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Parvovirus 4 in Individuals with Severe Hemophilia A and Matched Control Group

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ABSTRACT

Background: Hemophilia is a well-known bleeding disorder with worldwide distribution. Replacement therapy, using plasma-derived or recombinant coagulation factors, comprises a gold standard regimen for the treatment. Regardless of the advancements made in viral inactivation methods in the production of plasma-derived coagulation factors, the possibility of transmission of new viral infections remained as a noticeable concern yet. The aim of the current study was to investigate the status of parvovirus 4 (PARV4) in severe hemophilia A, von Willebrand disease (vWD), and healthy control.

Materials and Methods: In the current case-control study, 76 patients with hemophilia and vWD and 60 individuals from their family members entered the study. Nested PCR used to determine the presence of PARV4 in study subjects (76 cases). To characterize the PARV4 genotype, positive samples subjected to sequencing and phylogenetic analysis.

Results: PARV4 genome detected in 11 (14.47%) patients with bleeding disorders. Among whom, nine patients (14.75%) were with severe hemophilia A and two (13.33%) patients with vWD. Only five healthy controls (8.33%) were positive for PARV4. All PARV4 sequences were found to be genotype 1.

Conclusion: PARV4 infection in patients with hemophilia and vWD was higher than the control group. While detection of PARV4 DNA in patients with bleeding disorders may not necessarily reflect a clinical urgency, future investigations are needed to define the clinical significance of PARV4. It seems the detection of the virus immune signature of PARV4 infection, particularly in the context of acute and persistent infections, needs to focus on cellular and tissue targets.

Keywords: Hemophilia; Parvovirus 4; von Willebrand disease; Viral infection

INTRODUCTION

Hemophilia A is an X-linked bleeding disorder, which occurs due to lack or insufficient levels of coagulation factors VIII and IX in plasma. Replacement therapy using plasma-derived or recombinant coagulation factor concentrates is the mainstay treatment in these patients[1, 2]. While introducing plasma-derived coagulation factors

have brought new hopes in the 1980s, contaminated blood product with human immunodeficiency virus (HIV), hepatitis C virus (HCV), and hepatitis B virus (HBV) underscored the importance of such products in patients with hemophilia. The introduction of virus inactivated plasma-derived products as well as recombinant coagulation factors has revolutionized the management of these patients afterward. However, regardless of the advancements made in viral inactivation methods, the possibility of transmission of new viral infections remained as a noticeable concern. As such, continued surveillance of the patients who receive blood products remains an important ongoing issue, especially in developing countries.

Parvoviruses are non-enveloped DNA viruses that can infect a vast range of hosts like vertebrates and insects. These small viruses are relatively resistant to viral inactivation processes[3]. Among different members of this family, human parvovirus 4 (PARV4) has gained much attention in this regard. PARV4 is an emerging human virus, which originally identified in 2005[4]. Infection with parvoviruses including PARV4 is generally acute with rapid resolution of symptoms and clearance of the viral genome[5]. Although fewer data are available about the risk of PARV4 transmission to blood product recipients, the virus appears parenterally to transmit primarily between members of a family[6]. It has detected in high-risk groups such as injecting drug users (IDUs) particularly those co-infected with HIV, HCV, and even HBV[7, 8].

Recent evidence indicates that PARV4 can also have a relatively high frequency in the general population[9]. Although, the frequency of PARV4 in healthy blood donors and general population varies considerably[10-14] *Simmons et al.* demonstrated the acute onset of PARV4 infection in patients with the absence of HIV, HBV, and HCV viruses[15]. In a study conducted by *Sharp et al.* in 2012, 43% of patients with hemophilia were seropositive for IgG antibody against PARV4[16]. Although, viral loads found to be generally low[17]. There is a similar survey on PARV4 detection in patients with hemophilia from other region of Iran that showed eight (9.3%) patients among 86 patients with hemophilia A and B were positive for PARV4[18]. Detection of PARV4 in plasma-derived products such as factor VIII concentrates highlights the potentiality of the virus for being resistant to viral inactivation procedures[3, 16]. As such, some countries decided to use recombinant clotting factor instead of plasma-derived products in order to prevent the risk of virus transfer to patients receiving these products[19].

Now, no association was found between clinical syndrome and parvovirus 4 infections. In most of the cases, the infection is an asymptomatic and self-limiting event[20, 21].

