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RESEARCH ARTICLE

# A GENOTYPIC STUDY OF SEN VIRUS INFECTION IN HEALTHY BLOOD DONORS AND THALASSEMIA PATIENTS: WITH OR WITHOUT HCV INFECTION AND ITS CLINICAL IMPORTANCE

**BASHAR M. KHAZAAL*[1], ARWA H. AL-HAMDANI[2], ASHNA J. FAEQ[3]**

[1] MSc Student in Department of Microbiology, College of Medicine, Al-Mustansiriyah University. [2] Lecturer, Department of Microbiology, College of Medicine, Al-Mustansiriyah University. [3] Head of Molecular biology department / Central Public Health Laboratory (CPHL). Iraq, Baghdad.**Email:**basho_oo86@yahoo.com

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**ABSTRACT**

**Background:** SEN-Virus (SEN-V-D and SEN-V-H) is a DNA virus which associated with acute post transfusion hepatitis and blood transfusion is the most common mode of transmission of this virus like HCV, HBV and HIV among population. Beta thalassemia is a disease need continuous blood transfusions to manage the patient's life; so these patients are at increased risk of infection with SEN-V. **Aims of this study:** This study was designed to search the prevalence of SEN-V among thalassemia patients and blood donors and to evaluate the clinical importance of SEN-Virus in thalassemia patients with or without HCV infection in Iraq and to detect the exact genomic characterization of SEN-V-D and SEN-V-H genotypes in Iraq and study of similarity of these genomes with other countries especially the neighboring countries and the homology between each isolate. **Methods:** One hundred and fifty eight thalassemia patients (57.6% male, 42.4% female), with mean age of 16.8±8.5 year, and one hundred and fifty healthy blood donors with randomly selected persons (58.7%male, 41.3% female), with mean age of 16.7±8.6 year; all these samples involved in this study. SEN-V and HCV had been identified by nested conventional PCR. Liver transaminases (Aspartate Transaminase and Alanine Transaminase) had been determined, in addition of measure of serum ferritin levels by VIDAS. Gene sequencing and phylogenetic analysis had been studied of randomly selected amplified SEN-V D and H DNA samples. **Results:** SEN-V was detected in 68 from 158 (43%) of thalassemia patients and 16 from 150 (10.7%) of blood donors. HCV prevalence was (11.4%) in thalassemia patients. There was significant increase in prevalence of SEN-V or HCV infection with age but there was no significant difference in prevalence in both with gender. SEN-V and HCV co-infection significantly increases AST level above normal range. SEN-V significantly increases ALT level above normal range and has a great significant ALT level increase with HCV co-infection. Serum ferritin has no significant relation with SEN-V or SEN-V and HCV co-infection. The results from the study of gene sequencing and phylogenetic analysis of samples of amplified SEN-V-D DNA and samples of amplified SEN-V-H DNA that selected randomly from blood donors and thalassemia patients infected with D or H genotypes alone or together (co-infection) were concluded that the most transmission route of SEN-V D and H was blood transfusion, because there was (99%) gene similarity between blood donors and thalassemia patients, furthermore SEN-V-D or SEN-V-H sequences of the co-infected persons are the same sequences of D or H genotypes alone with the observations of similarity with other neighboring countries**. Conclusion:** SEN-V (D & H) can be transmitted via blood transfusion and cause acute hepatitis with or without HCV co-infection. The most countries had similar sequences to Iraqi SEN-V-D genotype sequence are Iran, USA and Brazil; while the most countries have similar sequences to Iraqi SEN-V-H genotype sequence are China and Iran. SEN-V-D or SEN-V-H sequences of the co-infected persons are the same sequences of D genotype or H genotype of persons that have infection with SEN-V-D or H alone.

**Key words:** sen-virus, hcv, thalassemia, ast, alt, ferritin, phylogenetic analysis.

## INTRODUCTION

Viruses that transmitted via blood transfusion are an important health issue like HBV, HCV and HIV [1], so beta thalassemia patients are at increased risk of infection with these viruses [2]. In 1999, SEN-virus had been discovered as blood borne and post-transfusion hepatitis virus; study group led by Dr. Daniele Primi produced that SEN-V firstly discovered in patient suffering from HIV-1 infection, this patient was injection drug user [3]. SEN-V is non-enveloped, round, icosahedral symmetry with single stranded DNA genome [4] belongs to *Circoviridae* family, *Circovirus* genus and *Circo* indicates the circular shape of the virus genome [5]. SEN-V had been sub grouped into 9 genotypes (A to I) [6]. It had been suggested that genotypes C and H as well as D and F could be combined due to similarities in ORF 1 [7]; therefore, with some other studies reported that the most important hepatotropic genotypes are D and H [8]. The transmission modes of SEN-V infection mostly parenteral, e.g. blood transfusion, drug addiction by injection uses, hemodialysis and organ or hematological progenitor cells transplantation [9].

## PATIENTS AND METHODS

### Blood samples

Samples had been selected as two groups; group I was patients of thalassemia (beta thalassemia patients received regular blood transfusion) that was composed of 158 patients (91 male, 67 female), aged from 3 to 50 years; These patients were collected from the thalassemia center of Ibn-baladi maternity and children's hospital / Baghdad. At this time of blood collection of this group, the patients were receiving (Exjade) iron chelating agent. Group II, healthy blood donors and randomly selected persons to be - as possible as - age and sex matched with the group I, this group composed from 150 person (88 male, 62 female) were collected from Iraqi national center of blood transfusion and randomly selected from community. Sample collection and the practical part of this study took time from January to June 2015.

