ORIGINAL ARTICLE

# Characterization of Drug –resistance Profiles to Directly Acting Agents in Hepatitis C virus Naive Patients

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## ABSTRACT

***Background***: *The development of direct-acting antiviral agents (DAAs) has revolutionized the treatment of HCV infection. The main challenge to HCV effective treatment with daas is the emergence of HCV drug-resistant variants. Detection of resistance associated variants is of importance in clinical settings in order to optimize DAA regimens, maximize success rates and reduce the impact of treatment failure.* ***Objectives:*** *The prevalence of possible mutations expected to induce potential directly acting antiviral agent (DAA) resistance was investigated in twenty DAAs naïve HCV infected patients.* ***Methodology***: *The twenty HCV isolates were genotyped using the full length NS3/4A, NS5B, and two third of the carboxy terminal region (including ISDR) of NS5A gene sequences.* ***Results:*** *Eighteen (90%) out of 20 strains were diagnosed as subgenotype 4a while 2 (10%) were of subgenotype 4n, Amino acid frequencies at each position in the NS3 protease sequence were determined with the VESPA software program. Twenty four Genotype4-specific amino acid signatures were present in almost all of our sequences, but were absent from all other genotypes. Among the twenty four amino acid signatures only one mutation at position 41 (T/S) reported to be associated with resistance to protease inhibitors. Compared to the wild type HCV GT-4; nine mutations were detected among our isolates at a frequency ranging from 27% to 100%. None of these mutations were associated with resistance to protease inhibitors. Forty three mutations were detected among our isolates at a much lower frequency ranging from 5.5% to 16.6%. Only 5 out of them were associated with protease inhibitor resistance. Amplification of domain II and III including the interferon sensitivity-determining region and the interferon/ribavirin resistance-determining region of the NS5a region showed a number of mutations exceeding 4 in all isolates and 82.3% of them had from 10-30 mutations. Thirty two Genotype 4-specific amino acid signatures were present in almost all of our sequences and absent from all other genotypes. The primary NS5B nucleoside polymerase inhibitors (NPIs) resistance variant 282T was not detected in our isolates.* ***Conclusion:*** *The large number of natural polymorphism of HCV 4 isolates as well as the large number of mutations detected in this study and different from those associated with DAA resistance makes it more practical to detect resistance associated mutations in DAA treatment failure then to look for these mutations in naïve patients.*

## INTRODUCTION

Hepatitis C virus (HCV) infection is a major cause of mortality and morbidity worldwide and is a main cause of end-stage liver disease, hepatocellular carcinoma and liver transplantation in developing countries. [1] In 2015, the prevalence of HCV in Egypt was found to be 10.0% in the 15–59 year age groups. [2] The national goal of the HCV management program in Egypt is to reduce the prevalence <2% within 10 years and to near elimination of the disease (prevalence <1%) by 2030. [3]

HCV has seven major genotypes and the genetic diversity of HCV has been clearly linked to the geographic distribution of the virus. The clinical significance of HCV genotype is due to its impact on the response to HCV therapy.[4,5] In Egypt, HCV genotype 4 (GT4) accounts for approximately 90% of infections, with subtype 4a predominating. [6]

Peginterferon/ribavirin (pegifn/RBV) combination has been used for the treatment of HCV infection. However, a sustained virological response was not always achieved in GT4. [7]

The development of direct-acting antiviral agents (DAAs) has revolutionized the treatment of HCV

infection. They specifically target non-structural viral proteins involved in viral replication; HCV NS3 protease, NS5B polymerase and NS5A protein. [8-10]

The main challenge to HCV effective treatment with daas is the emergence of HCV drug-resistant variants. The high replication rate of HCV, the low fidelity and poor proofreading of its RNA polymerase, generate a highly variable virus population, "viral quasispecies', and the creation of variants encoding amino acid substitutions which may lead to decreased susceptibility to daas. [11, 12]

Detection of resistance associated variants is of importance in clinical settings in order to optimize DAA regimens, maximize success rates and reduce the impact of treatment failure. Thus, the aim of this study was to determine the prevalence of possible mutations in NS3, NS5A and NS5B domains in DAA-naive HCV patients.

## METHODOLOGY

DAA-naive HCV patients referred to the Medical Research Institute, Alexandria University between December 2015 and June 2016 were enrolled in the current study. Serum samples were collected following approval of the study by the Ethics Committee of the institute and after obtaining written informed consents from patients.