In line with previous findings together with the concerns raised with regards to the possibility of virus transmission in blood products, we aimed to investigate the prevalence of PARV4 in plasma samples taken from Iranian patients with bleeding disorders including 61 patients with severe hemophilia type A, 15 cases with von Willebrand Disease, and 60 participants as healthy controls.

MATERIALS AND METHODS

Studied subjects

This case-control study performed on patients with hemophilia and healthy subjects. Having coagulation factor VIII or IX below ≤1 % and an age of above 18 years old was considered as inclusion criteria. There are about 1150 patients with bleeding disorders in the northeastern part of Iran (Khorasan Province). As we had access to more than 500 patients[20]. An invitation was sent to patients with severe hemophilia A and members of their family to participate for the purpose of this study. Among invited cases, only 76 cases with bleeding disorders and 60 members of their relatives, which are considered as a healthy control group, were referred to the Ghaem hospital where the blood samples were taken. Among them, 61 patients had severe hemophilia A and 15 cases with von Willebrand disease (vWD). All patients were male with a mean age of 41 ± 0.3 SD years. The control group represented of healthy male persons among the relatives of the patients with a similar age range (Mean ± SD: 38.18 ± 11.07) minimum 19 and maximum age was 61 years old. The patients with hemophilia were on-demand therapy using mainly plasma-derived coagulation factor VIII concentrates.

The patients with vWD in our province have not done vWD profile tests (vWF activity, vWF ristocetin cofactor activity assay, vWF multimer analysis) to detect subtypes of the disorder due to lack of obligatory specific coagulation tests and hemostasis laboratory.

All study subjects were given a description about the aim of the study before signing the consent form. This study was approved by the local ethical committees of both Tehran University of Medical Sciences (No: 22988) and Mashhad University of Medical Sciences (No: 910923).

Clinical symptoms related to PARV4

The patients under study asked about clinical symptoms, including the existence of skin rash, gastrointestinal (GI) or respiratory symptoms, encephalitis (fever and convulsion) and unexplained hepatitis (with minimal fluctuation in results of the liver function tests). Moreover, hemorrhagic symptoms, including nose bleeding, skin bruising; bleeding from minor wounds, oral cavity bleeding, post-dental extraction bleeding, GI hemorrhage, post-surgery bleeding, muscle hematoma, hemarthrosis and central nervous system bleeding asked at the time of blood sampling. Types of replacement therapy (prophylaxis or on-demand regimens) and kinds of the used coagulation factor concentrates (plasma-derived or recombinant) were registered.

Clinical symptoms related to hemophilia and vWD

Among 61 patients with severe hemophilia A, two cases had hemorrhagic symptoms (a case with mild hemarthrosis, a case with bleeding from finger wound). None of the patients with vWD had bleeding symptoms at the time of referring to us. The patient with severe hemophilia A who had hemarthrosis was positive for the parvovirus genome. Furthermore, patients under study did not have any symptoms related to parvovirus infection. A patients with severe hemophilia had elevated laboratory markers of liver function (SGPT=72, and SGOT=54). According to his medical records, this case was positive for HCV infection. All of the patients were on-demand coagulation therapy except that case with hemarthrosis who was under a prophylaxis regimen from the previous week of the sampling.

Laboratory assays

Around five ml of the blood sample took from all study subjects. The sera separated by 10 min centrifugation at 2000 rpm. The DNA extraction performed according to High Pure Viral NucleicAcid Kit (Roche Applied Science, Mannheim, Germany) manufacture's protocol. Nested PCR method was used to detect the presence of PARV4 using previously described the primer pair[21, 22]. Targeting a highly conserved 161 bp fragment within the open reading frame 1 (ORF1) related to NS1 of PARV4.The sequences of the primer pairs consist of PV4ORF1F1 (5'-AAGACTACATACCTACCTGTG-3'), PV4ORF1R2 (5'-GTGCCTTTCATATTCAGTTCC-3'), PV4ORF1F2 (5'-GTTGATGGYCCTGTGGTTAG-3') and PV4ORF1R2 (5'-CCTTTCATATTCAGTTCCTGTTCAC-3'). The first round of each PCR reaction was carried out in a 25µl reaction volume containing of 2.5µl Master mix buffer, 0.5µmol of each two primers (PV4ORF1F1 and PV4ORF1R1), 1U of Taq DNA polymerase (Roche), 0.2µmol dNTPs mix, 5µl DNA template and nuclease-free water, using the following PCR amplification conditions: 35 cycles, 94°C for 5 min, 94°C for 30 Sec, 55°C for 45 Sec, 72°C for 45 Sec and 1 cycle at 72°C for 5 min. The second round was also performed same as the first round with the same PCR amplification condition except for template PCR product (2.5µl) and primers specific for the second round (PV4ORF1F2 and PV4ORF1R2). Amplified PCR products with PV4ORF1F2 and PV4ORF1R2 primers were further sequenced using ABI prism 310 Automatic sequencers (Applied Biosystems, Carlsbad, CA) and multi-aligned with reference sequences strains of PARV4 available in GenBank.

