Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/11607
DOI URL: http://dx.doi.org/10.21474/IJAR01/11607

RESEARCH ARTICLE

IN *SILICO* ANALYSIS OF NS5B GENE OF HCV GENOTYPE 4 IN NORTH EGYPT

Amal Mahmoud[1,2], Tamer M. Roshdy[3]and Moustafa A. Sakr[4]

1. Department of Bioinformatics, Genetic Engineering and Biotechnology Research Institute (GEBRI),University of Sadat City, Egypt.
2. Department of Biology, College of Science, Imam Abdulrahman Bin Faisal University, P.O. Box 1982, 31441, Dammam, Saudi Arabia.
3. Department of Molecular Biology, Genetic Engineering and Biotechnology Research Institute (GEBRI),University of Sadat City, Egypt.
4. Department of Molecular Diagnostics and Therapeutics, Genetic Engineering and Biotechnology Research Institute (GEBRI),University of Sadat City, Egypt.

Manuscript Info

Manuscript History
Received: 30 June 2020
Final Accepted: 31 July 2020
Published: August 2020

Key words:-
HCV, Genotypes, Subtypes, Phylogenetic Analysis, Drug resistance mutations

Abstract

Background: Hepatitis C is a liver disease caused by the hepatitis C virus (HCV). HCV genotypes and subtypes are related to antiviral therapy response. Our purpose was to identify genotypes and subtypes of HCV isolates collected from Menoufia, Egypt and to analyze the effect of the known drug resistance mutations on the RdRp catalytic domain of HCV-NS5B protein.

Methods: Serum of 5 HCV samples was used for RNA extraction and amplification of NS5B gene, followed by direct DNA sequencing and sequence analysis of the partial NS5B sequence.

Results: Multiple sequence alignment and phylogenetic analysis of our isolates and those represent genotypes 1-6, retrieved from GenBank database showed that Menoufia-isolates 1, 2, 4 and 5 were classified as genotype 4a, however, isolate Menoufia-3 was grouped with genotype 4c. Four known mutations related to drug resistance were detected in different HCV genotypes; Q309R was detected in Menoufia-isolates 1, 2, 4 and 5; D244N, Q309R and D310N were common in genotype 3 (80-100%). Protein functional analysis indicated that the detected drug resistance mutations did not alter the RdRb catalytic domain of NS5B protein.

Conclusions: Our data showed that HCV genotype 4 is still the most prevalent genotype in Egypt and Subtype 4a is predominant.*In silico*detection of drug resistance mutations in HCV-infectedpatients before treatment is considered as a helpful tool could be used as a marker for clinical decisions to determine the suitable treatment.

Corresponding Author:-Amal Mahmoud
Address**:-**Department of Bioinformatics, Genetic Engineering and Biotechnology Research Institute (GEBRI),University of Sadat City, Egypt.

Introduction:-

Hepatitis C virus (HCV) is a major cause of liver diseases in the world (Mohd et al., 2013 and Mosaad et al., 2010). HCV genotypes are geographically distributed worldwide, Genotype 4 and subtype 4a are the most dominant in Egypt (Fakhr et al., 2013, Ray et al., 2000 and Youssef et al., 2009).

HCV belongs to the Flaviviridae family and consists of at least 10 distinct protein products (C, E1, E2, P7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) (Lyra et al., 2004 and Moradpour et al., 2007). HCV genotyping is important for prediction of the efficiency of antiviral therapy and for determining treatment duration (Cavalcante and Lyra, 2015). NS5B is a nonstructural protein encoded on the HCV genome, it is an RNA-dependent RNA polymerase (RdRp) responsible for HCV genome replication. Castilho et al. (2011) studied the genetic variability and the genotype's mutations of the RdRp catalytic domain that are typically associated with interferon/ribavirin (IFN/RIB) combination therapy and new non-nucleoside drugs.

In this study, we determined the genotypes and subtypes of HCV isolates, collected from North Egypt (Menoufia) and we detected4 known mutations related to drug resistance as this will influence the expected therapeutic response and could be used as a marker for clinical decisions.

