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Original Article

Xenotropic Murine leukemia related virus (XMRV) in Iranian HIV Patients

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Abstract

Background and Aims: Despite isolation of Xenotropic murine leukaemia related virus (XMRV) from the patients with prostate cancer in 2006 and patients with chronic fatigue syndrome in 2009, there have been controversial findings about its potential role in human diseases and frequencies in different population groups. The aim of this study was to determine the frequency of XMRV genome in Iranian HIV-infected patients for the first time.
Materials and Methods: We performed a cross-sectional study on the prevalence of XMRV nucleic acid among 150 patients diagnosed with HIV infection in Tehran's hospitals. After extracting the viral RNA from plasma samples, nucleic acid was amplified by Real-Time PCR using XMRV specific primers. HIV viral load testing was performed for all of the patients as well.
Results: Out of 150 patients, XMRV RNA was found in 13 (8.6 %) of patient's specimens, including 9 males (69/2%) and 4 females (30/8%). Average HIV viral load was 14471.92 and 17016.66 copies/ml in positive and negative XMRV patients, respectively.
Conclusions: Our results showed the presence of XMRV infection in HIV-infected patients. No other significant association was observed between XMRV with gender, age and HIV viral load of the patients. However, more studies are needed to demonstrate the actual prevalence of XMRV infection by geographical regions and different populations.
Keywords: XMRV, HIV, Iran, Real-Time PCR, Human Immunodeficiency Virus

Introduction

Xenotropic murine leukemia related virus (XMRV) was first described in 2006 by ViroChip technology, they conducted a study to find infectious agents in prostate cancer (PC) (1). Familial PC is associated with RNASEL gene mutation; This gene encodes RNase L, an endoribonuclease acting as a part of the innate immune response against viral infection (2, 3). Urisman used the isolated prostate tissue's RNA of patients with familial PC and placed them in a ViroChip probe which contained in oligonucleotides bearing conserved sequences of viral reference genomes. Majority of samples from the patients bearing a missense mutation in RNASEL could be hybridized to virus nucleotide sequences, particularly those associated with murine leukemia virus (MLV). Whole genome sequencing was performed and data showed homology with genomes of endogenous MLVs. Consequently, this new virus could be considered as a strain of xenotropic MLV (XMRV) which was the first potential human gamma retrovirus pathogen (1).

At the beginning, XMRV was isolated from PC patients. Samples contained patient's tissue with missense mutations in RNASEL gene. R462Q missense mutation normally results in a

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decreased enzyme activity (4) and the lack of functional RNase L in mice makes them highly susceptible to viral infection (5). It was suggested that RNASEL gene would be related to an increased risk of PC (6). However, association between XMRV and PC was demonstrated and published as a report in 2009 (7). In this study, severity of prostate cancer was correlated with the incidence of XMRV while there was no link to RNASEL mutation.

The role of XMRV in chronic fatigue syndrome (CFS) has been also investigated. Researchers have done extensive studies for viral causes of CFS. Yet, it has not been confirmed and the supposed correlation between CFS and XMRV has been taken the field by surprise. In the recent studies, XMRV was detected in respiratory secretions, blood and blood component of patients with CFS (8, 9). Furthermore, MLV-like gag sequences were detected in 86.5 % of CFS patient's PBMCs vs. 6.8 % of healthy volunteers (10). However, additional studies could not show any correlation between XMRV and CFS using quantitative PCR and neutralizing antibodies (11-14).

It was demonstrated that XMRV can infect peripheral blood mononuclear cells (PBMCs) and CD4+ T cells in vitro. Thus, XMRV is able to infect the same target cells as HIV-1 (9). However, recent studies has shown that productive replication of XMRV in PBMCs and spread in culture are strongly limited by APOBEC3 proteins, which known as inhibitor of XMRV infection and likely other host defense strategies (15). It is possible that target cells infected with HIV-1 might provide an optimal environment for XMRV replication by depleting cellular host restriction factors such as APOBEC3G (16, 17). The transmission of XMRV is similar to HIV, through sexual contact, blood transfusion and intravenous drug use (18, 19). These facts suggest a need for more studies on HIV infected risk groups who can be co- or superinfected with XMRV. HIV infected Patients experience more infections with other viruses due to their immunocompromised state which result in more efficient replication of other viruses, including XMRV. Hence, there are potential concerns about possible XMRV infections and corresponding outcomes in patients with HIV infection (20). So determination of the prevalence of XMRV in HIV-infected patients can be helpful to investigate the possible link between these two viral infections.