Exclusion criteria included patients with renal, cardiac, neoplastic diseases, immunological disorders and/or cirrhotic patients. All patients were negative for Hepatitis B surface antigen (HBsAg) to exclude HBV co-infection.

- **Viral RNA extraction:**

RNA extraction from serum samples was performed using Qiagen QIAamp viral RNA mini spin Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol._Elution was made in 50 µl AVE buffer, which was used as source for template RNA for viral load determination and cDNA synthesis.

- **HCV viral load determination:**

Quantitation of HCV viral load HCV-RNA was performed by (Artus HCV QS-RGQ-PCR Kit, µ) reagents on Stratagene Mx3000 P™ thermal cycler according to recommended protocol.

- **cDNA synthesis**

Ten µl of eluted RNA was used as a template for preparation of a cDNA using Thermo® Scientific Revert Aid First Strand cDNA Synthesis Kit with random hexamer primers according to the manufacturer recommendations.

The following thermal profile was used ; activation at 25°C for 5 minutes, reverse transcription at 42°C for 60 minutes and termination at 70°C for 5 minutes.

***Amplification of NS3, NS5A and NS5B genes:***

Amplification by conventional PCR in one step PCR for of NS3 protease and nested PCR for NS5A and NS5B domains on Veriti Thermal cycler (Applied Biosystem) using Hot start PCR master mix (Thermo Scientific) and pairs of specific primers (table 1) (Biosearch Technologies, Petaluma, CA).

**Table 1: primers used for amplification of NS3 protease, NS5A and NS5B genes.**[13-15]

| Primer | Nucleotide sequence (5'–3') | Target | Annealing Temp. (Ta) | Amplicon length by bases | Reference |
|---|---|---|---|---|---|
| **Forward**<br>**Reverse** | ATCTTGCTCGGGCCGGCCGA<br>GCGACCTGRTAGGTCTGRGGCA | **NS3** | 54°C | 650 | 13 |
| **Outer**<br><br>**Inner** | **F:**CTCAAYTCGTTCGTRGTGGGATC<br>**R**:CGAAGGTCACCTTCTTCTGCCG<br>**F**:ATGCGAGCCYGAGCCGGACGT<br>**R**:GCTCAGGGGGYTRATTGGCAGCT | **NS5A** | 50°C | 849 | 14 |
| **Outer**<br><br>**Inner** | **F**:GGA TCR GAG GAY GTM GTR TG<br>**R**:TGT GAT AAA TGT CYC CCC CG<br>**F**:CTG CCM ATY ARC CCC CTG AG<br>**R**:GGC AAT GGA GTG AGT YTG | **NS5B** | 52°C | 1559 | 15 |

***PCR product Sequencing***

Amplified DNA of NS3, NS5a and NS5b domains were sequenced after purification (ABI PRISM 310 genetic analyzer DNA Sequencer, Applied Biosystems, Foster City, CA, USA) and the BigDye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA)

***Detection of HCV genotypes and subtypes by sequence analysis of the NS5B gene:***

The HCV genotypes were identified by entering the sequencing data of the amplified NS3/4A gene, NS5B gene and the carboxy terminal region of NS5A gene (including ISDR) into HCV BLAST in GeneBank. (http://blast.ncbi.nlm.nih.gov).

**Sequence analysis of NS3, NS5A and NS5B genes and comparison with that of worldwide isolates for detection of mutations:**

BioEdit Sequence alignment editor version 7.2.5 was used in order to carry the alignment step with HCV GT-4 Reference Sequence (accession number (NC009825.1) with the sequenced samples.

**Viral Epidemiology Signature Pattern Analysis (VESPA) program**

Amino acid sequence variations and signature patterns were analyzed using the online VESPA. program (http://hcv.lanl.gov/content/sequence/VESPA/vespa.html) available in the HCV sequence database. It was used with nearly all HCV sub-genotypes other than GT-4 as background reference sequences and HCV GT-4 sequences included in this study as the query sequences.

