6	8.3	6.06	0.1644904	0.002578	3.4640711	0.169
T16243C	1	2.2	0	3.03	0	0	14.18184	0.2667
C16245G	3	6.6	8.3	6.06	0.1644904	0.002578	3.4640711	0.169
C16248T	1	2.2	0	3.03	0	0	14.18184	0.2667
T16249C	4	8.9	25	3.03	1.097715	0.0780658	62.90879	1
C16256T	1	2.2	0	3.03	0	0	14.18184	0.2667
A16258C	4	8.9	8.3	9.09	0.1002823	0.0017407	1.415948	0.05205
C16261T	2	4.5	0	6.06	0	0	1.864564	0.06667
A16265C*	5	11.1	8.3	12.1	0.0678609	0.0012391	0.8016635	0.01402
C16266T	1	2.2	0	3.03	0	0	14.18184	0.2667
A16269C	3	6.6	8.3	6.06	0.1644904	0.002578	3.4640711	0.169
C16270T	2	4.5	8.3	3.03	0.3535534	0.0042581	29.415806	0.4667
A16272T	1	2.2	0	3.03	0	0	14.18184	0.2667
G16273A	1	2.2	8.3	0	Inf	0.0093395	Inf	1
G16274A	1	2.2	8.3	0	Inf	0.0093395	Inf	1
A16275G	1	2.2	8.3	0	Inf	0.0093395	Inf	1
T16276A	4	8.9	2.2	6.6	0.1002823	0.0017407	1.415948	0.05205
T16288C	2	4.5	8.3	3.03	0.3535534	0.0042581	29.415806	0.4667
C16292T*	3	6.6	0	9.09	0	0	0.7995383	0.0155

A16293C	3	6.6	8.3	6.06	0.1644904	0.002578	3.4640711	0.169
C16294T	4	8.9	16.6	6.06	0.3324106	0.0214496	5.1345784	0.2859
C16296T*	6	13.4	16.6	12.1	0.136957	0.0105812	1.1456384	0.03541
T16298C	1	2.2	0	3.03	0	0	14.18184	0.2667
C16301A	2	4.5	0	6.06	0	0	1.864564	0.06667
T16304C	1	2.2	0	3.03	0	0	14.18184	0.2667
A16309G	2	4.5	0	6.06	0	0	1.864564	0.06667
T16311C	6	13.3	33.3	6.06	0.6957385	0.0837105	8.7989774	0.6503
A16316G	1	2.2	0	3.03	0	0	14.18184	0.2667
A16318T*	3	6.6	0	9.09	0	0	0.7995383	0.0155
G16319A	3	6.6	0	9.09	0	0	14.18184	0.2667
T16325C	2	4.5	8.3	3.03	0.3535534	0.0042581	29.415806	0.4667
C16327T	3	6.6	16.6	3.03	0.715478	0.0339994	45.63246	1
C16327A	1	2.2	8.3	0	Inf	0.0093395	Inf	1
C16355T	1	2.2	0	3.03	0	0	14.18184	0.2667
T16356C	2	4.5	0	6.06	0	0	1.864564	0.06667
T16357C	2	4.5	16.6	0	Inf	0.067035	Inf	1
T16359C	1	2.2	0	3.03	0	0	14.18184	0.2667
T16362C*	9	20	25	18.1	0.1074021	0.0133799	0.662486	0.00618
G16390A	2	4.5	16.6	0	Inf	0.067035	Inf	1
A16399G	1	2.2	0	3.03	0	0	14.18184	0.2667
C16519T*	12	26.7	33.3	24.2	0.075338	0.0104138	0.4248873	0.0007473
*Show statistically significant (P<0.05)								

Noticeably, Nucleotide variations C>T and C>A were the most frequent in transition and transversion groups, respectively. Transition presented 68.6% out of total, versus 31.4% transversion. Overall, sequence analysis indicated that most variations of mtDNA D-loop region were single nucleotide substitutions and most were transitions rather than transversions.

Table 3 Individual single nucleotide polymorphism loci in control group

mtDNA CR polymorphisms	N=463	Frequency in Controls (%)	mtDNA CR polymorphisms	N=463	Frequency in Controls (%)
G143A	5	1.07	C16082T	2	0.4
C186T	3	0.6	C16050T	11	2.4
T196C	4	0.8	T16172C	19	4.1
C198T	7	1.5	C16173T	15	3.2
T199C	15	3.2	C16187T	10	2.1
A200G	12	2.6	C16193T	9	1.9
A215G	3	0.6	A16235G	5	1.07
G228A	5	1.07	C16278T	26	5.6
T239C	4	0.8	C16290T	7	1.5
C242T	6	1.3	A16335G	5	1.07
G247A	3	0.6	T16352C	6	1.3
T250C	7	1.5	T16468C	4	0.8