Materials And Methods:-

Viral RNA extraction and partial NS5B gene amplification:

The method of Demetriou et al., (2009) was used to extract RNA, primer design and partial NS5B gene amplification. Viral RNA was extracted from plasma samples of HCV-infected patients collected in 2018 from Menufia governorate-Egypt using the QIAmp1 UltraSens1 Virus kit (Qiagen, Venlo, The Netherlands), 15 µl of the RNA was used in a one-step RT-PCR HiSenScript RH (-) RT PreMix kit (intron Biotechnology, Korea), following a heat-shock step at 70°C for 20 sec to denature the RNA secondary structure. RT-PCR was done in a 50 µl reaction with 20 pmol each of the outer sense and antisense degenerate primers correspond to the NS5B region of HCV genome. A nested PCR was performed using 3 µl of the RT-PCR product with 40 pmol each of the inner PCR primers, using 2x PCR master mix (I-Taq) (intron Biotechnology, Korea) in a 50 µl reaction. Amplified product was confirmed using a 2% agarose gel electrophoresis. DNA sequencing was carried out with the ABI 310 capillarity sequencer, sequences were edited using FinchTV version 1.4.0.

Sequence analysis:

Sequence similarity search was done using BLAST programs from National Center for Biotechnology Information (NCBI), USA, (http://www.ncbi.nlm.nih.gov/Blast). HCV isolates correspond to different genotypes and subtypes were obtained from GenBank database (NCBI). Sequence comparison between our HCV isolates and those of GenBank database was used to detect drug resistance mutations and to construct Neighbor-Joining phylogenetic tree.

GenBank Submission:

NS5B sequences were submitted to GenBank under the accession numbers MN068031-MN068035.

Phylogenetic analysis:

Molecular Evolutionary Genetics Analysis (MEGA) software version MEGA6 was used to construct the phylogenetic tree (Tamura et al., 2013).

Analysis of RdRp catalytic domain of NS5B protein:

PROSITE is a protein database for identifying protein domains, families and functional sites as well as associated patterns and profiles (Sigrist et al., 2012). ScanProsite tool was used to predict the effect of the drug resistance mutations on the RdRp catalytic domain of NS5B protein.

Results:-

Amplification of partial HCV NS5B gene and sequence analysis:

A total of 5 serum samples of HCV-infected patients collected in 2018 from Menufia governorate-Egypt were used to amplify HCV NS5B gene. PCR amplification of partial sequence of NS5B gene resulted in a product of 500bp, then PCR product of the 5 isolates was sequenced and the sequences were edited using FinchTV. Using BLAST,our

isolates showed 88.59%-95.11% identity and 91.80%-99.18% similarity to NS5B gene of HCV isolates in NCBI database for nucleotide and amino acid sequence, respectively.

Detection of the knowndrug resistance mutations in the RdRp catalytic domain of NS5B protein:
Mutations located in NS5B gene are typically associated with resistance to IFN/RIB and to new antiviral drugs. Multiple sequence alignment of our isolates and those represent genotypes 1-6, retrieved from GenBank database were used to examine the prevalence of these mutations(Fig. 1).Fourknown resistance mutations (D244N, Q309R, D310N and C316N) were detected as follow: (1) D244N was only detected in genotype 3 (80%), (2) Q309R was found in genotypes 1, 3, 4 and 5 (40%, 100%, 10% and 50% for genotype 1, 3, 4 and 5, respectively); Q309R was detected in isolates Menoufia-1, 2, 4 and 5 (80%), (3) D310N was detected in genotypes 2, 3 and 6 (20%, 100% and 90% for genotype 2, 3 and 6, respectively), and (4) C316N was detected in one isolate of genotype 2 (AAV36676.1) (10%).