### DNA and RNA Extraction

By using the ExiPrep™Plus Viral DNA/RNA Kit, which is designed for the extraction of viral DNA or RNA from various clinical samples, viral DNA or RNA was extracted from clinical samples by using a lysis buffer to disrupt viral structure. The DNA or RNA is bound to the surface of silica magnetic beads in binding buffer. Washing buffer rinses any impurities that may exist, and elution buffer extracts the pure DNA/RNA from the beads.

### DNA and RNA Concentration and Purity

DNA samples have been diluted 10 times, absorbance measured of DNA samples at 260 n.m wavelength and the concentration is calculated by the following equation (absorbance 260 n m × dilution factor × 50 µg/ml = µg/ml). Purity has been determined after 280 n m wavelength spectrophotometry absorbance taken and then calculated as follows (the purity of DNA = absorbance 260 / absorbance 280 = 1.8 ~ 2.0).

### SEN-V (SEV-V-D and SEN-V-H) and HCV Primers:

-SENV-AI-1F (5' to 3'): TTCCAAACGACCAGCTAGACCT
-SENV-AI-1F (5' to 3'): TACTCCAACGACCAGCTAGACCT
-SENV-AI-1R (5' to 3'): GTTTGTGGTGAGCAGAACGGA
-SENV-D-1148F(5'to 3'):CTAAGCAGCCCTAACACTCATCCAG
-SENV-D-1341R(5'to3'):GCAGTTGACCGCAAAGTTACAAGAG

-SENV-H-1020F (5' to 3'): TTTGGCTGCACCTTCTGGTTSENV
-H-1138R (5' to 3'): AGAAATGATGGGTGAGTGTTAGGG
-Forward-R1 (5' to 3'): CAGGCAGAAAGCGTCTAGCCATG
-Reverse-R1 (5' to 3'): TCGCAAGCACCCTATCAGGCAG
-Sense-R2 (5' to 3'): CCCCTGTGAGGAACTACTGTC
-Anti-sense-R2 (5' to 3'): TGCACGGTCTACGAGACCTC

**SEN-V (SEV-V-D and SEN-V-H) Amplification**

DNA of SEN virus had been amplified by nested conventional PCR; firstly the specific reaction contents have been added as follows:

12.5 µl of GoTaq®Green Master Mix.
1 µl of (SENV-AI-1F) primer.
1 µl of (SENV-AI-1R) primer.
7 µl of DNA.
3.5 µl DNase free sterile water.
The PCR protocol used for the first amplification was:
94 °C – 4 minutes for the initial denaturation in 1 cycle.
94 °C – 40 seconds for denaturation in 35 cycles.
55 °C – 50 seconds for annealing in 35 cycles.
72 °C – 50 seconds for extension in 35 cycles.
72 °C – 10 minutes for final extension in 1 cycle.
Secondly, the contents used for the other PCR run were:
12.5 µl of GoTaq®Green Master Mix.
1 µl of (SENV-D-1148F) primer.
1 µl of (SENV-D-1341R) primer.
1 µl of (SENV-H-1020F) primer.
1 µl of (SENV-H-1138R) primer.
8 µl of amplified DNA from the first PCR run.
0.5 DNase free sterile water.

The second run of PCR protocol was:

94 °C – 4 minutes for the initial denaturation in 1 cycle.
94 °C – 30 seconds for denaturation in 35 cycles.
55 °C – 50 seconds for annealing in 35 cycles.
72 °C – 50 seconds for extension in 35 cycles.
72 °C – 10 minutes for final extension in 1 cycle.

**Reverse transcription of extracted HCV RNA**

The extracted HCV RNA had been reversetranscripted into complementary DNA by GoScrip™ Reverse Transcription System by the following protocol steps (1-13):

4 µl of the extracted RNA.
1 µl of random primer from the system kit.
70 °C for 5 minutes.
Ice chilling for 5 minutes.
At this time, the listed contents below were prepared;
4 µl of (5×) buffer from the system kit.
1.2 µl Mgcl2 from the system kit.
1 µl of PCR Nucleotide Mix from the system kit.
0.5 µl of Recombinant RNase from the system kit.
1 µl GoScrip™ Reverse Transcriptase from the system kit.
7.3 µl of Nuclease free water from the system kit.
Then 15 µl prepared from step 5 to 10 above were mixed with the 5 µl prepared by step 1 and 2, then were undergone the following final steps:
25 °C for 5 minutes.
42 °C for 1 hour.
70 °C for 15 minutes.

**HCV Complementary DNA Amplification**

The complementary DNA of HCV had been amplified by nested conventional PCR; firstly the specific reaction contents had been added as follows:

12.5 µl of GoTaq®Green Master Mix.
1 µl of (Forward-R1) primer.
1 µl of (Reverse-R1) primer.
5 µl of DNA.
5.5 µl DNase free sterile water.

The PCR protocol used for the first amplification was

95 °C – 5 minutes for the initial denaturation in 1 cycle.
95 °C – 50 seconds for denaturation in 25 cycles.
55 °C – 40 seconds for annealing in 25 cycles.
72 °C – 50 seconds for extension in 25 cycles.