## RESULTS

The present study included 20 HCV naïve patients. Fourteen (70%) out of them were males and 6 (30%) were females. Their age ranged from 19 to 70 years. Among the 20 HCV patients included in this study 70.0% gave a history of dental intervention, followed by hospital admission 60.0%, surgical intervention 50.0% family history of a relative suffering from HCV infection 45.0%. The majority of the 20 HCV patients (95%) had a viral load ranging between $>10^5$ - $<10^7$ IU/ml. A single case (5%) had a viral load $>10^7$ IU/ml.

Demographic and laboratory characteristics of patients included in this study are summarized in

**Table**

**Table 2: Demographic and laboratory data of the patients**

| | Patients (n=20) | |
|---|---|---|
| Gender | | |
| Male | 14 | 70% |
| Female | 6 | 30% |
| Age | | |
| < 20 | 2 | 10% |
| 20-40 | 9 | 45% |
| >40-60 | 7 | 35% |
| > 60 | 2 | 10% |
| Viral Load IU/mL | | |
| $>10^5$-$<10^6$ | 9 | 45% |
| $>10^6$-$<10^7$ | 10 | 50% |
| $>10^7$ | 1 | 5% |
| Alanine Transaminase (ALT) | | |
| Normal | 10 | 50% |
| Abnormal | 10 | 50% |

Among the 18 sequences of NS3 and NS5A which have been successfully amplified; 16 (88.88%) were found to be HCV4a as shown by the HCVBLAST and 2(11.11%) were HCV 4n. While 18(90%) out of the 20 NS5B sequences were HCV4a and 2 (10%) were HCV4n, as shown in table3

**Table 3 Genotyping by direct sequencing of NS3, NS5A and NS5B gene regions:**

| Genotype | NS3 | | NS5A | | NS5B | |
|---|---|---|---|---|---|---|
| | Number of isolates | % | Number of isolates | % | Number of isolates | % |
| **HCV4a** | 16 | 88.88 | 16 | 88.88 | 18 | 90 |
| **HCV 4n** | 2 | 11.11 | 2 | 11.11 | 2 | 10 |
| **Total** | 18 | 100 | 18 | 100 | 20 | 100 |

**Mutations in the NS3 region:**

Mutations at the position 61 (A/S), 102 (A/S), 134 (I/T) were detected in all our 18 isolates, while mutations at position 101(S/A) and 114(I/V) were detected in 17 and 16 isolates respectively. Mutations at positions 18(V/I), 92(R/K), 95(A/T, S, V) and 150(R/A, C) were detected among 5-14 of our HCV isolates in comparison with genotype 4 reference strain using BIOEDIT program. (table 4 and fig. 1)

**Table 4: Common Mutations in the NS3 region:**

| Position | A.A in wild type | Mutation | No. of isolates | % |
|---|---|---|---|---|
| **18** | V | I | 5 | 27.77 |
| **61** | A | S | 18 | 100.0 |
| **92** | R | K | 7 | 38.88 |
| **95** | A | T/S/V | 10 | 55.55 |
| **101** | S | A | 17 | 94.44 |
| **102** | A | S | 18 | 100.0 |
| **114** | I | V | 16 | 88.88 |