Materials and methods

Study population and specimen collection. A total of 150 Iranian patients with HIV-1 infection from Tehran's hospitals were enrolled in this study from February 2015 to April 2016. The exclusion criteria considered as patients who had received anti-retroviral therapy. This study followed the principles of the Declaration of Helsinki and was approved by the local ethics committee of the Iran University of Medical Sciences, Tehran, Iran. The patients were informed about the current study, and Informed consent was obtained from all the patients prior to their enrollment. About 5 ml of peripheral blood specimen from each participant was collected into a sterile EDTA-containing Vacutainer tube, blood plasma was separated by centrifugation and stored at -70 oC until use.

XMRV RNA Extraction. XMRV RNA was extracted from 200 µl plasma with High Pure Viral Nucleic Acid kit (Roche Diagnostics, Germany). RNA pellets were resuspended in 100µl of pre-warmed Elution buffer and stored at -70°C until use.

cDNA Synthesis. For detection XMRV RNA, reverse transcription real time PCR (rReal Time PCR) was carried out, using the first strand cDNA synthesis kit by Revert AidcDNA synthesis kit (Thermoscientific, USA). In a nutshell, RNA samples were heated in 65°C for 10 minutes, then chilled on ice. The uniform suspension of bulk first-strand cDNA reaction mix was added according to the manufacturer's protocol, then One µl of DTT solution, and 1 µl of random hexamer (1)6 primer (0.2 µg) were added to the heat-denatured RNA. Samples were mixed properly by pipetting up and down for several times and then incubated for an hour at 42°C. For Real time PCR, the QuantiTect Probe PCR Kit

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(Qiagen, Germany) was used, based on its instruction kit.

HIV Viral load. COBAS TaqMan 48 (Roche Diagnostics, Hacienda Drive Pleasanton, CA, USA) kit was used for quantifying HIV RNA in the patient's plasma samples (500 µl) and high pure extraction was used according to the manufacture's recommendation. This method was a Real-Time PCR based on dual-labeled hybridization probe which targeted the highly conserved region of HIV-1 gag gene. Detection limit of the COBAS TaqMan was 48 copies/ml.

Real time PCR for XMRV. A Real Time PCR assay was developed to screen the XMRV's RNA. The primers amplify a 102 bp region containing the 3´ variable regions of the envelope. Primers and probe's sequences and their position with melting temperature are shown in Table 1. So, after alignment of complete genomes, XMRV consist of Nucleotide sequences based on pubmed database. Reactions contained 5µl of cDNA, 2.5 mmol/L MgCl2, 800 mmol/L of dNTPs, 100 ng of each primer,30 ng of probe and 1.5 units of QuantiTect Probe PCR Kit (Qiagen, Germany) to a total volume of 15 µL. Thermal cycling conditions were as follows: 95°C for 10 min; 40 cycles of 95°C for 15 sec, 60°C for 40 sec, Quantitative determination of the amplified products have done by the BioRad CFX-96 instrument (BioRad, USA). In order to synthesis our ideal genes, tests should be done by two pairs of forward and reveres primers. Our specific probes were designed by different fluorescent labels to track our targets separately.

Statistical analysis. The statistical analysis was performed using the Statistical Package for Social Sciences software version 21 (SPSS Inc, Chicago, IL, USA). Categorical variables were compared by Fisher's exact test or the chi-square test as appropriate. Continuous variables was analyzed using Student's t test. Data are presented as absolute counts, proportions [95% binomial exact confidence intervals (CI)], medians [interquartile range (IQR)], and means [standard deviation (SD)]. For all comparisons, p-value less than 0.05 was considered statistically significant.

Results

A total of 150 participants with HIV-1

Table 1: Sequences of primers, probes and their position in addition of melting temperature

	Sequence	Melting temperature	Position
Forward primer	CCTGTCTATCTACTACTGTG	59.8	725
Reverse primer	GGCCATAAACATAATTAGGG	59.9	826
XMRV probe	CCAGAGTTCAACCAGGACACAGTA	69	786

Table 2: The relationship between XMRV positive patients and gender

XMRV	Male	Female	p.value (Fisher exact test)
Positive	9 (69.2%)	4 (30.8%)	.79
Negative	86 (63.2%)	49 (36.8%)	

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Table 3: The relationship between XMRV positive patients and age

XMRV	N	Mean	Std. Deviation	p.value
Positive	13	40.9231	12.47305	.18
Negative	137	35.8759	12.95492	

Table 4: The relationship between XMRV positive patients and HIV viral Load (copies/ml)

XMRV	N	Mean (HIV viral load)	Std. Deviation	p.value
Positive	13	14471.92	16488.765	.61
Negative	137	17016.66	22785.581	

infection were enrolled in this study. The mean age of the patients was 36.51 ± 12.75 (range 4–64) years. Among 150 participants, 95 (63.3 %) were male and 53 (35.3 %) were female. Real-Time PCR assay for XMRV nucleic acid detection resulted in 13 (8.6%) positive samples out of 150 subjects, including 9 males (69/2%) and 4 females (30/8%). Based on the analysis by Fisher exact test, no significant association was observed between XMRV and gender of the patients (p value= 0.79) (Table 2).