Secondly, the contents used for the other PCR run were:

12.5 µl of GoTaq®Green Master Mix.
1 µl of (Sense-R2) primer.
1 µl of (Anti-sense-R2) primer.
3 µl of amplified DNA from the first PCR run.
7.5 DNase free sterile water.

The second run of PCR protocol was:

95 °C – 4 minutes for the initial denaturation in 1 cycle.
95 °C – 40 seconds for denaturation in 35 cycles.
64 °C – 60 seconds for annealing in 35 cycles.
72 °C – 40 seconds for extension in 35 cycles.
72 °C – 10 minutes for final extension in 1 cycle.

**Gel Electrophoresis**

Tris-Acetic EDTA buffer (10 ×) had been diluted 10 times to be (1 ×) by adding 100 ml of Tris-Acetic EDTA buffer (10 ×) with 900 ml distilled water, then 3 grams of Agarose powder was dissolved in 150 ml of (1 ×) Tris-Acetic EDTA buffer to be 2% Agarose solution, the last solution had been boiled at 300 °C; after boiling, the solution was cooled to 50 °C and after adding 2 µl Ethidium bromide, poured into the gel tray which be supported with comb.

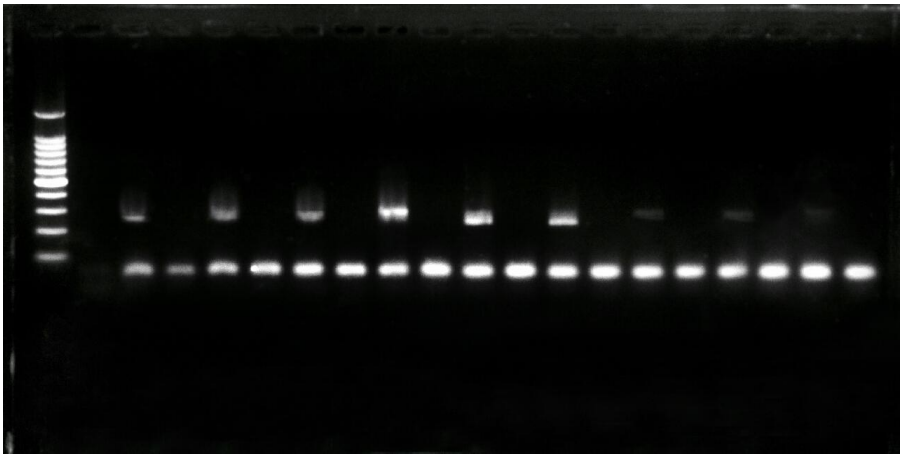

**Fig. 1: HCV complementary DNA bands picture, 298 b.p bands appeared by gel electrophoresis.**

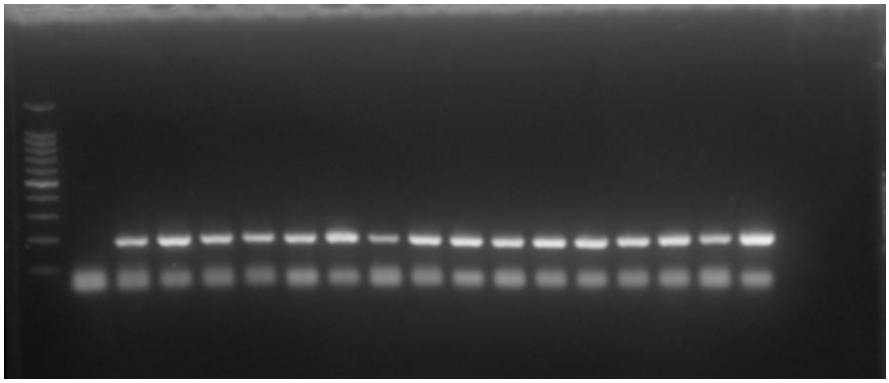

**Fig. 2: SEN-V-D genotype DNA bands picture, 193 b.p bands appeared by gel electrophoresis**

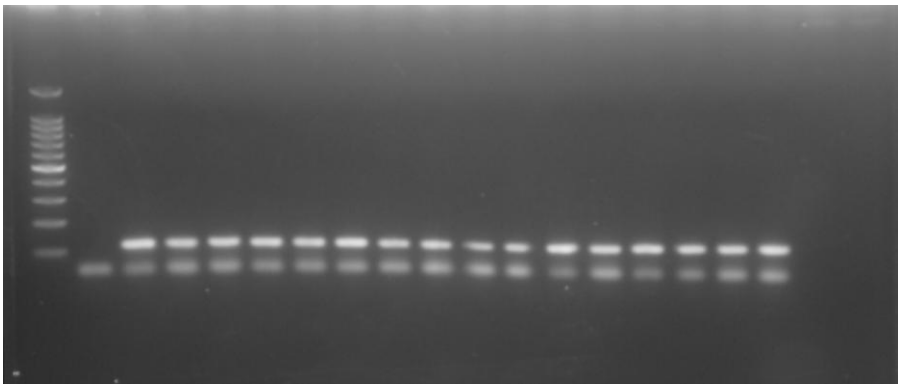

**Fig. 3: SEN-V-H genotype DNA bands picture, 118 b.p bands appeared by gel electrophoresis.**

**Biochemical Investigations**

**A.Liver Enzymes**

**Serum Alanine Transaminase (ALT):**

Colorimetric determination according to the following reaction:

Alanine + α Keto glutarate GPT ⇌ pyruvate + glutamate

The pyruvate formed is measured in its derivative form 2, 4 dinitrophenylhydrazone. The normal value in serum is < 45 units/l.

