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Original Article

# Investigation of SEN virus prevalence in hemophilia patients

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ABSTRACT

*Background:* Hemophilia and transfusion-dependent patients are at high risk of a wide range of blood-borne agents. Among these, SEN virus (SENV) stands out as a significant concern due to its association with transfusion-induced non-A to non-E hepatitis. This study, therefore, aimed to investigate the prevalence of this virus in hemophilia patients, focusing on potential complications and risk factors.

*Method:* This was a cross-sectional study conducted in a hemophilia center in the east of Iran. Blood samples were taken from patients and healthy people, and demographic and clinical information was collected. The sera samples were then subjected to DNA extraction. PCR-based methods detected SENV and its genotype, and then phylogenetic analysis was performed. The collected data were analyzed and interpreted by SPSS22 software.

*Results:* The mean age of patients and the healthy group was 26.18 ± 14.97 and 41.69 ± 14.05, respectively. Among the patient and healthy groups, 94.5 % and 36.4 % were male, and the rest were female, respectively. Most of the participants in the patient group had hemophilia type A (85.5 %), then type B (7.3 %), VWD type (3.6 %), and F and plt type (1.8 %) were in the next categories. SENV-DNA was detected in 58.2 % of patients and 20 % of healthy groups (P-value: 0.00). Among these, H and D genotypes were found in 35 % and 23.7 % of patients and 12.7 % and 7.3 % of healthy groups, respectively. The prevalence of the virus was significantly related to minor elevation of AST and was higher in hemophilia type A (63.8 %) and severe type of disease (63.2 %).

*Conclusion:* This study underscore the significant prevalence of the SENV virus in hemophilia patients, a particularly noteworthy finding compared to the healthy population. With the limited information available about this virus, our findings highlight the importance of continuous monitoring and follow-up of high-risk groups in relation to blood-borne pathogens, providing reassurance about the ongoing efforts in the field.

## 1. Introduction

Hemophilia is a genetic disorder characterized by impaired blood clotting, resulting from mutations in the genes encoding factor VIII (FVIII) and factor IX (FIX), which are critical components of the intrinsic coagulation pathway [1]. Severe hemophilia is defined by factor levels of 1 % or less, moderate by levels between 2 % and 5 %, and mild by levels ranging from 6 % to 40 % [2]. The therapeutic landscape for hemophilia has evolved significantly since the introduction of plasma infusion from healthy donors in 1948, marking the first milestone in hemophilia treatment [3]. Subsequently, the most widely adopted therapeutic approach has used plasma-derived factors obtained through cryoprecipitation of pooled plasma. However, this method is not without risks, as the potential for contamination with blood-borne pathogens, particularly hepatitis viruses, remains a concern [4]. Despite advancements in screening and purification techniques, numerous studies have documented instances of HIV, HBV, and HCV transmission within the hemophilia population through contaminated factor concentrates [5–7].

The 1990s witnessed significant advancements in molecular medicine that elucidated the genetic underpinnings of coagulation disorders. These breakthroughs facilitated the therapeutic production of recombinant coagulation factors VIII and IX, marking a pivotal moment in hemophilia treatment. Concurrently, the incorporation of virucidal steps in the manufacturing processes of plasma-derived coagulation products substantially enhanced their safety profile [5,8].

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In a parallel development, researchers identified a DNA virus, subsequently designated as SEN virus (SENV), in the serum of a patient infected with human immunodeficiency virus [9]. Subsequent phylogenetic analysis revealed the existence of eight distinct SENV strains [10], and recently a ninth genotype has been characterized [11]. Among these variants, two strains—SENV-D and SENV-H—have been in particular attention due to their significant association with transfusion-associated non-A-E hepatitis [12,13]. Further investigations have highlighted the potential clinical implications of SENV-D and SENV-H beyond transfusion-related complications. These strains have been detected with increased frequency in patients suffering from chronic liver disease and hepatocellular carcinoma (HCC), as compared to healthy adult populations [14,15].