In our study, half of the HIV-1 infected patients were over 30 years of age, while the other half were under 30 years. The mean age for 13 XMRV positive patients and 137 XMRV negative ones was 40.9 and 35.8 years respectively. There was no significant correlation between age and XMRV's RNA positivity (p value= 0.18) (Table 3).

By measuring the viral load, we could examine the relationship between HIV viral load and XMRV infection. The average HIV viral load in positive and negative XMRV patients was 14471.92 and 17016.66 respectively but t-test analysis showed no association between XMRV-positive RNA and HIV Viral Load (p value= 0.61) (Table 4).

Discussion

Our study is the first attempt in order to evaluate the presence of XMRV in blood samples which have been collected from HIV-1-infected patients in Tehran, Iran. In this study we used Real-Time PCR method mainly because it is more sensitive and reliable than other assays for detection of infection. Since XMRV could be potentially inhibited by anti-retroviral therapy (ART), especially AZT (21), none of our patients received AZT treatment. Thus, our results is not affected by the suppression of antiretroviral therapy.

For the first time, researchers in the United States have found a frequency of 10% to 27% of XMRV in prostate cancer patients (1, 7, 22). Prevalence of XMRV is 40% in prostate cancer patients carrying a homozygous R462Q mutation in the RNaseL gene (1). Also it was suggested that XMRV has a high prevalence (67%) in CFS patients in the United States (9).

Our data showed 13 (8.6%) studied patients of 150 individuals who were infected with HIV-1 had XMRV nucleic acid which was demonstrated by Real-Time PCR as 3´ variable regions of envelope gene. It is notable that negative test results would happen for some reasons. For instance, the potential genetic variation of XMRV could lead to “false-negative” results (23). However, the present sequenced data indicate that XMRV is highly conserved (9, 24) and it is likely that different strains of XMRV may exist (23, 24). In addition, some of the HIV-infected patients would have been infected with XMRV, but with short-term viremia or without any notable blood infection. Also, XMRV infection has a restricted geographic distribution and focal outbreaks of XMRV infection can occur in the specific areas and populations. For example,

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XMRV was exclusively detected in patients with prostate cancer in the United States (25) and CFS patients in one US study at the present time (9), but occasionally XMRV infection was found in 2.3% to 9.9% of patients with respiratory tract infections in Europe, with the exception of a recent German study (8). Finally, XMRV is a recently discovered human virus and its epidemiologic data are limited. Hence, comprehensive studies about the worldwide incidence of XMRV infection using well-standardized assays is needed.

Conclusion

In summary, we tested 150 blood samples from HIV-1-infected patients in Tehran, Iran for presence of XMRV nucleic acid using sensitive Real-Time PCR method. Thirteen samples were found positive and the prevalence of XMRV nucleic acid in HIV RNA positive patients was 8.6 %. Nevertheless, it should be considered that the small sample size can limit the statistical power of the study and the results. Therefore, more studies are needed to demonstrate the global epidemiologic and geographic distribution of XMRV.

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Conflicts of interest

There are no conflicts of interest.