**Serum Aspartate Transaminase (AST)**

Colorimetric determination according to the following reaction:

Aspartate + α Keto glutarate GOT ⇌ pyruvate + glutamate

The pyruvate formed is measured in its derivative form 2, 4 dinitrophenylhydrazone. The normal value in serum is < 40 units/l.

**B. Determination of serum ferritin**

The principle of this test depends on enzyme immunoassay sandwich method with final fluorescent detection. The Solid Phase Receptacle (SPR) serves as the solid phase (monoclonal anti-ferritin immunoglobulins of mice), and all the contents of the kit are ready to use (dilution buffer, calibrator, and control). The tested ferritin in serum reacts with (SPR) and activates the enzyme which hydrolyzes the substrate (4-Methylumbelliferyl phosphate) into fluorescence product (4-Methylumbelliferone) that measured at (450 n.m). All the test steps are automated by the instrument in relation to the calibration curve stored in memory, and then printed out.

**Sequencing**

Sequencing of PCR product above carried out by NICEM Company (South Korea) by sending the PCR DNA products with their specific primers by freezer bag. The sequencing study was conducted between the sequence of standard gene BLAST program which is available at National Center of Biotechnology Information (NCBI) online at (http://www.ncbi.nlm.nih.gov) and using BioEdit program. Evolutionary analysis was conducted in MEGA5 (Tamura, 2011).

**Statistical Analysis**

Analysis of data was carried out using the available statistical package of SPSS-22 (Statistical Packages for Social Sciences-version 22). Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range. The significance of difference of different means (quantitative data) were tested using Students-t-test for difference between two independent means or Paired-t-test for difference of paired observations (or two dependent means), or ANOVA test for difference among more than two independent means. The significance of difference of different percentages (qualitative data) were tested using Pearson Chi-square test ($\chi^2$-test). Statistical significance was considered whenever the P value for the test of significance was equal or less than 0.05.

**RESULTS**

One hundred and fifty eight thalassemia patients(57.6% male and 42.4% female), with mean age of 16.8±8.5 year, and 150healthy blood donors with randomly selected persons considered as controls (58.7% male and 41.3% female), with mean age of 16.7±8.6 year. Statistical analysis shows a significant (P<0.05) increase in SEN-V prevalence in thalassemia patients comparing to controls (Table 1).

**Table 1: Prevalence of SEN-V DNA among thalassemia patients and controls.**

| | | Thalassemia | | Controls | | P value |
|---|---|---|---|---|---|---|
| | | No | % | No | % | |
| SEN Virus | Positive | 68 | 43.0 | 16 | 10.7 | 0.0001* |
| | Negative | 90 | 57.0 | 134 | 89.3 | |

***Significant difference between proportions using Pearson Chi-square test at 0.05 levels.**

Moreover, as shown in table 2 and figure 4 there is a significant (P<0.05) increase in SEN-V prevalence in thalassemia patients than the controls regardless of HCV infection.

**Table 2: Prevalence of SEN-V among thalassemia patients with and without HCV infection.**

| | | Thalassemia HCV RNA +ve | | Thalassemia HCV RNA –ve | | Controls | | P value |
|---|---|---|---|---|---|---|---|---|
| | | No | % | No | % | No | % | |
| SEN Virus | Positive | 11 | 61.1 | 57 | 40.7 | 16 | 10.7 | 0.0001* |
| | Negative | 7 | 38.9 | 83 | 59.3 | 134 | 89.3 | |

***Significant difference between proportions using Pearson Chi-square test at 0.05 level.**

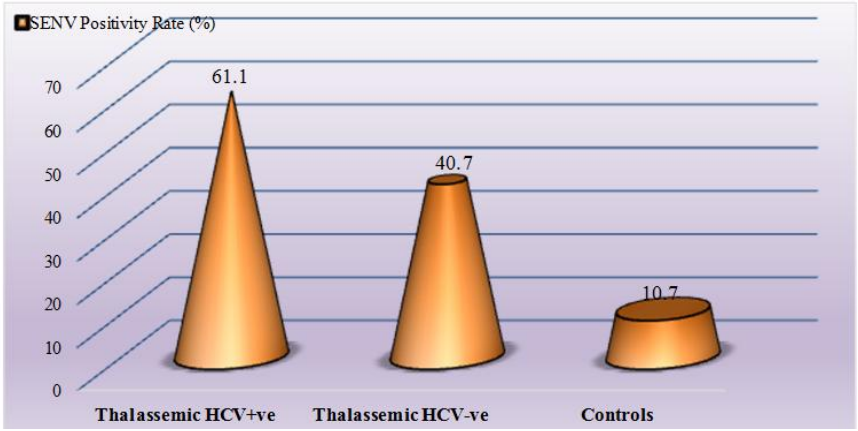


**Fig. 4: Prevalence of SEN-V in the thalassemia patients with or without HCV infection and controls.**

Table 3 shows the comparison of SEN-V genotypes prevalence among thalassemia patients with or without HCV co-infection and controls. The results show there is significant (P<0.05) increase in SEN-V-H in controls and thalassemia patients without HCV infection than those in thalassemia patients with HCV infection.