Our positive samples were almost not in order (ie: not in the same run). For quality management, all positive samples rechecked with a second technician to exclude any possible contamination. Moreover, we randomly selected positive samples and outsourced for sequencing. All necessary precautions considered to minimize the cross-contamination. We also tried our best to find a commercial ELISA kit to measure the prevalence of

PARV4 IgG; however, an appropriate kit was not available commercially.

Given the lack of available commercial kits and the technical challenges with regards in-house assays to test the antibodies directed against PARV4 (VLPs, NT, etc), we were not able to test the prevalence of antibodies directed against PARV4 at this stage.

Phylogenetic analysis

Amplified PCR products with PV4ORF1F2 and PV4ORF1R2 primers were further sequenced using ABI prism 310 Automatic sequencers (Applied Biosystems, Carlsbad, CA) and multi-aligned with reference sequences strains of PARV4 available in GenBank. Multiple sequence alignment performed with BioEdit software using the Clustal W method[23]. And the phylogenetic tree was constructed using the Maximum Likelihood model (MEGA X)[24]. The tree measured by the bootstrap method with 1000 replicates.

Statistical analysis

GraphPad Prism software (GraphPad Software, Inc, La Jolla, California) used for statistical analysis and P-value ≤ 0.05 was regarded as significant. Data presented either with median or mean ± SEM.

RESULTS

Looking at the presence of PARV4 genome, 11 (14.47%) patients were positive for PARV4 infection. Among which, nine (14.75%) patients were severe hemophilia A and two (13.33%) were suffering from vWD. Checking healthy group, only five (8.33%) samples found to be positive for PARV4 infection. Although not significant, the rate of PARV4 infection in patients with bleeding disorders found to be higher than healthy controls (Table 1). There was no relation between PARV4 infection and age of patients and control groups (P> 0.05).

Moreover, out of 76 patients included in this study, 33 patients had a history of HCV infection according to the patient's profile. Out of 11 patients with bleeding disorders, who were positive for presence of PARV4 genome, seven patients found to be co-infected with HCV infection while the other four patients had no history of HCV co-infection (P< 0.05). Although, the prevalence of PARV4 was higher among HCV co-infected patients than HCV-negative patients, the difference found was not statistically significant (P>0.05).

To further characterize the circulating genotypes of PARV4 in the studied subjects, samples positive for the presence of PARV4 genome was randomly selected, sequenced, and multiple alignment sequences were subject to phylogenetic analysis using MEGA X software (http://www.megasoftware.net/), and the evolutionary history was inferred using the Maximum Likelihood method. As indicated in Figure 1, the phylogenetic analyses for amplicon 161 *bp* of the *NS1* region showed that all identified sequences in individuals under study (both the case and the control groups) were clustered as genotype 1 when analyzed with reference sequences of available and described genotypes in GenBank.

Moreover, evaluating the relation between PARV4 DNAemia and type of coagulation therapy (on-demand therapy and prophylaxis regimens) was not possible due to all of the patients with hemophilia are under on-demand coagulation therapy. Only patients with hemophilia and inhibitors are under a prophylaxis regimen, which did not recruit in the study. In addition, PARV4 DNAemia and type of coagulation factor (plasma-derived or recombinant factors) did not evaluate statistically because of fluctuation in type of coagulation factors concentrates.