Despite the potential implications of SENV infection, its precise relationship with liver cell damage remains ambiguous, necessitating further comprehensive investigations to elucidate its clinical significance globally. The present study seeks to address this knowledge gap by examining the prevalence of the SEN virus in a specific high-risk population—hemophilia patients—in comparing with healthy individuals in South Khorasan province.

## 2. Material and methods

### 2.1. Study design and participants

This descriptive study was conducted in collaboration with the hemophilia society in South Khorasan province, eastern Iran. The primary objective was to investigate the molecular characteristics of the SEN virus (SENV) among patients with bleeding disorders and a group of healthy individuals. The study adhered to the principles of the Helsinki Declaration, and written informed consent was obtained from all participants.

Participants with bleeding disorders were recruited from the hemophilia ward, while the healthy group comprised companions of patients from other wards of the same hospital. Both groups were sampled using identical procedures and questionnaires. Demographic data and clinical information, including hemophilia type and severity, blood type, and hepatic enzyme levels, were extracted from medical records for the patient group.

### 2.2. Samples, DNA extraction and quality assessment

Whole blood samples were collected from the patient and healthy groups in EDTA-containing tubes. Plasma was subsequently separated and stored at −80 °C until further analysis. Viral DNA was extracted from 200 μL of plasma using the FavorPrep Viral Nucleic Acid Extraction Kit I (FAVORGEN, Taiwan), following the protocol of manufacturer. The yield and purity of the extracted DNA were assessed using a NanoDrop spectrophotometer (Thermo Scientific, USA). To verify the integrity of the extracted DNA, an internal PCR targeting the human beta-globin gene was performed as previously described [16].

### 2.3. SEN virus detection

The presence of SENV was determined through a nested polymerase chain reaction (PCR) approach, targeting a partial region of the ORF-1 gene. The protocol was adapted from methods reported by Hosseini and Bouzari (2016) and Kojima et al. (2003) [17,18].

The first round of PCR employed outer primers AI-1F (5′-TWCYCMAACGACCAGCTAGACCT-3′) and AI-1R (5′-GTTTGTGGTGAGCAGAACGGA-3′), amplifying a 349-bp region conserved across all SENV genotypes (A-I). The nested PCR step utilized genotype-specific primers for SENV-D and SENV-H. For SENV-D, primers D-1148F (5′-CTAAGCAGCCCTAACACTCATCCAG-3′) and D1341R (5′-GCAGTTGACCGCAAAGTTACAAGAG-3′) were used, generating a 195-bp amplicon. SENV-H detection employed primers H-1020F (5′-TTTGGCTGCACCTTCTGGTT-3′) and H-1138R (5′-AGAAATGATGGGTGAGTGTTAGGG-3′), producing a 119-bp fragment.

### 2.4. DNA sequencing and phylogenetic analysis

PCR products were purified and sequenced using a 3730XL DNA analyzer (Applied Biosystems) at Bioneer Co., South Korea. Sequences were edited using BioEdit software version 7.2.5 and compared to previously reported sequences using the BLAST search tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Phylogenetic analysis was conducted using MEGA6 software [19]. Sequence alignment was used to construct a phylogenetic tree using the neighbor-joining method [20] with the Kimura 2-parameter (K2P) model of evolutionary distance [21]. The analysis employed pairwise deletion and 1000 bootstrap replicates to ensure statistical robustness.

### 2.5. Statistical analysis

Analysis of the data was made by using SSPS software (version SPSS 22). The statistical significance of the difference in mean of variables between the two groups was assessed by qi-square or Fisher's exact test. P values $\leq 0.05$ were considered statistically significant.

## 3. Results

### 3.1. Demographic and clinical characteristics of participants

This study encompassed 135 participants, comprising 80 hemophilic patients and 55 healthy individuals. In the hemophilia group, the gender distribution was predominantly male (93.7 %, n = 75), with only 6.25 % (n = 5) female participants. The mean age of this group was 27.4 ± 15.6 years, ranging from 8 to 83 years. Conversely, the healthy group exhibited a more balanced gender distribution, with 63.6 % (n = 35) female participants. The mean age in healthy group was 41.6 ± 13.9 years, spanning from 20 to 71 years.