**Table 3: Distribution of SEN-V genotypes among the thalassemia patients with or without HCV infection and controls**

| | | Thalassemia HCV RNA+ve | | Thalassemia HCVRNA-ve | | Controls | | P value |
|---|---|---|---|---|---|---|---|---|
| | | No | % | No | % | No | % | |
| SENV genotype | H | - | - | 7 | 5.0 | 11 | 7.3 | 0.0001* |
| | D | 7 | 38.9 | 40 | 28.6 | 2 | 1.3 | |
| | D+H | 4 | 22.2 | 10 | 7.1 | 3 | 2.0 | |

***Significant difference between proportions using Pearson Chi-square test at 0.05 level.**

Data then had been divided into six subgroups as shown in table 4 and figure 5

**Table 4: Thalassemia and control subgroups prevalence**

| | | No | % |
|---|---|---|---|
| **I** | Thalassemia HCV +ve SEN-V +ve | 11 | 7 |
| **II** | Thalassemia HCV +ve SEN-V –ve | 7 | 4.4 |
| **III** | Thalassemia HCV -ve SEN-V +ve | 57 | 36.1 |
| **IV** | Thalassemia HCV -ve SEN-V-ve | 83 | 52.5 |
| **V** | Controls SENV +ve | 16 | 10.7 |
| **VI** | Controls SENV –ve | 134 | 89.3 |

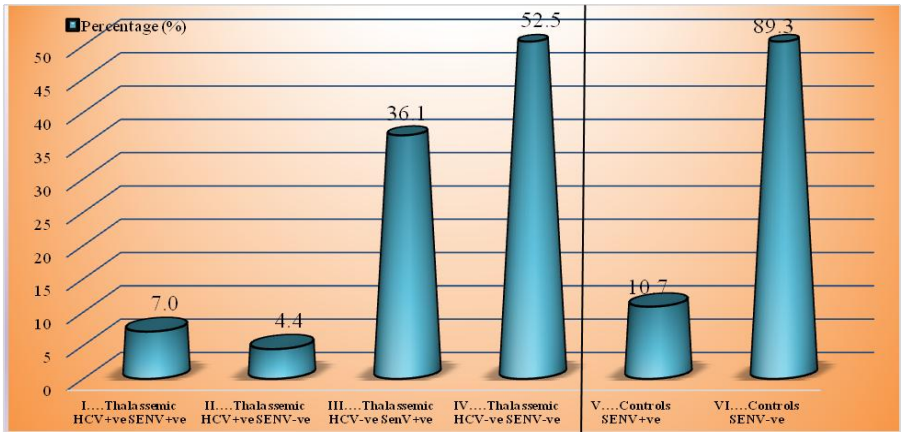


**Figure 5: Prevalence of thalassemia and control subgroups**

Moreover, there was a significant (P<0.05) increase in AST level in subgroup (I) than the other subgroups (Tables 5). This increase was significant only with thalassemia HCV and SEN-V co-infection but it was not significant in thalassemia patients with SEN-V infection alone.

**Table 5: Levels of AST in thalassemia patients and controls subgroups.**

| | | AST (U/l) | | | |
|---|---|---|---|---|---|
| | | <50 | | =>50 | |
| | | No | % | No | % |
| **I** | Thalassemic HCV+ve SENV+ve | 5 | 45.5 | 6 | 54.5 |
| **II** | Thalassemic HCV+ve SENV-ve | 4 | 57.1 | 3 | 42.9 |
| **III** | Thalassemic HCV-ve SenV+ve | 46 | 80.7 | 11 | 19.3 |
| **IV** | Thalassemic HCV-ve SENV-ve | 69 | 83.1 | 14 | 16.9 |
| **V** | Controls SENV+ve | 16 | 100 | - | - |
| **VI** | Controls SENV-ve | 134 | 100 | - | - |
| P value | | 0.0001* | | | |

***Significant difference between proportions using Pearson Chi-square test at 0.05 level**

In addition, there was a significant (P<0.05) increase in ALT level in subgroups (I) and (II) than other subgroups (Table 6).

**Table 6: Levels of ALT in thalassemia patients and controls subgroups.**

| | | ALT (U/l) | | | |
|---|---|---|---|---|---|
| | | <40 | | =>40 | |
| | | No | % | No | % |
| **I** | Thalassemic HCV+ve SENV+ve | 2 | 18.2 | 9 | 81.8 |
| **II** | Thalassemic HCV+ve SENV-ve | 3 | 42.9 | 4 | 57.1 |
| **III** | Thalassemic HCV-ve SenV+ve | 31 | 54.4 | 26 | 45.6 |
| **IV** | Thalassemic HCV-ve SENV-ve | 55 | 66.3 | 28 | 33.7 |
| **V** | Controls SENV+ve | 13 | 81.3 | 3 | 18.8 |
| **VI** | Controls SENV-ve | 134 | 100 | - | - |
| P value | | 0.0001* | | | |

***Significant difference between proportions using Pearson Chi-square test at 0.05 level.**

However, the significant (P<0.05) difference in table 7 comes from the 100% levels of serum ferritin among all thalassemia subgroups that resulted from iron overload of blood transfusion and there is no relation with HCV or SEN-V infection.