Table 1: The rate of PARV4 infection in patients with severe hemophilia type A and healthy controls

	PARV4 infection		
	Positive	**Negative**	**P**
Hemophilia A (n= 61)	*9 (14.75)	52 (85.25)	P > 0.05
Healthy individuals (n= 60)	5 (8.33)	55 (91.67)	
vWD (n=15)	2 (13.33)	13 (86.67)	

* Indicates number (percentage), vWD: von Willebrand Disease

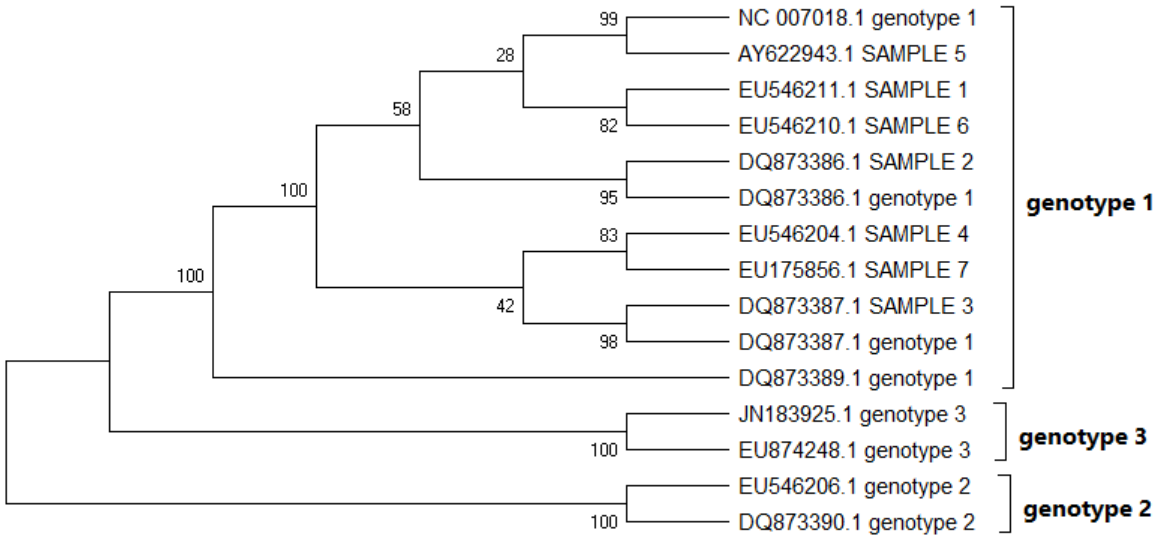

Figure 1. Evolutionary analysis by Maximum Likelihood method

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model [1]. The tree with the highest log likelihood (-11275.18) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. This analysis involved 15 nucleotide sequences. There were a total of 5273 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [2].

1. Tamura K. and Nei M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10:512-526.
2. Kumar S., Stecher G., Li M., Knyaz C., and Tamura K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35:1547-1549.

DISCUSSION

In the present study, the prevalence of parvovirus 4 in patients with hemophilia A and vWD found to be relatively high (14.47%) when compared to healthy controls (8.33%). This difference did not reach statistically significant. The number of recruited patient was not high, it may be due rarity of disorder in part, so we cannot demonstrate if the non-significant difference is due to the small numbers or it is truly no different. Hence, the number of patients entered the study was one of our limitations. Moreover, the number of the healthy control group was lower than recruiting patients (60 versus 76), this was due to the low number of children in families. In some families, there was only an affected child with a bleeding disorder without any sister or brother. There were two children of both sexes in some families, so finding a family with an affected patient and the other member of the same sex was a big challenge. In a study conducted by *Sharp et al*. 43% of hemophilia patients, receiving replacement therapy found to be positive for the PARV4 IgG compared to only 3% positivity in untreated family members. This may suggest a higher risk of seroconversion among patients on replacement therapy.

Detection of PARV4 in healthy blood donors[25, 26] or general population[9] indicates a subclinical infection.

However, the higher frequency of PARV4 in high-risk groups[7, 9], even up to 95%[7, 25] and potential clinical associations of PARV4 infection[27, 28] might indicate the pathogenic nature of PARV4 infection in highly susceptible patients[29]. Moreover, specific genetic polymorphisms may associate with viral specific immune responses[30]. Disease association of PARV4 might also reflect a higher rate of prior exposure to blood-borne viruses with chronic infections[31-33].

Although, the rates of PARV4 DNAemia have been considerably lower in some studies[34], still there are some reports indicating higher rates of PARV4 DNAemia, which is in agreement with our previous findings. In this regard, the detection rate of PARV4 DNA in healthy blood donors was 16.7% (16/96)[35] and 22.2% (43/193)[36, 37] have also reported higher PARV4 DNAemia in healthy controls at the frequency of 24 % and 30%, respectively.