Table 4 Comparison of SNPs in the population infected with HTLV-I based on different age groups

SNPs	Patients with HTLV-I Case(n=45)		AGE					P- Value
	N	%	20-30 Case (n=8)	30-40 Case (n=6)	40-50 Case (n=16)	50-60 Case (n=10)	60-70 Case (n=5)	
A73G	36	80	8	3	12	9	4	0.1722
C78A	1	2.2	0	0	1	0	0	1
A93G	1	2.2	0	0	0	0	1	0.1111
C96T	2	4.5	0	1	1	0	0	0.5879;
G100A	1	2.2	0	0	0	1	0	0.6444

G103A	2	4.5	0	0	2	0	0	0.7091
G109T	1	2.2	0	0	0	1	0	0.6444
C110A	1	2.2	0	0	0	1	0	0.6444
A111G	1	2.2	0	0	0	1	0	0.6444
C112A	1	2.2	0	0	0	1	0	0.6444
C113A	1	2.2	0	0	0	1	0	0.6444
C114A	1	2.2	0	0	0	1	0	0.6444
C122A	1	2.2	0	0	0	1	0	0.6444
T146C	8	17.8	2	0	3	2	1	0.8709
C147T	1	2.2	0	0	0	1	0	0.6444
C150T	4	8.9	0	1	2	1	0	0.884
C151T	3	6.6	1	0	1	1	0	1
T152C	13	28.9	2	2	4	4	1	0.902
T152G	1	2.2	0	0	1	0	0	1
A153G	1	2.2	0	0	1	0	0	1
A156G	1	2.2	0	0	0	1	0	0.6444
A189G	5	11.1	2	0	1	2	0	0.4597
C194T	3	6.6	1	0	0	2	0	0.2237
T195C	8	17.8	2	1	1	4	0	0.1817
T195A	1	2.2	0	1	0	0	0	0.2444
T204C	7	15.6	1	0	5	1	0	0.4274
G207A	6	13.3	2	0	3	1	0	0.7139
A210G	2	4.5	0	0	2	0	0	0.7091
T217C	3	6.6	0	1	2	0	0	0.5794
T233G	1	2.2	0	0	0	1	0	0.6444
A234G	1	2.2	0	0	0	1	0	0.6444
T236A	1	2.2	0	0	0	1	0	0.6444
C271T	1	2.2	0	0	0	0	1	0.1111
C285T	3	6.6	0	0	1	2	0	0.5794
C295T	2	4.5	0	1	0	0	1	0.08384
A297G	1	2.2	0	0	0	0	1	0.1111
C321T*	5	11.1	3	0	0	2	0	0.03439
T322G	2	4.5	1	0	0	1	0	0.4909
C325G	3	6.6	2	0	0	1	0	0.1772
C328A	1	2.2	1	0	0	0	0	0.4222
C333A	1	2.2	1	0	0	0	0	0.4222
A336T	1	2.2	1	0	0	0	0	0.4222
C339A	2	4.5	2	0	0	0	0	0.05354
C341T	2	4.5	2	0	0	1	0	0.1772
C344T	3	6.6	2	0	0	1	0	0.1772
C363A	4	8.9	2	0	0	2	0	0.1188
G367A	3	6.6	2	0	0	1	0	0.1772
C370A	1	2.2	1	0	0	0	0	0.4222
C376A	1	2.2	1	0	0	0	0	0.4222
G390A	3	6.6	2	0	0	1	0	0.1772
T392A	1	2.2	1	0	0	0	0	0.4222
C395T	1	2.2	1	0	0	0	0	0.4222
A403T	2	4.5	0	0	0	2	0	0.1697
C405T	3	6.6	2	0	0	1	0	0.1772
C412T	3	6.6	2	0	0	1	0	0.1772
A426T	2	4.5	2	0	0	0	0	0.05354
T453A	1	2.2	1	0	0	0	0	0.4222
C457T	1	2.2	1	0	0	0	0	0.4222
C463T	2	4.5	0	1	0	0	1	0.08384