| **134** | I | T | 18 | 100.0 |
|---|---|---|---|---|
| **150** | R | **A/C** | 14 | 77.77 |

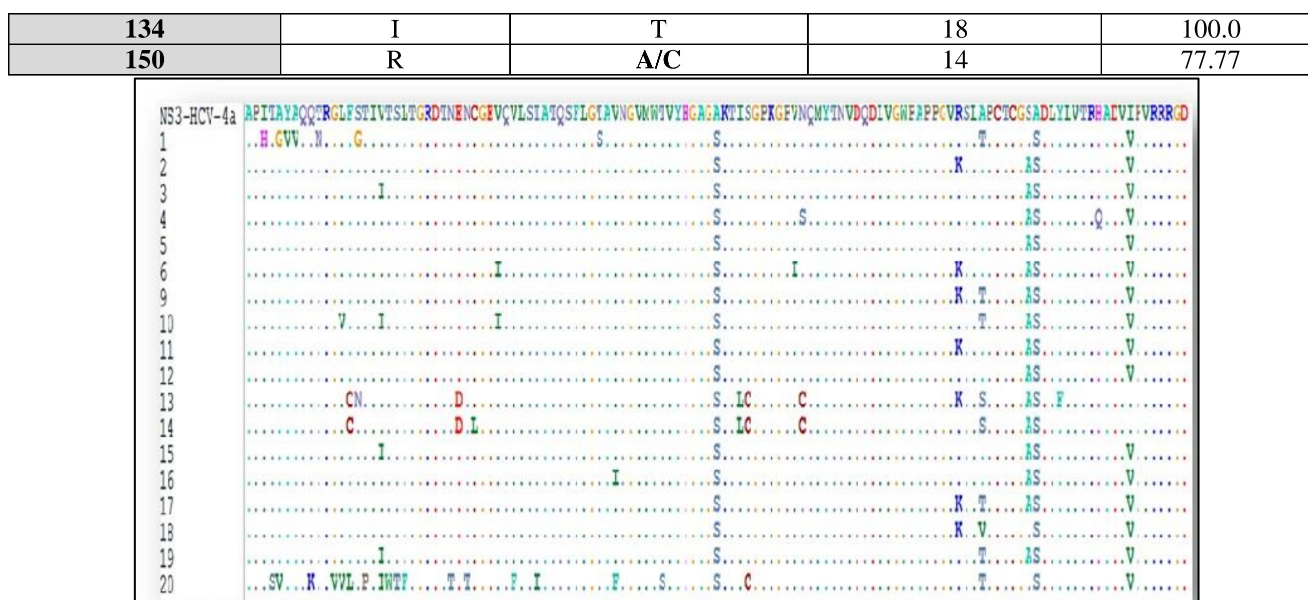

**Fig. 1:**Bioedit alignment showing amino acid substitutions in the NS3 region of our18 HCV GT-4 cases in comparison with GT-4 Reference Sequence accession number (NC009825.1).

On the other hand, mutations associated with resistance to protease inhibitors as reported in other studies were detected in only 5 of our isolates as seen in table 5

**Table 5: Mutations associated with resistance to NS3 protease inhibitors**

| Strain | Mutation |
|---|---|
| 3 | L175P |
| 10 | V170I |
| 14 | D168E |
| 16 | I153L, V170I |
| 20 | T54S, I153V, L175V |

Figure (2) shows the amino acids signature profile of our 18 HCV genotype 4 versus the non HCV genotype 4 background signatures. Nine amino acids were found only in our HCV genotype 4 isolates (Query signature) compared to other non HCV genotype 4 (Background signature) as shown in the VESPA software outlet. Moreover, mutation at position 41(T/S) found in all our isolates was reported in other studies to be associated with resistance to protease inhibitors.