Effect of the drug resistance mutationson RdRp catalytic domain of NS5B protein:
PROSITE database was used to analyze the effect of the detected drug resistance mutations on the NS5B-RdRp catalytic domain (Fig 2). ScanProsite tool recognized the full length of RdRp catalytic domain (P_{214}-D_{332}) of the reference isolate 430(accession number AUF37153.1) (Fig. 2a). Similarly, the prediction of the partial NS5B-RdRp catalytic domain of our isolates and those representHCV genotypes (represented in Fig. 1) confirm that no effect of the detected drug resistance mutations on the NS5B-RdRp catalytic domain (data not shown).The predicted partial RdRp catalytic domain (C_{243}-D_{332}) of NS5B protein of isolate Menoufia-1 is shown in Fig. 2b.

Phylogenetic classification of HCV isolates based on NS5B protein sequence:
To identify genotypes and subtypes of HCV isolates,our HCV isolates and those represent genotypes and subtypes of HCV-NS5B, retrieved from GenBank database were used to construct the phylogenetic tree (Fig. 3). Phylogentically, our isolates were grouped with HCV isolates of genotype 4 (Fig. 3a). In Fig. 3b, isolates Menoufia-1, 2, 4 and 5 were clustered with subtype 4a and showed the lowest divergence (0.00%-0.044) with 6 isolates from Egypt and one isolate from France. However isolate Menoufia-3 was clustered with subtype 4c where divergence was 0.00% with 2 isolates from Gabon, and one isolate from Canda.

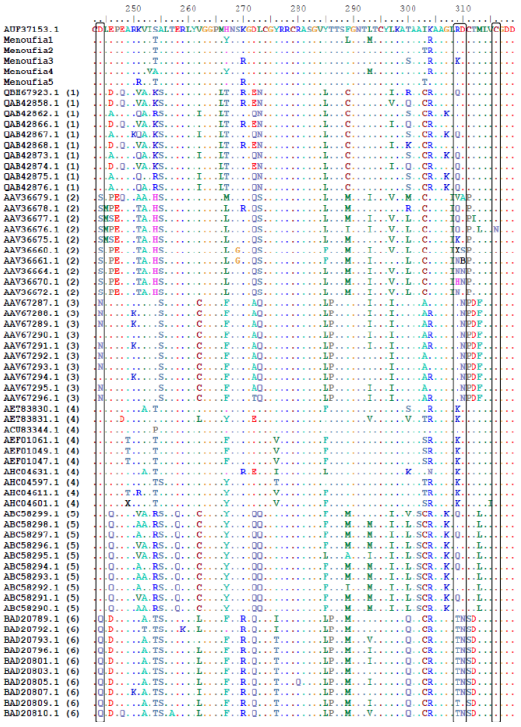

Fig.1:-Multiple sequence alignment of NS5B protein of HCV genotypes. Amino acids positions correspond to HCV-NS5Bisolate 430(accession number AUF37153.1). The detected drug resistance mutations (D244N, Q309R, D310N and C316N) are boxed.

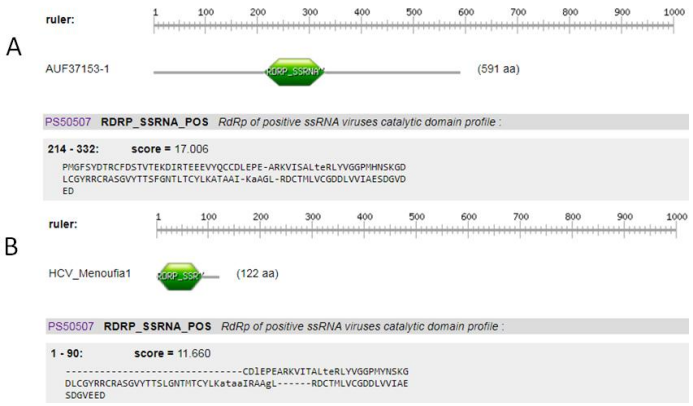

Fig.2:- Prediction of RdRp catalytic domain of NS5B proteinusing ScanProsite tool.(A)corresponds to the reference isolate 430 (AUF37153.1) and(B)corresponds to Menoufia-1 isolate.