C470T	1	2.2	0	0	0	1	0	0.6444
T483C	2	4.5	1	0	1	0	0	0.8384
C487T	1	2.2	0	0	0	0	1	0.1111
T490C	6	13.3	1	1	0	2	2	0.09165
C492A	3	6.6	2	0	0	1	0	0.1772
C498T	1	2.2	0	0	1	0	0	1
A509G	2	4.5	0	1	1	0	0	0.5879
T594C	1	2.2	0	0	0	0	1	0.1111
A16051G	3	6.6	0	1	2	0	0	0.5794
C16069T	2	4.5	0	1	0	0	1	0.08384
C16071T	1	2.2	0	0	1	0	0	1
T16086C	3	6.6	0	1	0	2	0	0.1574
T16093C	3	6.6	0	0	1	1	1	0.6899
C16111T	2	4.5	0	0	1	1	0	1
C16114T	1	2.2	1	0	0	0	0	0.4222
T16124C	1	2.2	0	1	0	0	0	0.2444
T16126C	13	28.9	3	2	3	3	2	0.7825
G16129C	2	4.5	0	1	1	0	0	0.5879
G16145A*	3	6.6	0	1	0	0	2	0.01029
C16148T	1	2.2	0	0	1	0	0	1
A16163G	1	2.2	0	0	0	1	0	0.6444
C16179T	1	2.2	1	0	0	0	0	0.4222
A16182C	1	2.2	1	0	0	0	0	0.4222
A16183C	3	6.6	0	1	2	0	0	0.5794
C16184T	2	4.5	1	0	0	0	1	0.1242
C16186T	1	2.2	0	0	0	1	0	0.6444
T16189C	7	15.6	2	0	4	1	0	0.5914
C16192T	1	2.2	0	0	0	1	0	0.6444
T16195G	1	2.2	0	0	1	0	0	1
C16197G	1	2.2	0	0	0	1	0	0.6444
C16197T	2	4.5	1	0	1	0	0	0.8384
C16201A	4	8.9	1	0	2	1	0	1
C16201T	1	2.2	0	0	1	0	0	1
T16209A	3	6.6	0	0	2	1	0	0.9098
C16211A	4	8.9	1	1	1	1	0	0.9356
C16214A	2	4.5	0	0	2	0	0	0.7091
T16217C	3	6.6	1	0	0	1	1	0.2519
T16217A	1	2.2	1	0	0	0	0	0.4222
C16223T	14	31.1	4	0	6	3	1	0.3463
C16234T	1	2.2	0	0	1	0	0	1
C16239T	3	6.6	1	0	2	0	0	0.8252
T16243C	1	2.2	0	0	1	0	0	1
C16245G	3	6.6	1	0	1	1	0	1
C16248T	1	2.2	0	0	1	0	0	1
T16249C	4	8.9	0	0	3	1	0	0.6752
C16256T	1	2.2	0	0	1	0	0	1
A16258C	4	8.9	1	0	2	1	0	1
C16261T	2	4.5	0	1	0	0	1	0.08384
A16265C	5	11.1	1	0	1	2	1	0.6708
C16266T	1	2.2	0	0	1	0	0	1
A16269C	3	6.6	0	0	2	1	0	0.9098
C16270T	2	4.5	0	0	1	0	1	0.4909
A16272T	1	2.2	1	0	0	0	0	0.4222
G16273A	1	2.2	0	0	1	0	0	1

G16274A	1	2.2	0	0	1	0	0	1
A16275G	1	2.2	1	0	0	0	0	0.4222
T16276A	4	8.9	1	0	2	1	0	1
T16288C	2	4.5	0	0	1	0	1	0.4909
C16292T	3	6.6	1	0	1	1	0	1
A16293C	3	6.6	0	0	2	1	0	0.9098
C16294T	4	8.9	0	1	1	1	1	0.6013
C16296T	6	13.4	3	0	1	1	1	0.2048
T16298C	1	2.2	0	0	0	0	1	0.1111
C16301A	2	4.5	0	0	1	1	0	1
T16304C	1	2.2	0	1	0	0	0	0.2444
A16309G	2	4.5	0	0	1	1	0	1
T16311C	6	13.3	0	0	4	1	1	0.4599
A16316G	1	2.2	0	0	1	0	0	1
A16318T	3	6.6	1	0	1	1	0	1
G16319A	3	6.6	1	0	0	0	0	0.4222
T16325C	2	4.5	0	0	0	2	0	0.1697
C16327T	3	6.6	0	0	1	1	1	0.6899
C16327A	1	2.2	1	0	0	0	0	0.4222
C16355T	1	2.2	0	0	0	1	0	0.6444
T16356C	2	4.5	0	0	1	1	0	1
T16357C	2	4.5	0	0	1	0	1	0.4909
T16359C	1	2.2	0	0	1	0	0	1
T16362C	9	20	4	0	1	3	1	0.06371
G16390A	2	4.5	1	0	0	0	1	0.1242
A16399G	1	2.2	0	1	0	0	0	0.2444
C16519T	12	26.7	1	3	3	4	1	0.4209
*Show statistically significant (P<0.05)								