Regarding to the hemophilia classification, the majority of patients were diagnosed with hemophilia type A (86.3 %), followed by type B (6.3 %), with other coagulation disorders being relatively rare. Liver function tests revealed mean ALT and AST levels in the hemophilia group of 24.5 ± 13.7 and 26.4 ± 9.6, respectively. In comparison, the healthy group demonstrated mean ALT and AST levels of 22.6 ± 14.2 and 20 ± 10.3, respectively. (Fig. 1).

### 3.2. Prevalence and molecular characterization of SENV

Polymerase Chain Reaction (PCR) assays were employed to detect SENV-DNA, identified by a 350 bp PCR band (Fig. 2). The molecular prevalence of SENV differed significantly between the hemophilia and healthy groups, with rates of 47/80 (58.8 %) and 11/55 (20 %), respectively ($P < 0.001$). This marked disparity underscores a potentially heightened susceptibility to SENV infection among hemophilic individuals.

More analysis revealed significant demographic and clinical differences between SENV-positive and SENV-negative samples. Notably, the mean age was substantially lower among SENV-negative individuals ($p < 0.05$, Table 1). Liver enzyme levels exhibited a marked disparity between the two groups. In overall, AST levels were significantly higher in individuals with SENV viremia ($p < 0.01$, Table 1). Besides, SENV-positive patients demonstrated significantly elevated mean ALT and AST levels compared to their SENV-negative counterparts, following an age-related pattern in both groups (Fig. 3). Examination of hemophilia types revealed that SENV prevalence was highest among patients with hemophilia type A (65.2 %) and those with severe hemophilia, characterized by Factor VIII counts below 1 IU/dL (69 %, Table 2). Occupational analysis indicated a significant association between SENV prevalence and student status (57.1 %, p = 0.026). The distribution of

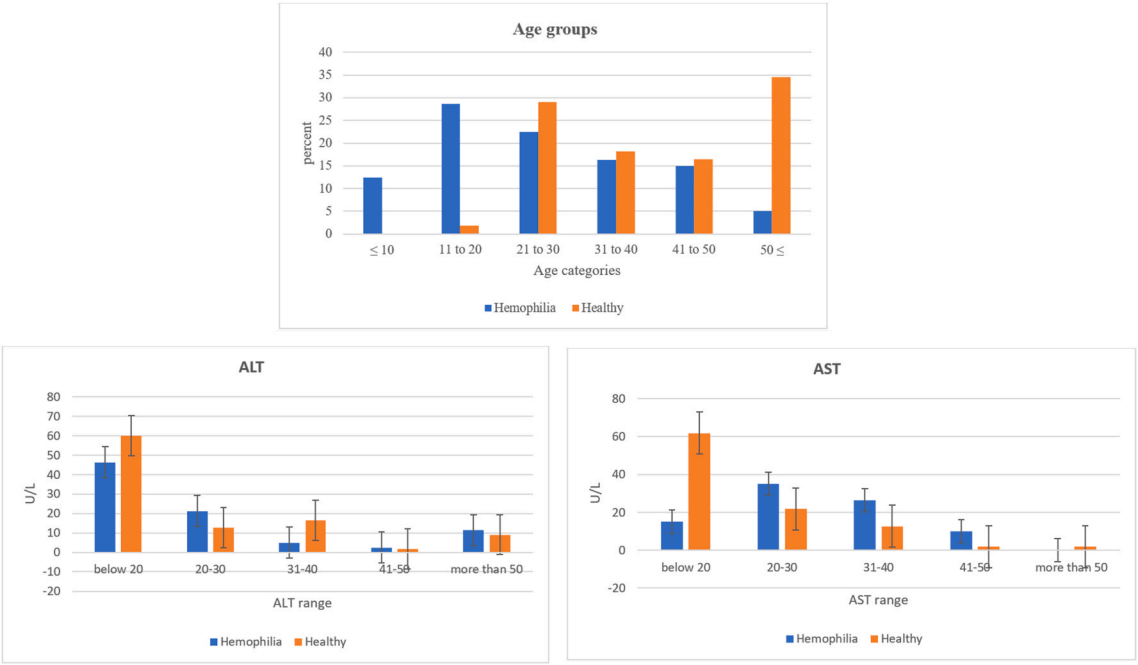


**Fig. 1.** The Number of hemophilia group participants was significantly higher in ages below 10; however, it was lower than in the healthy group in ages over 50 years (P<0.05). Levels of ALT and AST in the two groups were not statistically different (P>0.05).