**Table 7: Levels of serum ferritin in thalassemia patients and controls subgroups.**

| | | Serum ferritin (ng/ml) | | | | | |
|---|---|---|---|---|---|---|---|
| | | **<30M or <25F** | | **30-350M&20-250F** | | **>350M &>250F** | |
| | | **No** | **%** | **No** | **%** | **No** | **%** |
| **I** | Thalassemic HCV+ve SENV+ve | - | - | - | - | 11 | 100 |
| **II** | Thalassemic HCV+ve SENV-ve | - | - | - | - | 7 | 100 |
| **III** | Thalassemic HCV-ve SenV+ve | - | - | - | - | 57 | 100 |
| **IV** | Thalassemic HCV-ve SENV-ve | - | - | - | - | 83 | 100 |
| **V** | Controls SENV+ve | - | - | 16 | 100 | - | - |
| **VI** | Controls SENV-ve | - | - | 133 | 99.3 | 1 | .7 |
| P value | | 0.0001* | | | | | |

***Significant difference between proportions using Pearson Chi-square test at 0.05 level.**

According to SEN-V genotypes in thalassemia patients and controls, mean levels of AST, ALT and serum ferritin show no significant differences. Moreover, AST and ferritin mean levels were statistically studied in SEN-V genotypes among thalassemia (with and without HCV infection) and control show no significant differences, while ALT shows significant (P<0.05) increase in comparing of HCV RNA + (co-infection) with the thalassemia HCV RNA – and controls (Table 8).

**Table 8: Mean levels of ALT in SEN-V genotypes among thalassemia patients (with and without HCV infection) and controls**

| **SENV genotype** | | **ALT (U/l)** | | **P value** |
|---|---|---|---|---|
| | | **No** | **Mean±SD (Range)** | |
| H | Thalassemic HCV+ve | - | - | 0.091 |
| | Thalassemic HCV-ve | 7 | 43.57±24.05 (15-87) | |
| | Controls | 11 | 28.64±11.10 (14-44) | |
| D | Thalassemic HCV+ve | 7 | 79.43±51.14 (16-149) | 0.037* |
| | Thalassemic HCV-ve | 40 | 43.03±32.07 (12-124) | |
| | Controls | 2 | 29.00±15.56 (18-40) | |
| D+H | Thalassemic HCV+ve | 4 | 110.50±44.64 (56-148) | 0.006* |
| | Thalassemic HCV-ve | 10 | 43.50±29.60 (9-99) | |
| | Controls | 3 | 32.33±4.04 (28-36) | |

**Molecular study and phylogenetic analysis of SEN-V**

**SEN-V-D genotype**

Twelve samples of amplified SEN-V-D DNA had been sent for sequencing that were carried out by NICEM company (South Korea); two from blood donor (controls) (no.1 was infected with D genotype only, and no.2 was infected with D and H genotypes), six from thalassemia patients group (no.3, 4, 5, 6, 7 and 8 were infected with D genotype only), and four from thalassemia patients group again but these four (no.9, 10, 11, and12) were co-infected with D and H genotypes. Alignment study of samples sent revealed there were four countries had the most DNA sequence compatibility (Table 9), by the study of the comparison of each SEN-V-D DNA sent with AH2 clone that was had the most sequence compatibility (99%) and by the study of the differentiation between each SEN-V-D DNA

sample, results and phylogenetic tree had been obtained (Table 10, figure 6 and 7).

**Table 9: Compatibility of SEN-V-D DNA characteristics with the most compatible SEN-V-D DNA of other countries**

| | Accession no. | Strain | Country | Clone | Compatibility |
|---|---|---|---|---|---|
| 1 | dbj\|AB856067.1\| | SEN-V-D | Iran | AH2 | 99% |
| 2 | dbj\|AB856069.1 | SEN-V-D | Iran | AH4 | 97% |
| 3 | dbj\|AB062297.1\| | SEN-V-D | USA | RP90 | 95% |
| 4 | : gb\|AY449524.1\| | SEN-V-D | Brazil | 5-A | 85% |

**Table 10: The characteristics of SEN-V-D clinical samples.**

| | Virus | Sequence ID | Compatibility | No. Nucleotide |
|---|---|---|---|---|
| 1 | SEN virus D | dbj\|AB856067.1\| | 99% | 1-197 |
| 2 | SEN virus D | dbj\|AB856067.1\| | 96% | 62-197 |
| 3 | SEN virus D | dbj\|AB856067.1\| | 95% | 63-197 |
| 4 | SEN virus D | dbj\|AB856067.1\| | 97% | 55-197 |
| 5 | SEN virus D | dbj\|AB856067.1\| | 98% | 61-197 |
| 6 | SEN virus D | dbj\|AB856067.1\| | 99% | 63-197 |
| 7 | SEN virus D | dbj\|AB856067.1\| | 96% | 70-197 |
| 8 | SEN virus D | dbj\|AB856067.1\| | 99% | 62-197 |
| 9 | SEN virus D | dbj\|AB856067.1\| | 97% | 55-197 |
| 10 | SEN virus D | dbj\|AB856067.1\| | 96% | 55-197 |
| 11 | SEN virus D | dbj\|AB856067.1\| | 97% | 55-197 |
| 12 | SEN virus D | dbj\|AB856067.1\| | 97% | 63-197 |