DISCUSSION

Many studies demonstrated that mitochondrial DNA abnormalities play an important role in cancer and neurodegenerative diseases [5,19-22]. As Di Mauro reported in 2001, the mitochondrial genome is extremely susceptible to mutations because of the low level of mismatch repair (MMR) and the high level of reactive oxygen species (ROS) produced in the organelle [23]. In addition, many somatic mutations with unclear implication identified silent or occur in non-coding regions of mtDNA [24]. The non-coding D-loop region involves in many function including replication, transcription, and organization of the mitochondrial genome. It has been established that D-loop mutations resulting in instability of mitochondrial genome. Mutations in coding region of mtDNA change protein synthesis, and eventually affect the respiration chain function which confines the cell energy and ROS production [25,26]. ROS leads to genome injury and then induces cancer progression and/or neurodegenerative disorders such as Huntington's disease, Alzheimer's disease, Parkinson's disease, and Multiple sclerosis [5,27-30]. Although mutations occur throughout the entire mitochondrial genome, D-loop is the most variable region of human mitochondrial genome [31].

Iran, especially northeast part, is one of the main endemic areas for HTLV-1infection with prevalence of 3.4% in the general population [17]. The origin of such geographical distribution in Iran is not well understood and is still the matter of many hypotheses. Hence, the study of accumulation of mtDNA nucleotide variations may provide an explanation for association with HTLV-I in this region.

In the present study, we evaluated the variation of the mtDNA D-loop region in HTLV-I patients and healthy controls. Using sequence alignment, 164 CR polymorphisms were found in both the HTLV-I group and the control group, according to the diseases-associated mtSNPs in the D-loop locus in Mitomap database (http://www.mitomap.org). Remarkably, 89 of them were found to have asignificantly higher frequency in patients with HTLV-I compared to the control cohort (P<0.05). According to the previous studies, a high rate of mutations has been reported in the D-loop region in some types of cancers including Medulloblastoma [32], prostate [33], gastric [15] and colorectal carcinomas [34], and also various diseases such as Parkinson [35] and Neurofibromatosis type-1 [36]. Studies demonstrated

that 20% to 70% of somatic mtDNA mutations were found in non-coding regions [36]. Our findings presented that the majority of the base substitution variations were either C to T (32.14%) or T to C (17.85%) followed by C to A (12.85%). In this study, the incidence of C150T transition in the women population infected with HTLV-1 was higher than that of men. Researches have been reported that C150T polymorphism in mtDNA D-loop could be an important factor in respiratory morbidity among children. Besides, the C150T variant is associated with an increased risk of cervical cancer caused by HPV infection [37,38]. This polymorphism has been reported in prostate [33] and thyroid tumours as well as hepatocellular carcinoma [33,39,40].

Schmuczerova, et al. in 2009 showed the frequency of T152C variant (19.7%) plays an important role in the respiratory diseases in children [38]. Moreover, T152C variant has been reported in ovarian cancers [41], as well as Alzheimer's disease by Coskun, et al. in 2004 [42]. These findings are consistent with our result that T152C variantwas the second most common polymorphism among the patients with HTLV-1 (28.9%).

Here, we reported the other polymorphisms in the D-loop region of patients with HTLV. For example, 204T/C, 207G/A, 16051A/G, 16069C/T and 16145G/A polymorphisms of the mtDNA control region showed significant differences in HTLV-1 patients group ($P<0.05$). The 16069C/T polymorphism has been reported in bladder cancer [43], endometrial cancer [44], breast cancer [45], pancreatic cancer, prostate cancer [46] and age-related macular degeneration [47]. Hence, it has been suggested that, mitochondrial 16069T polymorphism plays an effective role in carcinogenesis.

Recent studies have shown multiple deletions in non-D-loop mtDNAin familial mitochondrial myopathy [48]. On the other hand, in 2006 Kamalidehghan reported a8.9 kb deletion in mtDNA of gastric cancer tumoral cells [19,48,49]. But, we could not find deletion in mtDNA of HTLV-1 infected group. More investigation needs to be done to clarify the association of mtDNA deletion and pathogenesis of various types of diseases.

According to our hypothesis, the occurred mutations in the D-loop region of the HTLV-1 infected individuals may interfere with the transcription of the whole mtDNA, and likely cause significant changes in the mitochondrial function; in other words, the increase in ROS may result from mutations in the D-loop region in HTLV-1 patients and lead to development of the related cancer. Regarding HTLV-1 is an oncovirus, it might lead to adult T-cell lymphoma leukaemia (ATLL), although the role of mitochondrial mutations has not been determined yet in the progression of ATLL.

CONCLUSION

In this study, we presented high frequency of D-loop mutations in HTLV-I infected individuals compared to control group. Several polymorphisms were reported that some of them presented for the first time. These findings could be important in understanding of molecular mechanisms in recognition of acquired and/or inherited mitochondrial dysfunction that might be correlated with HTLV-I related diseases.

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