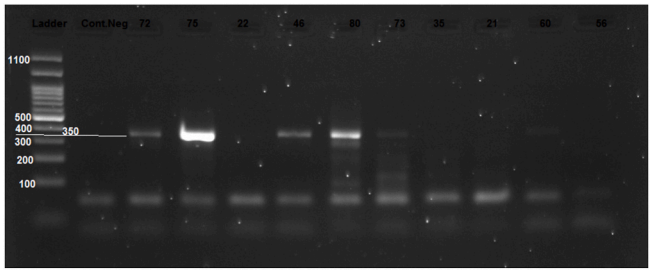


**Fig. 2.** The amplicon of the first run PCR showed a 350bp product following electrophoresis.

**Table 1**
Comparison of the mean age, ALT, and AST among subjects with SENV infection and opposing groups.

| | SENV | Number | Age | ALT | AST |
|---|---|---|---|---|---|
| *Hemophilia* | Positive | 47 | 26.4 ± 12.5 | 26.3 ± 16.8 | 27.6 ± 9.8 |
| | negative | 33 | 28.9 ± 19.4 | 24.6 ± 9.2 | 24.6 ± 9.2 |
| *Healthy* | Positive | 11 | 36.8 ± 12.8 | 22.5 ± 15.1 | 22.6 ± 10.8 |
| | negative | 44 | 42.9 ± 14.2 | 22.6 ± 14.1 | 19.3 ± 10.1 |
| *total* | Positive | 58 | 28.4 ± 13.1 | 25.5 ± 16.4 | 26.6 ± 10.2 |
| | negative | 77 | 36.9 ± 17.9 | 22.3 ± 11.7 | 21.3 ± 10.1 |
| *p-value* | | | 00 | 0.461 | 0.0 |

SENV prevalence concerning gender and living situation was also assessed, with detailed results presented in Table 3. These findings suggest complex interactions between SENV infection and various demographic and clinical factors in the studied population, highlighting the need for further investigation into the epidemiological patterns and potential clinical implications of SENV in hemophilia patients.

### 3.3. Determination of SENV genotype

As previously described (Fig. 4), SENV-positive samples were genotyped using specific primers for types H and D. Analysis of the 58 SENV-positive samples revealed that 35 (60.3 %) were identified as type D. In contrast, 23 (39.7 %) were classified as SEN virus genotype H. This distribution pattern was relatively consistent across both study groups (Fig. 5). Among the samples, H and D genotypes were found in 35 % and 23.7 % of patients and 12.7 % and 7.3 % of healthy groups, respectively.

Co-infection with both SENV genotypes (mixed infection) was observed in 11 out of 58 (19 %) positive samples. The prevalence of mixed infections was varied between the hemophilia and healthy groups, with 17 % and 27.3 % of SENV-positive samples, respectively (Fig. 3). Notably, individuals with mixed infections were significantly older than those with single-type infections (mean age 32.4 ± 13.4 years vs. 25.2 ± 12.1 years, p = 0.016).

### 3.4. Phylogenetic analysis

In the phylogenetic analysis, the PCR product from the outer step underwent direct sequencing utilizing the Sanger method with big dye termination, performed by Bioneer CO, South Korea. The resulting sequence data was trimmed and aligned against the reference sequence within the corresponding open reading frame. Subsequently, the curated sequences were submitted to the GenBank database for public access and future reference (GenBank MK251574-MK251586). The aligned sequence file was then analyzed using Mega7 software, where model selection was conducted to determine the most appropriate evolutionary model. Through this process, the Hasegawa-Kishino-Yano model emerged as the optimal choice when employing the maximum likelihood program, providing a robust framework for further phylogenetic inference and evolutionary relationship analysis among the studied sequences.