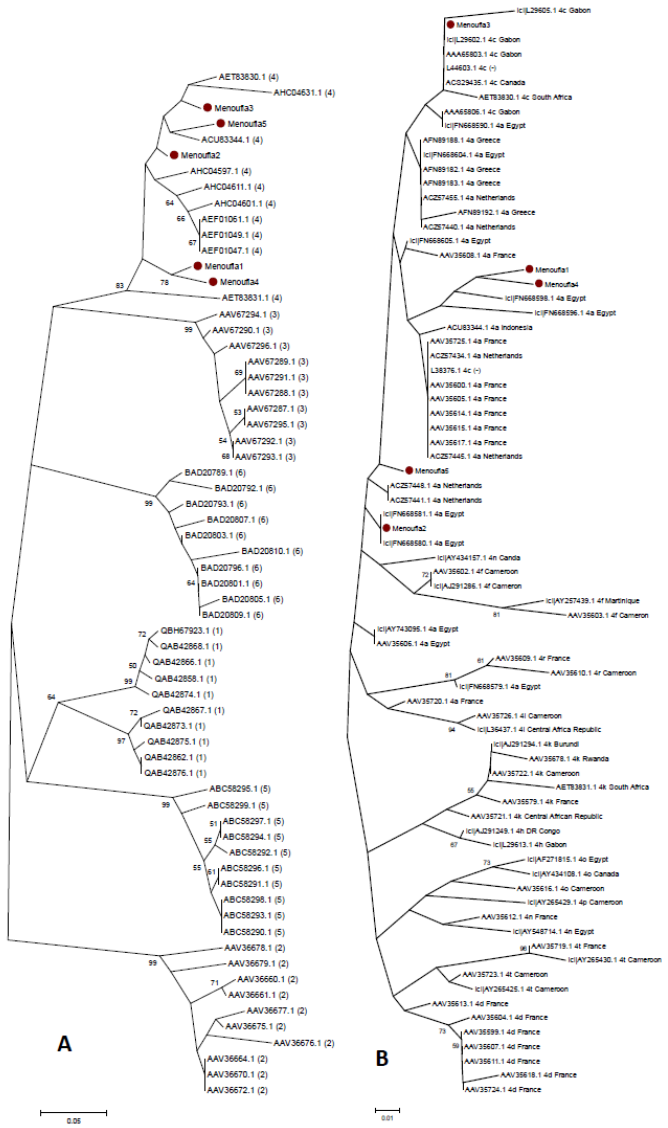

Fig.3:- Neighbor-joining phylogenetic analysis of NS5B proteinof HCV genotypes (a) and subtypes (b).

Discussion:-

It is important to classify HCV genotypes, since HCV genotypes respond differently to several treatment regimens (Kumar et al., 2018 and Omata et al., 2012). The objective of this study was to classify HCV isolates collected from HCV-infected patients from Egypt based on HCV NS5B gene and to predictthe presence and the effect of the known drug resistance mutations on the RdRp catalytic domain of NS5B protein.

As reported previously, genotype 4a is the most predominant HCV genotype in Egypt (Fakhr et al., 2013, Rost et al., 2004 and Youssef et al., 2009). This was confirmed in the current study, in which genotype 4a was observed in 4/5 of our isolates (Menoufia-1, 2, 4 and 5). Isolate Menoufia-3 was clustered with subtype 4c from Gabon; similarly, Mahmoud and Hashem (2012) found a close relationship between an Egyptian isolate of HCV from Sohag and an isolate from Gabon. It is possible that subtype 4c circulates in Egypt and originated from Gabon (Central Africa). Iles et al. (2014) reported that genotype 4 originated in central Africa before disseminating elsewhere.