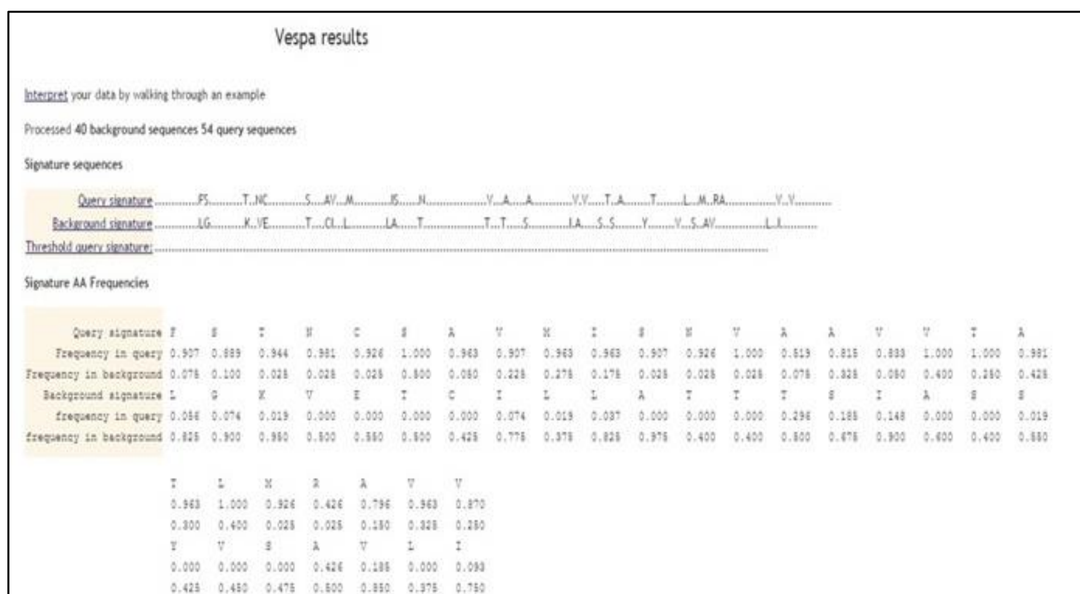


**Fig. 2:** NS3 protease signature pattern using VESPA program

**Mutations in NS5A region among the 17 HCV cases:**

Fourteen (82.3%) out of 17 isolates had from 10 -30 mutations as compared to HCV genotype 4 reference strain using BIOEDIT program. While only one isolate had 51 mutations (table 6). The application of VESPA for comparing the NS5A sequence of our HCV genotype 4 isolates (Query signature) with other non HCV genotype 4 (Background signature) was not possible due to the high heterogeneity of this region in HCV genotype 4.

**Table 6: Frequency of mutations in NS5A region among the 17 HCV cases:**

| Number of mutations | Number of isolates | % |
|---|---|---|
| 10- 20 | 6 | 35.29 |
| 21-30 | 8 | 47.05 |
| 31-40 | 2 | 11.76 |
| 41-50 | - | - |
| >50 | 1 | 5.88 |

**NS5B mutations detected among 20 HCV cases:**

Nineteen common mutations were detected in a number of isolates ranging from 55% to 100% (table 7). Meanwhile, 26 less common mutations were detected in the NS5B region using BIOEDIT program as compared to reference HCV4 in a number of strains ranging from 10% to 55%. (table 8 and fig 3)

**Table 7 NS5B common mutations detected among 20 HCV isolates**

| Position | A.A in wild type | Mutation | Number of isolates | % |
|---|---|---|---|---|
| 58 | S | N | 15 | 75 |
| 76 | P | A | 20 | 100 |
| 94 | R | K | 11 | 55 |
| 116 | S | N | 16 | 80 |
| 120 | K | E | 20 | 100 |
| 124 | D | E | 17 | 85 |

| 163 | S | V | 20 | 100 |
|---|---|---|---|---|
| 177 | K | Q | 13 | 65 |
| 178 | T | L | 13 | 65 |
| 208 | N | K | 15 | 75 |
| 231 | V | A | 11 | 55 |
| 254 | D | E | 19 | 95 |
| 278 | T | S | 19 | 95 |
| 303 | A | G | 19 | 95 |
| 372 | V | A | 19 | 95 |
| 392 | V | A | 20 | 100 |
| 420 | I | V | 15 | 75 |
| 484 | A | S | 18 | 90 |
| 513 | R | K | 15 | 75 |

**Table 8 NS5B Less common mutations detected among the 20 HCV isolates**

| Position | Mutation | Number of isolates | % |
|---|---|---|---|
| 53 | L/V | 2,5 | 10 |
| 53 | L/M | 6,9 | 10 |
| 55 | V/M | 6,7,9,19,20 | 25 |
| 62 | E/D | 13,14,15 | 15 |
| 62 | E/A | 10,12,19 | 15 |
| 64 | L/F | 5,8,17,18 | 20 |
| 73 | R/T | 13,14 | 10 |
| 73 | R/K | 12,18 | 10 |
| 81 | T/I | 9,10 | 10 |
| 81 | T/V | 13,14 | 10 |
| 112 | I/V | 2,3,5,10,11,14 | 30 |
| 131 | P/S | 2, 5,10,11,15,16,19 | 35 |
| 172 | H/I | 3,13,14,16,18 | 25 |
| 179 | A/P | 5,7,8,11,12,15,16,17,18,19,20 | 55 |
| 179 | A/T | 1,2,3,4,6,9,10,14 | 40 |
| 199 | F/I | 2,5,8,10,11,13,14,16,17 | 45 |
| 209 | D/T | 6,7,10,11,15,12,16,17,18,19,20 | 55 |
| 209 | D/V | 9,13,14 | 15 |
| 233 | E/A | 2,5,8 | 15 |
| 233 | E/G | 12,15,16 | 15 |
| 240 | D/N | 3,14,15 | 15 |
| 250 | T/A | 1,4,12,16,19,20 | 30 |
| 266 | K/R | 13,14,16,17 | 20 |
| 268 | D/E | 3,10,11 | 15 |
| 281 | Y/F | 8,10,11,13,14,16 | 30 |
| 289 | L/M | 9,12,15 | 15 |
| 299 | I/T | 13,14,16,18 | 20 |
| 300 | R/K | 5,8,11,12,15 | 25 |
| 305 | R/K | 5,11,13,14,15,16,17,18 | 40 |
| 329 | N/S | 2,5,9,13 | 20 |
| 329 | N/A | 8,10,11 | 15 |
| 329 | N/K | 19,20 | 10 |
| 373 | T/A | 9,11 | 10 |
| 373 | T/V | 10,18 | 10 |
| 376 | K/R | 13,14,20 | 15 |
| 410 | V/I | 3,6,9 | 15 |
| 410 | V/L | 13,14 | 10 |