## 4. Discussion

Hemophilia types A and B are among the most prevalent congenital bleeding disorders, necessitating routine administration of clotting factors and, in many cases, transfusion of whole blood or plasma. This reliance on blood products exposes patients to significant biohazards, particularly the transmission of blood-borne pathogens such as HIV and viral hepatitis. In recent years, a newly identified agent, SENV (SEN

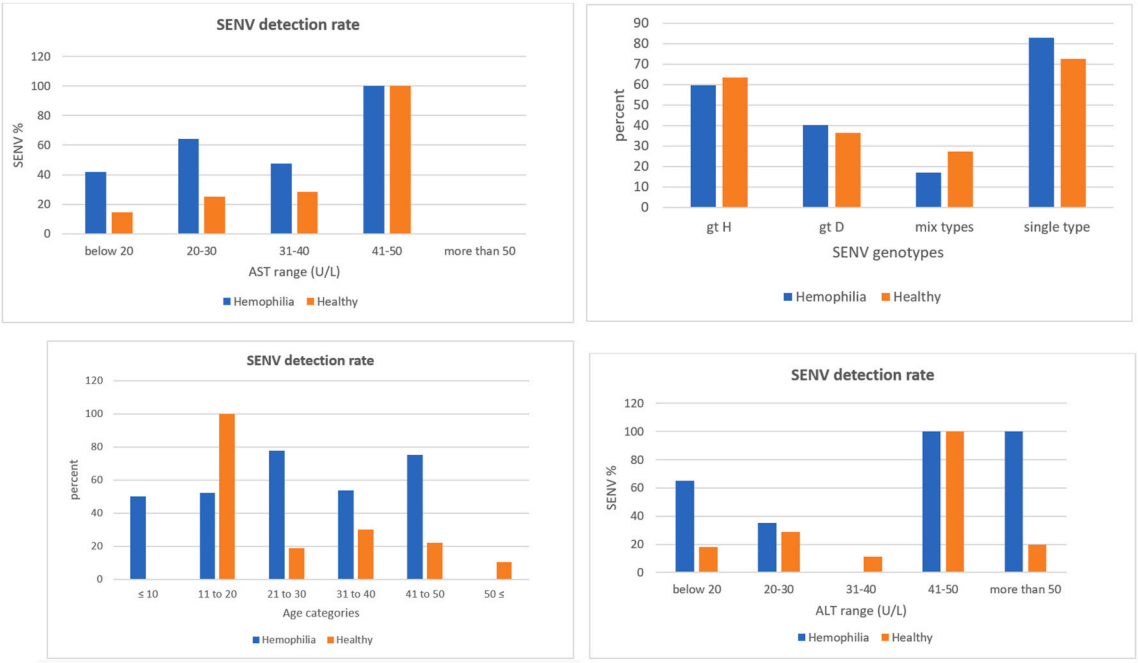


**Fig. 3.** The above figures show the prevalence of SENV in different age categories, according to the ALT/AST levels, as well as the percentage of SENV genotypes in two groups. In both groups, the mean ALT and AST were significantly higher among SENV-positive cases than negatives in ages over 40 years (P<0.05).

**Table 2**
Prevalence of SENV in association with hemophilia type, severity, and job.

| *SENV-PCR positive rate* | Hemophilia type | | | | P-value |
|---|---|---|---|---|---|
| | Type A | Type B | VWD | Rare types | |
| | 45/69 (65.2) | 0/5 | 0/4 | 2/2 (100)0 | 0.044 |
| | VIII factor count | | | | |
| | | Below 1 | 1–5 | 5–10 | |
| | | 20/29 (69) | 25/38 (65.8)) | 0/6 | 0.003 |

**Table 3**
Prevalence of SENV in association with gender and living situation.

| | number | SENV prevalence SENV positive/total (%) | | | |
|---|---|---|---|---|---|
| | | Male | female | urban | rural |
| *Hemophilia* | 80 | 45/75 (60) | 2/5 (40) | 17/33 (51.5) | 28/45 (62.2) |
| *Healthy* | 55 | 3/20 (15) | 8/35 (22.9) | 6/30 (20) | 5/25 (20) |
| *Total* | 135 | 48/95 (50.5) | 10/40 (25) | 23/63 (36.5) | 33/70 (47.1) |
| *P-value* | | | 0.003[a] | | 0.118 |

[a] In regarding total population, it is a significant value.