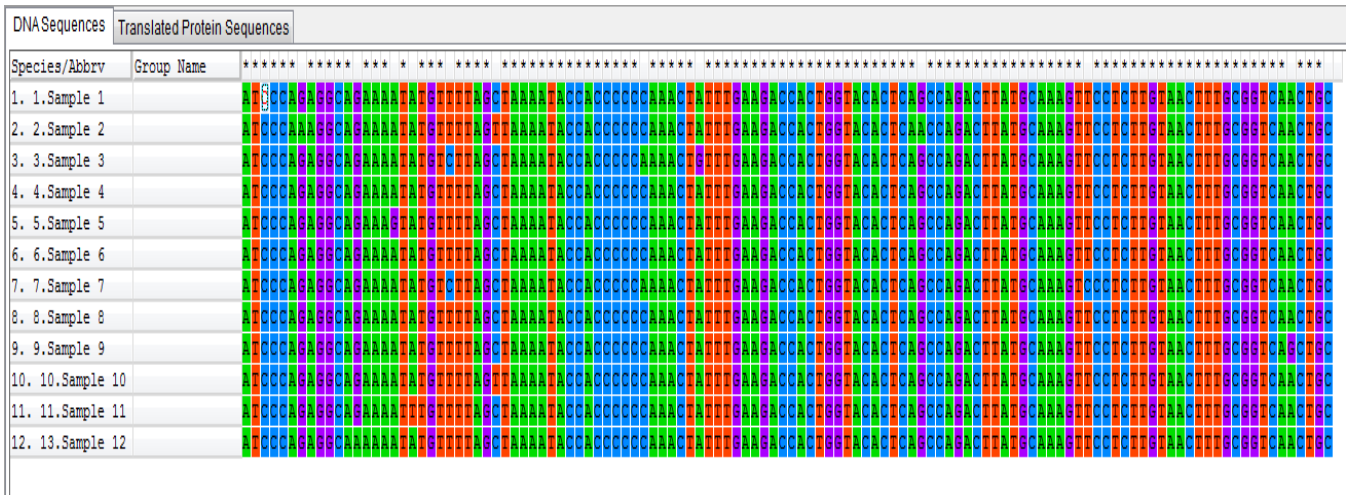


**Fig. 6: Differentiation between SEN-V-D clinical samples.**

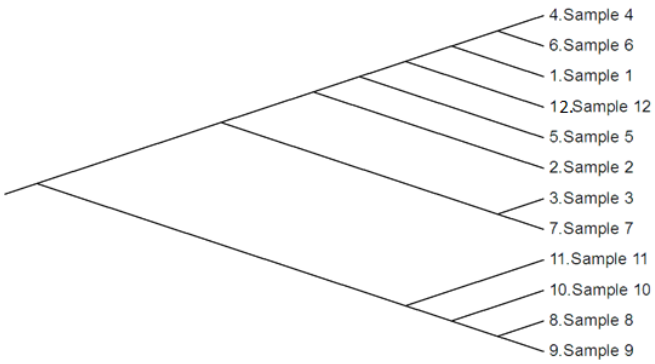


**Figure 7: Phylognetic tree of SEN-V-D.**

**SEN-V-H genotype** With the same squeal of events and numbers of SEN-V-D, SEN-V-H genotype amplified DNA samples had been studied and results had been obtained (Table 11, 12, figure 8 and 9)

**Table 11: Compatibility of SEN-V-H DNA characteristics with the most compatible SEN-V-H DNA of other countries.**

| | Accession | Strain | Country | Clone | Compatibility |
|---|---|---|---|---|---|
| 1 | gb\|AY206683.1\| | SEN-V-H | China | SENV-H Orf2 | 99% |
| 2 | dbj\|AB856066.1\| | SEN-V-H | Iran | AH1 | 96% |
| 3 | dbj\|AB856068.1\| | SEN-V-H | Iran | AH3 | 92% |

**Table 12: The characteristics of SEN-V-D clinical samples.**

| | Organism | Sequence ID | Compatibility | No. Nucleotide |
|---|---|---|---|---|
| 1 | SEN-V-H | gb\|AY206683.1\| | 99% | 908-976 |
| 2 | SEN-V-H | gb\|AY206683.1\| | 88% | 931-867 |
| 3 | SEN-V-H | gb\|AY206683.1\| | 95% | 900-979 |
| 4 | SEN-V-H | gb\|AY206683.1\| | 96% | 55-197 |
| 5 | SEN-V-H | gb\|AY206683.1\| | 93% | 900-979 |
| 6 | SEN-V-H | gb\|AY206683.1\| | 95% | 900-976 |
| 7 | SEN-V-H | gb\|AY206683.1\|\| | 99% | 909-976 |
| 8 | SEN-V-H | dbj\|AB856066.1\| | 90% | 50-122 |
| 9 | SEN-V-H | gb\|AY206683.1\| | 95% | 901-976 |
| 10 | SEN-V-H | gb\|AY206683.1\| | 95% | 906-979 |
| 11 | SEN-V-H | gb\|AY206683.1\| | 94% | 894-979 |
| 12 | SEN-V-H | gb\|AY206683.1\| | 94% | 900-980 |