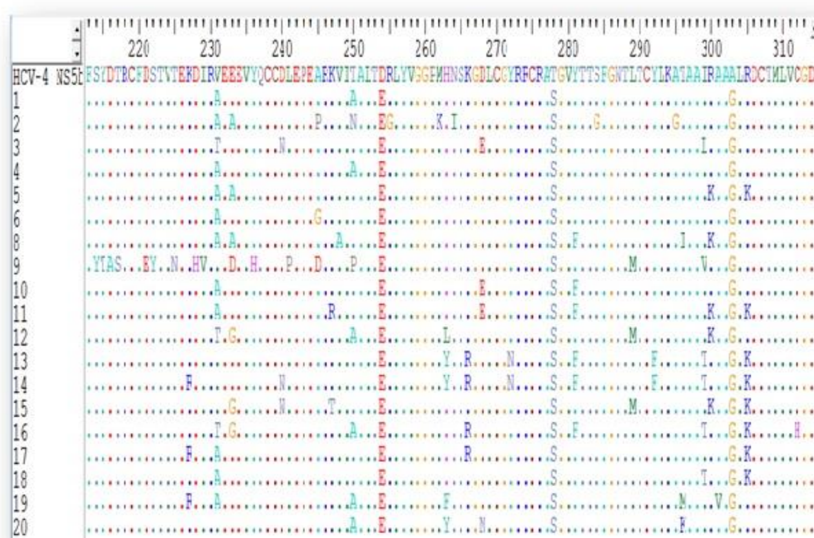


**Fig. 3:** Bioedit alignment showing amino acid substitutions in the NS5B region of the 20 HCV GT-4 cases in comparison with GT-4 Reference Sequence accession number (NC009825.1).

Table 9 and fig. 4 show the amino acid signature (32) of our 20 HCV isolates (Query signature) as compared to non HCV4 isolates (background signature)

**Table 9 NS5B signature pattern using VESPA program.**

| Position | Background signature | Query signature |
|---|---|---|
| 6 | I 0.808 | V 1.00 |
| 13 | E 0.731 | S 1.00 |
| 18 | N 0.846 | S 1.00 |
| 30 | L 0.432 | M 1.00 |
| 34 | S 0.654 | A 1.00 |
| 69 | A 0.538 | R 1.00 |
| 97 | Y 0.615 | F 1.00 |
| 102 | E 0.577 | D 1.00 |
| 106 | L 0.615 | H 1.00 |
| 168 | M 0.654 | R 1.00 |
| 268 | S 0.423 | L 1.00 |
| 284 | M 0.577 | F 1.00 |
| 292 | I 0.423 | L 1.00 |
| 306 | P 0.423 | C 1.00 |
| 319 | S 0.462 | A 1.00 |
| 329 | A 0.462 | R 1.00 |
| 330 | N 0.230 | A 1.00 |
| 376 | R 0.346 | V 1.00 |
| 430 | A 0.654 | S 1.00 |
| 432 | D 0.500 | E 1.00 |
| 433 | Q 0.462 | A 1.00 |
| 439 | N 0.500 | D 1.00 |
| 441 | E 0.692 | D 1.00 |
| 445 | A 0.577 | V 1.00 |
| 446 | V 0.500 | T 1.00 |
| 449 | V 0.654 | I 1.00 |
| 468 | S 0.885 | T 1.00 |
| 471 | S 0.385 | G 1.00 |
| 483 | A 0.500 | G 1.00 |
| 489 | A 0.577 | V 1.00 |
| 538 | R 0.308 | A 1.00 |
| 539 | R 0.423 | K 1.00 |