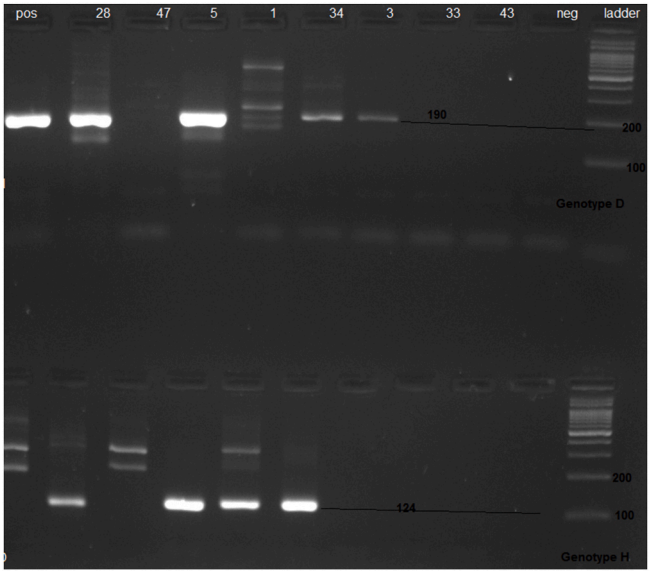


**Fig. 4.** The result of type-specific PCR and gel electrophoresis showed PCR divers bands, 198bp for type D and 124 bp for type H.

virus), has been introduced concerning non-A non-B hepatitis, demonstrating high communicability through blood routes. To our knowledge, this study represents the first investigation of SENV prevalence among patients with hemophilia in Iran. Given the high prevalence rates of this virus previously demonstrated in various populations, we aimed to assess its prevalence among patients with bleeding disorders in Iran. This research is particularly pertinent due to the increased vulnerability of hemophilia patients to blood-borne infections causing from their frequent exposure to blood products. This investigation revealed a significant association between the high prevalence of SENV and hemophilia compared to the healthy population (58.8 % vs 20 %). Nearly 60 % of hemophilia subjects in our study demonstrated viremic evidence for SENV. Populations with bleeding disorders are particularly interesting for assessing blood-borne viral infections, including emerging pathogens. For instance, previous studies in Iran have identified human pegivirus-1 (HPgV-1) and torque teno virus (TTV) among hemophilia patients, with prevalence rates of 4.4 % and 17.4 %, respectively [22]. HPgV-1, formerly known as GB virus C or hepatitis G virus (HGV), is a novel non-A non-B agent belonging to the Flaviviridae family [23]. TTV, discovered in 1997, is a small single-stranded DNA virus within the Anelloviridae family, sharing significant similarities with SENV regarding genomic organization and structure. This virus has been associated with a high global prevalence and potential implications

