Determination of Sero- epidemiological of HTLV-1 and HTLV-2 among Blood Donors in Guilan Province of Iran

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The unknown cases of serological diagnosis of HTLV-1, 2 virus are of the issues in blood transfusion centers. So in this study, we investigated and determined the sero-epidemiological prevalence of HTLV-1 and HTLV-2 among blood donors in Guilan province of Iran. In this experimental study, 200 serum samples of blood donors were evaluated in terms of HTLV-1, 2 viruses by ELISA screening. Repeatability of positive cases was confirmed using Western blot analysis. In Western blot analysis, the number of 50 persons of the donors was reported as negative and there were significant differences between donors with negative and positive results in terms of age and sex. Uncertain cases in the serology of HTLV infection are of the problems of blood transfusion centers around the world. The prevalence of unknown cases among blood donors in Guilan province of Iran was determined more than non-endemic areas and lower than endemic regions.

Key words: HTLV-1 and 2, blood donors, prevalence, ELISA

The human T-lymph tropic virus or human T-cell lymph tropic virus (HTLV) family of viruses are a group of human retroviruses that are known to cause a type of cancer called adult T-cell leukemia/lymphoma and a demyelinating disease called HTLV-I associated myelopathy and tropical spastic par paresis (HAM or TSP)[13, 23, 24]. The HTLVs belong to a larger group of primate T-lymph tropic viruses (PTLVs)[15].

Members of this family that infect humans are called HTLVs, and the ones that infect old world monkeys are called Simian T-lymph tropic viruses (STLVs)[6]. To date, four types of HTLVs (HTLV-I, HTLV-II, HTLV-III, and HTLV-IV) and four types of STLVs (STLV-I, STLV-II, STLV-III, and STLV-IV) have been identified [11]. The HTLVs are believed to originate from interspecies transmission of STLVs[3]. The original name for HIV, the virus that causes AIDS, was HTLV-III; this term is no longer in use[4]. The HTLV-1 genome is diploid, composed of two copies of a single-stranded RNA virus whose genome is copied into a double-stranded DNA form that integrates into the host cell genome, at which point the virus is referred to as a prover[22].

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A closely related virus is bovine leukemia virus BLV[17].

HTLV-I is an abbreviation for human T-cell lymphoid tropic virus type 1, also called human T-cell leukemia type 1, a virus that has been seriously implicated in several kinds of diseases, including HTLV-I-associated myelopathy, and as a virus cancer link for leukemia (see adult T-cell leukemia/lymphoma)[7]. HTLV was discovered by Robert Gallo and colleagues in 1980[20]. Between 1 in 20 and 1 in 25 infected persons are thought to develop cancer as a result of the virus[6]. A virus closely related to HTLV-I, also discovered by Robert Gallo and colleagues[19], HTLV-II shares approximately 70% genomic homology (structural similarity) with HTLV-I[10].

When HIV, the virus that causes AIDS, was characterized in 1984 by Robert Gallo [14], he named it HTLV-III. HTLV-III is currently the name used to describe another virus related to HTLV-I and HTLV-II. [Citation needed] "HTLV-IV" has been used to describe recently characterized viruses [2]. These viruses were discovered in 2005 in rural Cameroon, and where, it is presumed, transmitted from monkeys to hunters of monkeys through bites and scratches[21].

HTLV-III is similar to STLV-III (Simian T-lymph tropic virus 3)[16]. Multiple strains have been identified[2]. It expresses gag, poll, and envoy, among other proteins[13]. HTLV-IV does not resemble any known virus[4]. It is not yet known how much further transmission has occurred among humans, or whether the viruses can cause disease[22].

The use of these names can cause some confusion, because the name HTLV-III was one of the names of HIV in early AIDS literature, but has since fallen out of use[11]. The name HTLV-IV has also been used to describe HIV-2 [22]. A large Canadian study documented this confusion among healthcare workers, where >90% of HTLV tests ordered by physicians were actually intended to be HIV tests[7].

HTLV-I and HTLV-II can be transmitted sexually[1] and via breast feeding. Screening of blood donors for these two viruses from the middle decade 1980 Gregorian in Japan started and gradually in other regions of the world such as the United States of America, Canada, France, Holland and Denmark became common[8]. In our country, also of the year 1994 Gregorian, screening of blood donors in Gilan province that is the endemic area about contamination with this virus has been done[12] using anti-HTLV antibody detection via ELISA[26]. Positive cases were evaluated more by confirmatory procedures such as western blotting[25]. In this method the possibility of the virus type detection has provided using specific recombinant proteins. According to Iranian Blood Transfusion Organization standards, donors' products for which ELISA test is positive are destroyed and they are exempted permanently from donating blood. However, the verification test can be done for them [9]. Considering that in Gilan province in which contamination is endemic with this virus, so far a study has not been performed in this field, it was the aim of this cross sectional study [18] to determine the sero-epidemiological prevalence of HTLV-1 and HTLV-2 among blood donors in Guilan province of Iran, as well as serological, demographic and blood group characterization and then comparison of results with healthy donors and positive donors in terms of HTLV-1,2 [5].