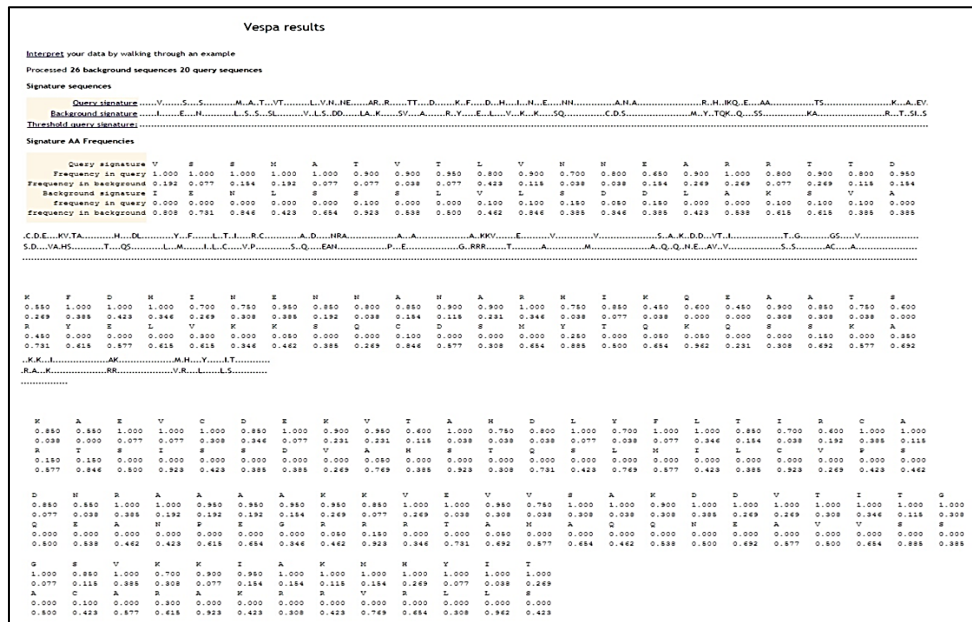


**Fig. 4:** NS5B signature pattern using VESPA program.

## DISCUSSION

The 2016 Guidelines provide recommendations on the preferred and alternative DAA regimens by HCV genotype. [16] Therefore knowing patient genotype is still important for determining the most appropriate treatment regimen.

In the present study, HCV genotyping of the 20 HCV isolates was tried using the full length NS3/4A, NS5B, and the two third of the carboxy terminal region including ISDR (interferon resistance determining region) of NS5A gene sequences. Full concordance was observed in 18 out of the 20 isolates of which 16 were diagnosed as subgenotype 4a while 2 (10%) were of subgenotype 4n. The remaining 2 isolates were successfully sequenced only in the NS5b region and were diagnosed as subgenotype 4a.

High subtype concordance between the three target sequences was also reported by Schnell *et al*, [17] among their patients infected with subtype 4a.

DAAs have been developed to target non-structural viral proteins involved in viral replication. However, due to the low fidelity of HCV polymerase, the high HCV replication rate and the strong selective pressures on the virus, a collection of HCV quasispecies develops within an infected patient before the start of treatment. [18, 9]

Only few studies report the prevalence of DAA-naive patients with viral populations predominantly resistant to DAAs. [19, 20] The aim of this study was to detect the presence of NS3, NS5A, or NS5B polymerase inhibitor-resistant variants in 20 HCV patients who are naive to DAA treatment regimen.

NS3 protease gene was successfully sequenced in 18 (90%) out of the 20 HCV isolates. The catalytic site on NS3 protease is located in a shallow substrate binding groove which does not facilitate tight binding to the inhibitors.[21] Therefore, inhibitors depend on few interactions with the enzyme and only a few critical mutations in the enzyme may be enough to confer significant resistance to these drugs.[22]Several mutations in different positions at the NS3 protease have been associated with loss in susceptibility to Protease Inhibitors.[23]

In the present study, 2 types of mutations were observed with different frequencies. Amino acid frequencies at each position in the NS3 protease sequence were determined with the VESPA software program. Twenty four Genotype 4 -specific amino acid signatures were present in almost all of our sequences, but were absent from all other genotypes. Among the 24 amino acid signatures only one mutation at position 41 (T/S) was reported to be associated with resistance to protease inhibitors was detected in this study. This resistant mutation was detected for the first time in vivo by Vermehren *et al*, [24] in 3 out of 26 patients, during treatment with Boceprevir (which was withdrawn from the market due to the overwhelming superiority of newer direct-acting antiviral agents). [25]

The second type of mutations was detected by nucleotide and amino acid sequences of the NS3 region aligned with Clustal X and BIOEDIT version 7.2.5 software and analyzed for the presence of previously reported substitutions conferring resistance to NS3 protease inhibitors among our isolates compared to the wild type HCV GT-4. Mutations at positions 18, 61, 92, 95, 101, 102, 114, 134, and 150 were detected at a high frequency ranging from 27% to 100%; however, none of these mutations were reported to be associated with resistance to protease inhibitors.