References

1. Urisman A, Molinaro RJ, Fischer N, Plummer SJ, Casey G, Klein EA, et al. Identification of a novel Gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant. PLoS pathogens. 2006;2(3):e25.
2. Hassel BA, Zhou A, Sotomayor C, Maran A, Silverman RH. A dominant negative mutant of 2-5A-dependent RNase suppresses antiproliferative and antiviral effects of interferon. The EMBO journal. 1993;12(8):3297-304.
3. Zhou A, Hassel BA, Silverman RH. Expression cloning of 2-5A-dependent RNAase: a uniquely regulated mediator of interferon action. Cell. 1993;72(5):753-65.
4. Casey G, Neville PJ, Plummer SJ, Xiang Y, Krumroy LM, Klein EA, et al. RNASEL Arg462Gln variant is implicated in up to 13% of prostate cancer cases. Nature genetics. 2002;32(4):581-3.
5. Zhou A, Paranjape J, Brown TL, Nie H, Naik S, Dong B, et al. Interferon action and apoptosis are defective in mice devoid of 2',5'-oligoadenylate-dependent RNase L. The EMBO journal. 1997;16(21):6355-63.
6. Silverman RH. A scientific journey through the 2-5A/RNase L system. Cytokine & growth factor reviews. 2007;18(5-6):381-8.
7. Schlaberg R, Choe DJ, Brown KR, Thaker HM, Singh IR. XMRV is present in malignant prostatic epithelium and is associated with prostate cancer, especially high-grade tumors. Proceedings of the National Academy of Sciences of the United States of America. 2009;106(38):16351-6.
8. Fischer N, Schulz C, Stieler K, Hohn O, Lange C, Drosten C, et al. Xenotropic murine leukemia virus-related gammaretrovirus in respiratory tract. Emerging infectious diseases. 2010;16(6):1000-2.
9. Lombardi VC, Ruscetti FW, Das Gupta J, Pfost MA, Hagen KS, Peterson DL, et al. Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome. Science. 2009;326(5952):585-9.
10. Lo SC, Pripuzova N, Li B, Komaroff AL, Hung GC, Wang R, et al. Detection of MLV-related virus gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors. Proceedings of the National Academy of Sciences of the United States of America. 2010;107(36):15874-9.
11. Erlwein O, Kaye S, Robinson M, McClure M. Chronic fatigue syndrome: xenotropic murine leukemia virus-related virus, murine leukemia virus, both, or neither? Proceedings of the National Academy of Sciences of the United States of America. 2010;107(43):E161; author reply E3-4.
12. Groom HC, Boucherit VC, Makinson K, Randal E, Baptista S, Hagan S, et al. Absence of xenotropic murine leukaemia virus-related virus in UK patients with chronic fatigue syndrome. Retrovirology. 2010;7:10.
13. Hohn O, Strohschein K, Brandt AU, Seeher S, Klein S, Kurth R, et al. No evidence for XMRV in German CFS and MS patients with fatigue despite

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[DOI: 10.21859/isv.10.1.8]

the ability of the virus to infect human blood cells in vitro. PloS one. 2010;5(12):e15632.
14. Switzer WM, Jia H, Hohn O, Zheng H, Tang S, Shankar A, et al. Absence of evidence of xenotropic murine leukemia virus-related virus infection in persons with chronic fatigue syndrome and healthy controls in the United States. Retrovirology. 2010;7:57.
15. Chaipan C, Dilley KA, Paprotka T, Delviks-Frankenberry KA, Venkatachari NJ, Hu WS, et al. Severe restriction of xenotropic murine leukemia virus-related virus replication and spread in cultured human peripheral blood mononuclear cells. Journal of virology. 2011;85(10):4888-97.
16. Groom HC, Yap MW, Galao RP, Neil SJ, Bishop KN. Susceptibility of xenotropic murine leukemia virus-related virus (XMRV) to retroviral restriction factors. Proceedings of the National Academy of Sciences of the United States of America. 2010;107(11):5166-71.
17. Paprotka T, Venkatachari NJ, Chaipan C, Burdick R, Delviks-Frankenberry KA, Hu WS, et al. Inhibition of xenotropic murine leukemia virus-related virus by APOBEC3 proteins and antiviral drugs. Journal of virology. 2010;84(11):5719-29.
18. Sharma P, Rogers KA, Suppiah S, Molinaro RJ, Onlamoon N, Hackett J, Jr., et al. Sexual Transmission of XMRV: A Potential Infection Route. Advances in virology. 2011;2011:965689.
19. Jahanbakhsh F, Hattori J, Matsuda M, Ibe S, Monavari SH, Memarnejadian A, et al. Prevalence of transmitted HIV drug resistance in Iran between 2010 and 2011. PloS one. 2013;8(4):e61864.
20. Jahanbakhsh F, Ibe S, Hattori J, Monavari SH, Matsuda M, Maejima M, et al. Molecular epidemiology of HIV type 1 infection in Iran: genomic evidence of CRF35_AD predominance and CRF01_AE infection among individuals associated with injection drug use. AIDS research and human retroviruses. 2013;29(1):198-203.
21. Sakuma R, Sakuma T, Ohmine S, Silverman RH, Ikeda Y. Xenotropic murine leukemia virus-related virus is susceptible to AZT. Virology. 2010;397(1):1-6.
22. Arnold RS, Makarova NV, Osunkoya AO, Suppiah S, Scott TA, Johnson NA, et al. XMRV infection in patients with prostate cancer: novel serologic assay and correlation with PCR and FISH. Urology. 2010;75(4):755-61.
23. Kean S. Virology. An indefatigable debate over chronic fatigue syndrome. Science. 2010;327(5963):254-5.
24. Coffin JM, Stoye JP. Virology. A new virus for old diseases? Science. 2009;326(5952):530-1.
25. Silverman RH, Nguyen C, Weight CJ, Klein EA. The human retrovirus XMRV in prostate cancer and chronic fatigue syndrome. Nature reviews Urology. 2010;7(7):392-402.

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