MATERIALS AND METHODS

From April 2012 to September 2013 a total of 200 blood samples was awarded by donors in Gilan province of Iran. The age range of donors was between 17 to 65 years old. Whole the donors were interviewed by physicians before donating and were eligible based on Iranian Blood Transfusion Organization standards. One third of these individuals were regular donors and the rest were donors with a history.

All donors were evaluated in terms of contamination with HTLV-1, 2 by ELISA screening. Repeatability of positive cases was confirmed using western blot analysis. Serological results and demographic characteristics of interest were extracted by software system and statistical analysis was performed by using the chi-square test. Characteristics of these individuals, including age, genus and blood group were extracted from donors information software.

The screening was perfomed using antibodies against HTLV-1,2 virus by an ELISA Kit (Gene labs HTLV-1/2 ELISA 3.0, Singapore) based on Kit instructions. Positive samples also

were tested with the same kit and positive results were repeatable. Obtained results were interpreted based on the kit regulations.

Accordingly, if GD21 and rgp46-1 were positive, the samples were reported as positive HTLV-1 and if GD21 and rgp46-2 were positive, the samples were reported as positive HTLV-2. Positive bands p19, p24 and GD21 without the presence of specific bands type rgp46-1 and rgp46-2 also were considered as positive HTLV.

The relative abundance of unknown western blot results was calculated by determining the confidence interval of 95%. Comparison of age, sex, blood group between the different groups and also a comparison of the relationship between western blot bands and any of age, sex and blood group results were performed by Chi-square test using the SPSS 11.5 software and the level of significant differences were placed <P 0/05.

RESULTS

200 serum samples of blood donors (containing 100 men and 100 women) were assessed at a time-interval of 18 Months in terms of HTLV-1,2 viruses by ELISA screening and western blot confirmatory method.

The highest percentage of frequency of the band rgp46-2 was 33.3, followed by the gangs GD21 and rgp46-1 28/3% and 8/6%, respectively. Only there was a relationship between blood groups and GD21 and not a significant relationship between the age of the donors and western blot gangs.

There was a significant correlation between the optical absorption ratio to cut off in an ELISA test so that the gp21, rgp46-1, and p19 bands were associated with higher optical density in ELISA.

A significant difference was not observed between HTLV-1,2 positive and unknown donors in terms of blood group. HTLV-1,2 positive persons compared with unidentified persons were older. Most of HTLV-1,2 positive samples were related to women and unknown cases were observed in men.

182 persons of blood donors were reported positive in terms of HTLV-1/2. In these cases 80 persons were positive for HTLV-1 and

Table 1. Western blot test results donors with positive ELISA repeatable to disaggregated by sex

P value	Positive		Unclear		Negative		
0/02	Percent	Number	Percent	Number	Percent	Number	
	48	152	23	87	17/5	120	Man
	10	120	1/5	59	2/5	58	Woman
	58	272	24/5	149	20	178	Total

Table 2. Distribution of healthy donors, *donors with result HTLV Unclear and HTLV Positive, based on blood group

P value	Positive		Unclear		Healthy		Blood group
0/28	Percent	Number	Percent	Number	Percent	Number	A+
	10/4	28	25	24	26/5	43	B+
	4/23	10	15	34	10	56	O+
	23/56	45	7	20	14	30	AB+
	10/9	23	9	16	5/5	20	A-
	9/12	13	23/3	40	15	30	B-
	15/5	23	15/9	34	20	10	O-
	24/57	40	6/56	23	3	11	AB-
	98//28	182	82/76	191	94	200	Total

* healthy donors people who ELISA test result is negative.

102 persons were positive for the HTLV-2.

There were 40 percent of donors from our own that 48% were male and 16% were female. Since in healthy donors the percentage of women was 7/2, with the comparison of these two amounts there was a significant difference in terms of genus between positive and healthy donors.

There was no significant difference between healthy and unknown groups in terms of blood group, age and genus.

DISCUSSION

In a total of 200 blood donors from Gilan province of Iran, were examined in terms of contamination with HTLV-1/2 by ELISA and western blot, the prevalence of uncertain cases was 19 study, which almost was suited to the amount obtained in other studies. The prevalence of uncertain cases of donors in endemic parts of Gilan province in terms of HTLV-1 , 2 compared with donors in non-endemic regions such as: French, United States is more, but compared with donors in endemic areas such as: Brazil and central areas of Africa is less. The Specific reason for this difference is not mentioned, but it should be also mentioned that each endemic region can have its own unique characteristics.