No evidence of clinical disorders has reported in patients with severe hemophilia A who exposed to PARV4. Higher frequency of PARV4 infection has been in patients with hemophilia co-infected with HIV and HCV[16]. In line with this finding, 63.7% (7/11) of PARV4 positive patients found to co-infect with HCV infection in this study. Hence, PARV4 infection might complicate the clinical assessments of the other blood-borne virus infections[38].

The remarkable sequence homogeneity of PARV4 nucleotide sequences shown in our study subjects may simply indicate the recent origin and spread of PARV4[39]. However, a wider geographic sampling of high-risk groups are warranted to broaden our understanding of genetic diversity of PARV4 infection. As replacement therapy comprises the mainstone of hemophilia treatment, coagulation factor concentrates can be as potential sources for PARV4 transmission.

CONCLUSION

Given the relatively high prevalence of PARV4 infection in hemophilia and vWD, further studies need to define the virus immune signature as well as the clinical significance of PARV4 infection particularly in the context of acute and persistent infections with the focus on cellular and tissue targets.

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CONFLICT OF INTEREST

The authors state that they have no conflicts of interest

REFERENCES

1. Mansouritorghabeh H. Clinical and laboratory approaches to hemophilia A. Iran J Med Sci 2015;40(3):194-205.

2. Mansouritorghabeh H, Rezaieyazdi Z, Bagheri M. Successful use of factor VIII concentrate and fresh frozen plasma for four dental extractions in an individual with combined factor V and VIII deficiency. Transfus Med Hemother. 2009;36(2):138-139.

3. Fryer J, Hubbard A, Baylis S. Human parvovirus PARV4 in clotting factor VIII concentrates. Vox Sang 2007;93(4):341-7.

4. Jones MS, Kapoor A, Lukashov VV, et al. New DNA viruses identified in patients with acute viral infection syndrome. J Virol. 2005;79(13):8230-6.

5. Touinssi M, Reynaud-Gaubert M, Gomez C, et al. Parvovirus 4 in French in-patients: A study of hemodialysis and lung transplant cohorts. J Med Virol. 2011;83(4):717-20.

6. Simmonds P, Manning A, Kenneil R, et al. Parenteral transmission of the novel human parvovirus PARV4. Emerg Infect Dis. 2007; 13(9):1386-8.

7. Simmons R, Sharp C, McClure CP, et al. Parvovirus 4 infection and clinical outcome in high-risk populations. J Infect Dis. 2012:205(12): 1816-1820.

8. Yu X, Zhang J, Hong L, et al. High prevalence of human parvovirus 4 infection in HBV and HCV infected individuals in shanghai. PloS One. 2012;7(11):e29474.

9. Asiyabi S, Nejati A, Shoja Z, et al. First report of human parvovirus 4 detection in Iran. J Med Virol. 2016;88(8):1314-8.

10. Maple PA, Beard S, Parry RP, et al. Testing UK blood donors for exposure to human parvovirus 4 using a time-resolved fluorescence immunoassay to screen sera