**Fig 8: Differentiation between SEN-V-H clinical samples.**

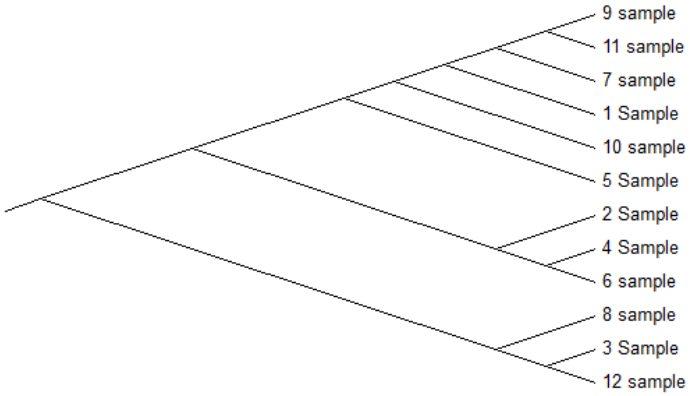


**Fig. 9: Phylogenetic tree of SEN-V-H.**

## DISCUSSION

There are several diseases that depend on blood transfusion as a way of disease management; one of these diseases is thalassemia especially β type, so regular blood transfusion considered as the only way for maintaining of patient's life [10], and there are several blood borne agents that infect these patients via blood transfusion and can cause severe complications, the most important infectious agents that transmitted via blood transfusion are HCV, HBV and HIV [11]. HCV infection has the major importance particularly in blood transfusion situations during last decades but there are other transmitted agents still unknown like SEN-V [12]. In our study, SEN-V and HCV infections had been identified by DNA and RNA extraction, HCV RNA reverse transcripted into complementary DNA, DNA amplification by PCR and then gel electrophoresis. The statistical study of results show In Iraq the prevalence rate of SEN-V DNA in thalassemia patients is 43% while in healthy blood donors is 10.7% which means there were approximately four folds increasing in the prevalence. Co-infection rate of SEN-V and HCV in Iraqi thalassemia patients is 61.1% and among thalassemia patients that not infected with HCV was 40.7%. Moreover, it had been observed that SEN-V-H was higher among controls than those with thalassemia patients while SEN-V-D was higher in thalassemia patients than controls. The percentage of SEN-V-H in thalassemia patients with HCV co-infection was consistent with the study of Sagir and others in which the co-infection with HCV may reduce the SEN-V-H replication [13]. SEN-V infection rate in thalassemia patients increases with age as a result of increasing frequency of blood pints taken. Gender appeared to be not significantly differed among SEN-V positive and SEN-V negative thalassemia patients. SEN-V and HCV co-infection significantly increases AST level above normal range. SEN-V significantly increases ALT level above normal range and has a great significant ALT level increase with HCV co-infection. The significant increasing was appeared in ALT levels above normal was inconsistent with the study of Abbas and others in Iran; he was noticed that there was no significant difference in the mean

of liver enzymes levels between SEN-V positive and SEN-V negative in healthy blood donors and thalassemia [14], also with the study of Chia-yen Dai and others in Southern Taiwan who concluded that SEN-V-D or SEN-H infection was not associated with ALT level [15]. Serum ferritin has no significant relation with SEN-V or SEN-V and HCV co-infection. On the other hand, there are no significant differences between SEN-V-D, SEN-V-H and SEN-V D and H co-infected samples in AST and ferritin among thalassemia HCV positive, thalassemia HCV negative patients and controls except the ALT levels that shows significant ($P<0.05$) increase in comparing of HCV RNA + (co-infection) with the thalassemia HCV RNA – and controls. After SEN-V detection and SEN-V D and H genotypes determination by PCR, the samples had been divided into two groups, D and H group for studying the sequencing with alignment and phylogenetic tree of each group. SEN-V-D DNA had been aligned and results that obtained showed there was 99% compatibility with the SEN-V-D DNA study of Bouzari and Hosseini in Iran [16], while 97% compatibility with the study of Bouzari and Hosseini again but another clone in Iran [16]. Moreover alignment results showed 95% compatibility with the study of Umemura and others in USA [17]. At last, Diniz-Mendes and others study in Brazil showed 85% compatibility with current study [18] (Table 9). In addition, study of the differentiation between each SEN-V-D DNA samples revealed that the compatibility between samples of healthy blood donors (controls) and thalassemia patients (blood recipients); this means the virus had been transmitted most probably via blood transfusion and D sequence of the co-infected persons with SEN-V D and H is the same sequence of D genotype of the SEN-V-D infected person (Table 10, Figure 6 and 7). Although SEN-V-H genotype DNA samples had been aligned and the results showed 99% compatibility with the study of Du J. and others in China [19], while 96% compatibility with the study of Bouzari and Hosseini in Iran [16]. Moreover, results showed 92% compatibility with the study of Bouzari and Hosseini in Iran again but another clone [16] (Table 11). Study of the differentiation between each SEN-V-H DNA samples showed that there were compatibility between samples healthy blood donor (control) and thalassemia patient (blood recipient), this means this genotype also had been transmitted most probably via blood transfusion. Also, H sequence of the co-infected persons with SEN-V D and H is the same sequence of H genotype of the SEN-V-H infected persons (Table 12, Figure 8 and 9). Finally, the current study concluded that the most probably mode of transmission was blood transfusion because of the sequence homology between donors and recipients and this conclusion is found to be consistent with the study of Umemura [8].

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