Four known drug-resistance mutations (D244N, Q309R, D310N and C316N) were detected in the analyzed HCV genotypes. D244N, Q309R and D310N were highly represented in genotype 3 in comparison to the other genotypes (80% for D244N and 100% for both Q309R and D310N). It was noticed that D244N was detected only in the isolates of genotype 3; in a study by Asahina et al., (2005), D244N was mainly represented in subtype 3a. Similarly, castilho et al., (2011) analyzed mutations related to non-response to IFN/RIB observed in subtypes 1a, 1b, 2b, 3a and 4 and found that D244N was detected only in subtype 3a. We detected Q309R with frequencies 40%, 100%, 10% and 50% for genotype 1, 3, 4 and 5, respectively); Q309R mutation was the most common mutation related to non-response to IFN/RIB in genotypes 1a, 1b, 3a and 4, except subtype 2b with frequency above 20% (castilho et al., 2011). Also, D310N was found to be associated with IFN/RIB resistance (Asahina et al., 2005). In our study, C316N was detected only in genotype 2 (10%), in castilho et al., (2011) study, it was observed in only 24% of isolates subtype 1b. The codon 316 implicated with re¬sistance to new NS5B inhibitors; C316N increased the replication fitness by 1.6-fold using AG-021541 inhibitor (Shi et al., 2008, Dutartre et al., 2006 and McCown et al., 2009). In our study, the S282T resistance substitution was not detected in any of the analyzed genotypes which was previously confirmed by castilho et al., (2011). Although S282T has been associated with NS5B polymerase inhibitor Sofosbuvir (SOF) resistance in vitro, it has very rarely been detected, both before and after SOF-based treatment failure, mainly because it seems to be a highly unfit substitution that is usually only detectable for very short periods of time (Dietz et al., 2018). Detection of drug-resistance mutations in HCV infected-patients before treatment indicates that the detection of these mutations could be used as a marker for clinical decisions. When a patient has a confirmed increase in HCV RNA levels while adhering to therapy, implying development of resistance, antiviral drug should be quickly discontinued to prevent evolution of viral population (Strahotin and Babich 2012).

Functional analysis of NS5B protein indicated that the detected drugresistance mutations did not alter the RdRpcatalytic domain of NS5B protein in all genotypes and in our isolates. Nachappa et al., (2018) did not find change in the motifs identified in the RpoB and KatG wild-type protein sequences compared with the RpoB Ser531Leu and KatG Ser315Thr mutants.However, single nucleotide polymorphism detected in the human proteome resulted in domain alteration led to loss of connectivity of cell signaling pathways (Liu and Tozeren 2010).

In conclusion, the data suggest that genotype 4a is still the predominant genotype in Egypt. Furthermore, the study indicates that isolate Menoufia-3, classified ad subtype 4c, could be introduced from Central Africa as new subtype of genotype 4 in Egypt. Also, the detection of NS5B drug-resistance mutations in HCV infected-patients before treatment could be used as a marker to determine the suitable treatment.

References:-

1. Asahina Y, Izumi N, Enomoto N, Uchihara M, Kurosaki M, Onuki Y, et al. Mutagenic effects of ribavirin and response to interferon/ribavirin combination therapy in chronic hepatitis C. J Hepatol. 2005; 43:623-629.
2. Castilho MCB, Martins AN, Horbach IS, Perez RM, Figueiredo FAF, Pinto PT A, Nabuco LC, et al. Association of hepatitis C virus NS5B variants with resistance to new antiviral drugs among untreated patients. Mem. Inst. Oswaldo Cruz. 2001; 106:968-975.
3. Cavalcante LN and Lyra AC. Predictive factors associated with hepatitis C antiviral therapy response. World J Hepatol. 2015; 7 (12):1617–1631.