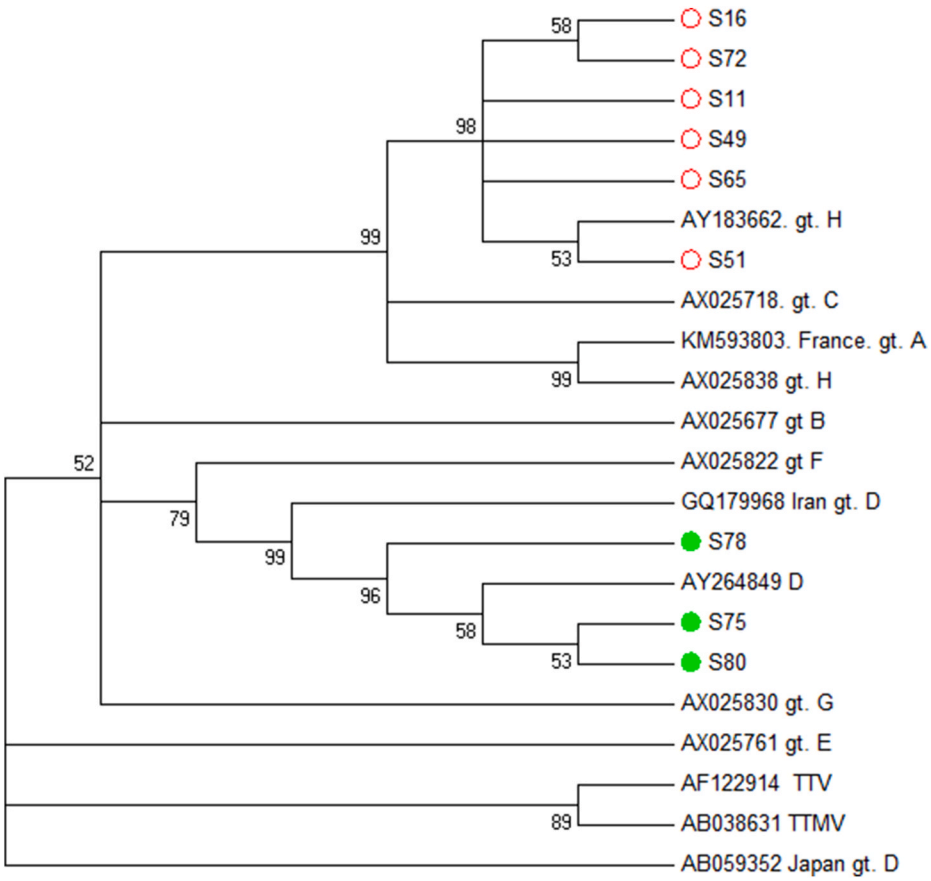


**Fig. 5.** The result of the phylogenetic analysis showed nucleotide divergence of the orf1-SENV region among isolates of the current region.

for liver diseases [24].

The high prevalence of SENV observed in this study raises significant concerns regarding the potential transmission of blood-borne viruses among patients with bleeding disorders. While the introduction of recombinant factors in hemophilia treatment has substantially reduced the risk associated with contaminated blood products, the findings of this study, consistent with previous reports, suggest that the risk of viral transmission remains a pressing issue [25]. Consequently, ongoing surveillance of this population is crucial, as hemophilia remains a high-risk condition for acquiring transfusion-transmissible infections.

SENV viremia has been associated with various clinical manifestations, with hepatitis and exacerbation of liver diseases in co-infection with other viral hepatitis being the most frequently reported concerns [26]. The current study partially corroborates these findings, as a slight elevation in liver enzymes was observed in patients with SENV viremia. In this population, lymphocyte proliferation abnormalities present another significant implication, potentially arising from viral infections. These abnormalities are thought to impair immune responses, subsequently leading to nonspecific immunosuppression conditions [27].

The prevalence of SENV infection in populations with bleeding disorders has been the subject of a few investigations. A study conducted in Taiwan among high-risk groups reported SENV prevalence rates of 68 %, 90 %, 68 %, and 54 % in hemophilia, thalassemia, hemodialysis, and intravenous drug users, respectively [13,15]. Similarly, a German study involving 12 hemophilia patients found that over 40 % were viremic for SENV [28].

SENV investigations mostly have been extended to other high-risk groups, with particular emphasis on hemodialysis patients. Reported prevalence rates in this population vary considerably across different geographical regions, ranging from 12.8 % in Germany [29], and 13 % in Pakistan [30], to 52.4 % in Egypt [31]. In Turkey, a study differentiated between SEN-D and SEN-H strains, reporting prevalence rates of 33 % and 22 %, respectively [32]. These rates are generally higher than those observed in healthy populations, suggesting an increased risk of SENV infection in hemodialysis patients. Furthermore, a positive correlation between SENV infection and transfusion history has been established [30], which is particularly relevant for patients with bleeding disorders who frequently require blood transfusions.