On the other hand, 43 mutations compared to the wild type HCV GT-4 were detected among our isolates at a much lower frequency ranging from 5.5% to 16.6%. Only 5 out of them were associated with protease inhibitor resistance.

Mutation at T54S detected in 1(5.5%) out of our 18 isolates was associated with Telaprevir and Boceprevir resistance (both drugs are discontinued) but not with Simeprevir and Grazoprevir. [26]

Mutation at I153 /L detected in only 2 (11.1%) of our isolates was reported at a much higher frequency (94.6%) by Vallet *et al,* [13] and was associated with resistance to protease inhibitors.

Moreover, mutation at D168 /E detected in 1 (5.5%) of our isolates was reported to confer high level resistance to Simeprevir. [26]Shindo *et al*, reported this mutation as the most prevalent protease inhibitor -resistance-proven mutations (1.5% out of their 261 patients). [27] Mutation at V170I was detected in 2(11.1%) out of our 18 isolates. Peres-da-Silva *et al*, [28] stated that although V170I substitution has not yet been associated with resistance, it was detected in 98% of their samples.

Naturally occurring mutation M175L in the NS3 protease region was observed by Costantino *et al*, [29] in 100 % of patients infected with genotype 4. Sixteen (88.8%) out of our 18 strains had this naturally occurring mutation (L) while mutation to P or V was detected in only 2(11.1%) out of our isolates.

In the present study, we amplified domain II and III of the NS5A region including the interferon sensitivity-determining region (ISDR) and the interferon/ribavirin resistance-determining region (IRRDR).Mutations in these regions are reported to be associated with sensitivity to interferon and ribavirin

Out of our 20 isolates 17 gave fairly good sequences that have been aligned with Clustal X and BIOEDIT and analyzed for the presence of mutations compared to the wild type HCV GT-4. All our isolates had a number of mutations exceeding 4(82.3% of them had from 10-30 mutations) and thus, should have been sensitive to interferon and ribavirin. However, in spite of the

presence of a high number of NS5A mutations reported by us and by others, sensitivity of HCV 4 to interferon and/ or ribavirin ranges only between 50-60%. [30]

Sofosbuvir is the first NS5B polymerase inhibitor (NPI) to become available. Resistance variants reported in the literature to this NPI included S282T, L159F and E341D. [31]In the present study, 2 mutations A179P/T and L289/M were detected in 95% and 15% of our isolates respectively in absence of S282T. Our results agree with Ludmerer *et al*, [32] who reported that the primary NPI resistance variant 282Twas not found among all their HCV sequences analyzed.

Similarly, Gane *et al*, [33] reported that when Sofosbuvir based regimen was administered, no S282T variant was detected at baseline, and that emergence of this variant was infrequent (1%) in subjects who had virological failure.

In the present study, 19 mutations were detected in the NS5B region among our 19 isolates ranging from 55% to 100% as well as less frequently detected mutations [26] ranging from 10% to less than 55% using BIOEDIT.

Amino acid frequencies at each position in the NS5B sequence were determined with the VESPA software program. 32 Genotype 4 -specific amino acid signatures were present in almost all of our sequences, but were absent from all other genotypes.

## CONCLUSION

None of the mutations reported to be associated with resistance were detected among our isolates, however other neighboring mutations such asV410/I, I420/V and V449/I were detected.

The difference in mutations reported in our study compared to other studies may be due to that our strains were isolated from DAA naïve patients or due to HCV genotype 4 heterogeneity. While mutations cited in the literature were associated with DAA resistance either in treatment failure or in DAA naïve patients infected with different genotypes. It seems more practical to detect resistance associated mutations in DAA treatment failure then to look for these mutations in naïve patients.

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