The observed difference between unclear

Table 3. Distribution with the result, donors HTLV Unclear According to the western blot bands

Percentage of positive cases	Number of positive cases	Band
32/1	110	Rgp46-2
27/9	102	GD21
9/6	25	Rgp46-1
3/8	9	P24
1/4	6	Gp21
1/2	5	p19
0/7	3	P28
4/5	15	Rgp46-1 and rgp46-2
2/9	12	Rgp46-1 and GD21
3/7	10	rgp46-2 and GD21
1/2	6	rgp46-2 and gp21
3/3	4	rgp46-2 and p24
1/1	13	rgp46-1, gp21, and GD21
2/2	9	rgp46-1, rgp46-2, and GD21

Table 4. Results obtained of those HTLV-1 and HTLV-2 infected in Blood donors

WB pattern	ELISA** Biokit	ELISA** vironostika	Sample code
Indet-P24	-	+	1
HTLV-1	+	+	2
HTLV-1	+	+	3
HTLV-1	+	+	4
HTLV-1	+	+	5
Indet-P24,P19, P26, P28, P36, P46-1	+	+	6
HTLV-1	+	+	7
HTLV-1	+	+	8
HTLV-1	+	+	9
HTLV-1	+	+	10
HTLV-1	+	+	11
HTLV-1	+	+	12
Indet, (HGIP)	+	+	13
HTLV-1	+	+	14
Indet.P19	+	+	15
HTLV-1	-	+	16
HTLV-1	+	+	17
HTLV-1	+	+	18
HTLV-1	+	+	19
HTLV-1	+	+	20
Indet.P24, Indet.P28	+	+	21
Indet.P19	+	+	22
HTLV-1	+	+	23
HTLV-1	-	+	24
HTLV-1	-	+	25
Indet (HGIP)	+	+	26
HTLV-1	+	+	27
HTLV	+	+	28
HTLV-1	-	+	29
HTLV-1	+	+	30
Indet.P24 P28	+	+	31
HTLV-1	+	+	32

Table 5. Demographic characteristics of HTLV-1/2

Age	Range	19-54
	Mean	34/6
	Female	35/2
	Male	36
Gender	Female	8
	Male	27
Origin	Arequipa	27
	Puno	5
	Cuzco	3

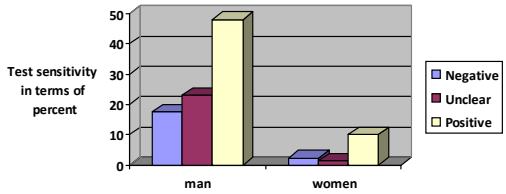

Fig. 1. Comparison of HTLV positive values between men and women.

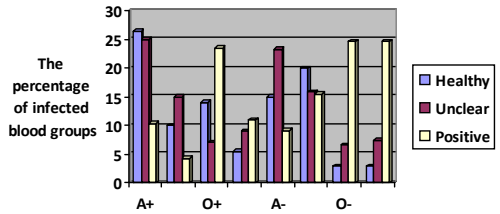

Fig. 2. Comparison of HTLV positive values between blood groups

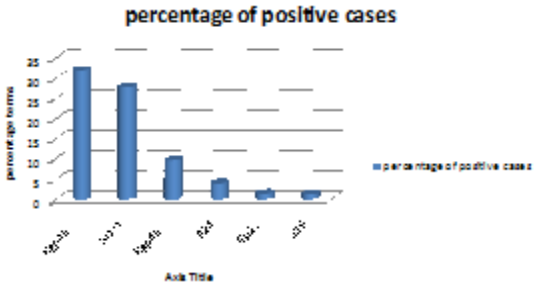

Fig. 3. The percentage of positive cases of HTLV in ELISA and Western blot

and positive individuals in terms of parameters of age and gender in our study was also reported in other studies. Though the reason for this difference is not mentioned. Of reviews 200 donated blood units in terms of contamination with HTLV-1 2 in Gilan province turned out, prevalence of that is 0/4% infection, in a previous study in the year 2003 in blood donors of Rasht city the prevalence of infection was reported 0/72%, that the reason for this the difference can be improved screening methods mentioned before donating blood.

CONCLUSION

Uncertain cases in the serology of HTLV infection are of the problems of blood transfusion centers around the world. Although the studies conducted show that most of the unclear cases are negative in terms of HTLV infection, but there is some seroconversions and even the HTLV genome existence in blood in unclear individuals, considering in some of serological and epidemiological characteristics in these individuals pre advertising and positivity, in terms of HTLV helps, performed more studies are needed, as well as the pursuit of the unclear appears.

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