and Western blot to confirm reactive samples. Transfusion. 2013;53 (10 Pt 2):2575-84.
11. Eis-Hübinger AM, Drexler JF, Reber U, et al. Absence of detection of novel human parvoviruses in German plasma donations. Transfusion. 2010;50(1):266-7.
12. Touinssi M, Brisbarre N, Picard C, et al. Parvovirus 4 in blood donors, France. Emerg Infect Dis. 2010;16(1):165-6.
13. Fryer JF, Delwart E, Hecht FM, et al. Frequent detection of the parvoviruses, PARV4 and PARV5, in plasma from blood donors and symptomatic individuals. Transfusion. 2007;47(6):1054-61.
14. Sharp CP, Vermeulen M, Nébié Y, et al. Epidemiology of human parvovirus 4 infection in sub-Saharan Africa. Emerg Infect Dis. 2010;16(10):1605-1607.
15. Simmons R, Sharp C, Levine J, et al. Evolution of CD8+ T cell responses after acute PARV4 infection. J Virol. 2013;87(6):3087-96.
16. Sharp CP, Lail A, Donfield S, et al. Virologic and clinical features of primary infection with human parvovirus 4 in subjects with hemophilia: frequent transmission by virally inactivated clotting factor concentrates. Transfusion. 2012;52(7):1482-9.
17. Schneider B, Fryer J, Oldenburg J, et al. Frequency of contamination of coagulation factor concentrates with novel human parvovirus PARV4. Haemophilia. 2008;14(5):978-86.
18. Javanmard D, Ziaee M, Ghaffari H, et al. Human parvovirus B19 and parvovirus 4 among Iranian patients with hemophilia. Blood Res. 2017;52(4):311-5.
19. Norja P, Lassila R, Makris M. Parvovirus transmission by blood products–a cause for concern? Br J Haematol. 2012;159(4):385-93.
20. Panning M, Kobbe R, Vollbach S, et al. Novel human parvovirus 4 genotype 3 in infants, Ghana. Emerg Infect Dis. 2010; 16(7): 1143-6.
21. Chaves A, Ibarra-Cerdeña CN, López-Pérez AM, et al. Bocaparvovirus, Erythroparvovirus and Tetraparvovirus in New World Primates from Central America. Transbound Emerg Dis. 2020; 67(1): 377-87.
22. Mansouritorghabeh H, Manavifar L, Banihashem A, et al. An investigation of the spectrum of common and rare inherited coagulation disorders in north-eastern Iran. Blood Transfus. 2013;11(2):233-40.
23. Lurcharchaiwong W, Chieochansin T, Payungporn S, et al. Parvovirus 4 (PARV4) in serum of intravenous drug users and blood donors. Infection. 2008;36(5):488-91.
24. Fryer JF, Kapoor A, Minor PD, et al. Novel parvovirus and related variant in human plasma. Emerg Infect Dis. 2006;12(1):151-4.
25. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W. Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 1994;22(22):4673-80.
26. Tamura K, Peterson D, Peterson N, et al. Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011;28(10):2731-9.
27. Sharp CP, Lail A, Donfield S, et al. High frequencies of exposure to the novel human parvovirus PARV4 in hemophiliacs and injection drug users, as detected by a serological assay for PARV4 antibodies. J Infect Dis. 2009;200(7):1119-25.
28. Slavov SN, Kashima S, Rocha-Junior MC, et al. Human parvovirus 4 in Brazilian patients with haemophilia, beta-thalassaemia major and volunteer blood donors. Haemophilia. 2015;21(1):e86-8.
29. Benjamin LA, Lewthwaite P, Vasanthapuram R, et al. Human Parvovirus 4 as Potential Cause of Encephalitis in Children, India. Emerg Infect Dis. 2011;17(8):1484-7.
30. Lavoie M, Sharp CP, Pépin J, et al. Human parvovirus 4 infection, Cameroon. Emerg Infect Dis. 2012;18(4):680-3.
31. Sancho-Shimizu V, de Diego RP, Jouanguy E, et al. Inborn errors of anti-viral interferon immunity in humans. Curr Opin Virol. 2011;1(6):487-96.
32. Delwart E. Human parvovirus 4 in the blood supply and transmission by pooled plasma-derived clotting factors: does it matter? Transfusion. 2012;52(7):1398-403.
33. Bernardin F, Operskalski E, Busch M, et al. Transfusion transmission of highly prevalent commensal human viruses. Transfusion. 2010;50(11):2474-83.
34. Yu X, Zhang J, Hong L, et al. High prevalence of human parvovirus 4 infection in HBV and HCV infected individuals in shanghai. PLoS One. 2012;7(1):e29474.
35. Touinssi M, Reynaud-Gaubert M, Gomez C, et al. Parvovirus 4 in French in-patients: A study of hemodialysis and lung transplant cohorts. J Med Virol. 2011;83(4):717-20.
36. Ma YY, Guo Y, Zhao X, et al. Human parvovirus PARV4 in plasma pools of Chinese origin. Vox Sang. 2012;103(3):183-5.
37. Lahtinen A, Kivela P, Hedman L, et al. Serodiagnosis of primary infections with human parvovirus 4, Finland. Emerg Infect Dis. 2011;17(1):79-82.
38. Schneider B, Fryer JF, Reber U, et al. Persistence of novel human parvovirus PARV4 in liver tissue of adults. J Med Virol. 2008;80(2):345-51.
39. Simmonds P, Manning A, Kenneil R, et al. Parenteral transmission of the novel human parvovirus PARV4. Emerg Infect Dis. 2007;13(9):1386-8.