In contrast, studies on relatively healthy populations, such as blood donors in Ahvaz, Iran, have reported lower SENV prevalence rates of 5.4 % [33]. However, our current study found a prevalence of 20 % in the healthy control group, which is notably higher. Other Iranian studies have reported similarly elevated rates: 37.33 % in healthy blood donors, 56 % in HIV-positive individuals [34], 28.6 % of hemodialysis patients [35], and 46 %, 66 %, and 90 % in patients with HCV, HBV, and healthy individuals, respectively [17]. These findings suggest that SENV prevalence in Iran may be higher compared to other countries. However, this observation remains controversial and may be influenced by various factors such as sample size, methodology, date/year and population characteristics.

The majority of studies, including ours, have employed a recently designed nested-PCR assay for SENV detection. While this method offers high sensitivity, it also raises concerns about the potential overestimation of prevalence rates. Our study initially used single-run PCR results for frequency detection, but further investigations, particularly among hemophilia patients, are necessary for more definitive conclusions.

Interestingly, our study revealed that patients with SENV viremia were generally younger, a finding consistent with previous research [36,37]. This age-related trend may be attributed to various factors, including parenteral transmission routes more common in younger individuals, increased sexual activity among youth, and the possibility of

congenital and perinatal transmission [38]. The prevalence of SENV infections in healthy young individuals may also be related to the biology of SENV replication, such as the distribution of receptors and permissive cells. Patients with SENV viremia in our study exhibited elevated levels of AST, although these elevations rarely exceeded the normal range. This observation warrants further investigation to elucidate the potential hepatic effects of SENV infection.

Regarding SENV genotyping, our study revealed that genotype D was the predominant type, accounting for 60.3 % of isolates, followed by genotype H at 39.7 %. This distribution pattern aligns with previous findings in populations with beta-thalassemia [37]. However, it is important to note that this pattern contrasts with observation reported in another study [39]. The variability in SENV genotype distribution across different geographic regions and populations underscores the need for further investigations to elucidate the influence of transmission routes, predisposing conditions, and risk behaviors on SENV genotype prevalence.

Our study also identified mixed infections with both SENV genotypes in 11 out of 58 (19 %) positive samples. Specifically, mixed infections were observed in 17 % of SENV-positive hemophilia patients and 27.3 % of SENV-positive individuals in the healthy population. In comparison, a study conducted in Gilan province of Iran reported a notably higher prevalence of mixed infections among thalassemic patients. In that study, the overall prevalence of types H and D was 93 % and 86 %, respectively, and 81 % of SENV-positive individuals harboring mixed infections [36]. Interestingly, in the Gilan study, type H was more prevalent than type D in mixed infections, which differs from our observations.

Conclusion: In conclusion, this study has demonstrated a high prevalence of the SENV virus among patients with hemophilia. While the findings revealed a relatively elevated level of AST in SENV-positive patients, it is imperative to note that more comprehensive investigations are necessary to draw more reliable conclusions. Future research should ideally be conducted in a cohort setting with a larger sample size to enhance the statistical power and generalizability of the results. Furthermore, the presence of SENV in the general population warrants additional virological studies, focusing on viral tropism, whole genome sequencing, genetic variations, and precise taxonomic classification to determine its appropriate viral family. Moreover, elucidating the immune signature associated with SENV infection is crucial for a more comprehensive understanding of the virus's pathogenesis and potential clinical implications. These proposed avenues of research will contribute to a deeper understanding of SENV and inform potential preventive and therapeutic strategies for managing infections in high-risk and general populations.

## Ethics approval

The local Ethics Committee of Birjand University of Medical Sciences reviewed and approved this study, code number IR. BUMS. REC.1395.119.

## CRediT authorship contribution statement

**Davod Javanmard:** Writing – review & editing, Project administration, Methodology. **Motahareh Mahi-Birjand:** Writing – review & editing, Writing – original draft. **Effat Alemzadeh:** Writing – original draft, Project administration. **Mahdie Mohammadi:** Methodology, Investigation, Data curation. **Masood Ziaee:** Writing – review & editing, Supervision, Project administration, Conceptualization.

## Declaration of competing interest

All the authors of above manuscript have acclaimed to have no declaration of interest for statement.

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