4. Demetriou VL and Van de Vijver DA. Molecular Epidemiology of Hepatitis C Infection in Cyprus: Evidence of Polyphyletic Infection. J Med Virol. 2009; 81:238–248.
5. Dietz J, Susser S, Vermehren J, Peiffer KH, Grammatikos G, Berger A, et al. Patterns of resistance-associated substitutions in patients with chronic HCV infection following treatment with direct-acting antivirals. Gastroenterology 2018; 154:976–988.
6. Dutartre H, Bussetta C, Boretto J, Canard B. General catalytic deficiency of hepatitis C virus RNA polymerase with an S282T mutation and mutually exclusive resistance towards 2'-modified nucleotide analogues. Antimicrob Agents Che. 2006;50:4161-4169.
7. Fakhr AE, Pourkarim MR, Maes P, Atta AH, Marei A, Azab, M, et al. Hepatitis C Virus NS5B Sequence-Based Genotyping Analysis of Patients From the Sharkia Governorate, Egypt. Hepat Mon. 2013; 13(12):1-5.
8. Iles JC, Raghwania J, Harrisonb A, GL, Pepinc J, Djokod CF, Tamoufe U, et al. Phylogeography and epidemic history of hepatitis C virus genotype 4 in Africa. Virol. 2014;464-465:233–243.
9. Liu Y, Tozeren A. Domain Altering SNPs in the Human Proteome and Their Impact on Signaling Pathways. PLOS ONE. 2010; 5(9): e12890.
10. Kumar A, Rajput MK, Paliwa D, Yadav A, Chhabra R, Singh S. Genotyping & diagnostic methods for hepatitis C virus: A need of low-resource countries. Indian J Med Res. 2018;147(5):445–455.
11. Lyra AC, Fan X, Bisceglie, AMD. Molecular biology and clinical implication of hepatitis C virus. Braz J Med Biol Res. 2004; 37:691–5.
12. Mahmoud A, Hashem MH. Phylogenetic subtyping of hepatitis C virus 5' UTR isolated in Egypt and the effect of 2 transitions in subdomain IIId on the apical loop structure. Life Sci J. 2012; 9 (1):903-909.
13. Nachappa SA, Neelambike SM, Amruthavalli C, Ramachandra NB. Detection of first-line drug resistance mutations and drug-protein interaction dynamics from tuberculosis patients in south india. Microbial Drug Resistance. 2018; 24(4):377-385.McCown MF, Rajyaguru S, Kular S, Cammack N, Nájera I. GT-1a or GT-1b subtype-specific resistance profiles for hepatitis C virus inhibitors telaprevir and HCV-796. Antimicrob Agents Ch. 2009; 53:2129-2132.
14. Mohd HK, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. Hepatology 2013; 57:1333–42.
15. Moradpour D, Penin F, Rice CM. Replication of hepatitis C virus. Nat Rev Microbiol. 2007; 5:453–63.
16. Mosaad YM, Farag RE, Arafa MM, Eletreby S, El-Alfy HA, Eldeek BS, Tawhid ZM. Association of human leucocyte antigen Class I (HLA-A and HLA-B) with chronic hepatitis C virus infection in Egyptian patients. Scand J Immunol. 2010; 72:548–553.
17. Omata M, Kanda T, Yu M-L, Yokosuka O, Lim S-G, Jafri, W, et al. APASL consensus statements and management algorithms for hepatitis C virus infection. Hepatol Int. 2012; 6:409–35.
18. Ray SC, Arthur RR, Carella A, Bukh, J, Thomas DL. Genetic epidemiology of hepatitis C virus throughout Egypt. J Infect Dis. 2000; 182(3):698–707.
19. Rost B, Yachdav G, Liu J. The predict protein server. Nucleic Acids Res. 2004; 32:W321-W326.
20. Shi ST, Herlihy KJ, Graham JP, Fuhrman SA, Doan C, Parge H, et al. In vitro resistance study of AG-021541, a novel non-nucleoside inhibitor of the hepatitis C virus RNA-dependent RNA polymerase. Antimicrob Agents Ch. 2008; 52:675-683.
21. Sigrist CJA, de Castro E, Cerutti L, Cuche BA, Hulo N, Bridge A, Bougueleret L, Xenarios I. New and continuing developments at PROSITE. Nucleic Acids Research 2013; 41:344-347.
22. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. MolBiolEvol. 2013; 30:2725-2729.
23. Youssef A, Yano, Y, Utsumi T, Serwah A-H, Hayashi Y, abd El-alah EM et al. Molecular epidemiological study of hepatitis viruses in Ismailia, Egypt. Intervirology 2009; 52(3):123–131.
24. Strahotin CS, Babich M. Hepatitis C Variability, Patterns of Resistance, and Impact on Therapy. Hindawi Publishing Corporation Advances in Virology 2012; 2012:1-10.