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Impact of HCV ns5a resistance associated substitutions on treatment outcome of chronic HCV in daclatasvir-based antiviral regimens

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> **Abstract**---Background: RASs have direct relation to the treatment outcome in chronic HCV patients receiving DAAs. Aim and objectives: to evaluate prevalence of NS5A HCV RASs and their impact on the treatment outcome in daclatasvir-based antiviral regimens in a cohort of Egyptian patients. Subjects and methods: This study was conducted on 40 Egyptian chronic HCV patients at the virology unit of Cairo Fatemya hospital. The study participants were recruited from cases attending the virology unit of Cairo Fatemya hospital in the period from 1/11/2019 to 1/6/2020. Those patients were divided to two equal groups: responders and non responders (20 patient each).Result:Significant difference was found between the two groups as regard RAS in NS5A region in amino acid position 332 (K substitution was detected in responders while E substitution was detected in non-responders), There was a significant difference

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between the two groups of studied patients as regard RAS in NS5A region in amino acid position 28 (L substitution was found in responders while M substitution was found in non-responders).Conclusion; Although our results showed that RASs against NS5A and NS5B inhibitors have impact on treatment outcome, this finding needs to be confirmed by a larger study.

Keywords---Hepatitis C virus; directing-acting antiviral; HCV; treatment response, RAS; Resistance associated substitutions.

Introduction

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease, with approximately 71 million chronically infected individuals worldwide. Clinical care for patients with HCV-related liver disease has advanced considerably thanks to an enhanced understanding of the pathophysiology of the disease, and because of developments in diagnostic procedures and improvements in therapy and prevention **(1)**.

For some newly produced viruses, however, the transcription errors result in changes in critical coding regions that may, by chance, change the susceptibility of the virus to 1 or more drugs used to treat the virus. The emergence of such drug-resistant viruses most often occurs when drug levels are subtherapeutic, thereby creating selective pressure for the resistant viruses to emerge as the dominant species. These newly formed resistant viruses have a selective growth advantage that allows them to replicate in the presence of antiviral drugs. In a subset of patients with chronic HCV infection, viral variants harboring substitutions associated with resistance to HCV directing-acting antivirals (DAAs) are detectable prior to antiviral therapy and, particularly in the case of NS5A inhibitor-containing regimens, may negatively impact treatment response. These substitutions often are referred to as baseline resistance-associated substitutions (RASs) (2).

In the case of HCV DAAs, resistant viruses are also selected for and/or enriched in patients for whom a DAA regimen fails.

These viruses contain substitutions that are designated as treatment-emergent (or treatment-selected) RASs. NS5A and NS3 RASs are frequently selected in patients with failure of NS5A or NS3 inhibitor-containing regimens, respectively. In contrast, NS5B nucleotide RASs are rarely detected (1% of failures) even after exposure to a failing DAA regimen containing a nucleotide inhibitor (3).

The magnitude of the negative impact of RASs, both baseline and selected, on treatment outcome varies according to regimen (coadministered drugs); patient factors that impact treatment response (cirrhosis); and the fold change decrease in potency conferred by the specific RAS(s). Given these considerations, RAS testing alone will not dictate optimal DAA regimen selection. In addition, a drug predicted to suffer a significant loss of potency in the presence of a RAS still may be used in specific clinical settings/regimens (2).

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Method

Patients and Methods

This study was conducted on 40 Egyptian chronic HCV patients at the virology unit of Cairo Fatemya hospital (sample size was calculated statistically). The study participants were recruited from cases attending the virology unit of Cairo Fatemya hospital in the period from 1/11/2019 to 1/6/2020.

Patients were classified into two groups: Group (1): Responders to Daclatasvir based regimens, group (2): Non responders to Daclatasvir based regimens Daclatasvir based regimens included Sofosbuvir and Daclatasvir (10 cases from each group) or Sofosbuvir, Daclatasvir& Ribavirin (10 cases from each group) for 12 weeks

Inclusion criteria: Age: 18-75 years old, positive HCV RNA within the past 6 months. If the patient has received HCV therapy during that period, a new test should be performed.

Exclusion criteria: Child C cirrhosis, manifest liver decompensation: uncontrolled ascites, history of hepatic encephalopathy, hepatorenal syndrome, serum albumin less than 2.8 g/dl, total bilirubin more than 3 mg/dl and INR 1.7 or more, platelets count less than 50,000/mm3, HCC, except 6 months after concluding an intervention aiming at cure with no evidence of activity by dynamic CT or MRI, extra-hepatic malignancy except after two years of disease-free interval. In lymphomas and chronic lymphatic leukemia, treatment can be initiated immediately after remission based on the treating oncologist's report and pregnancy or inability to use effective contraception.

Patient selection methodology in details: We randomly selected 20 non responder to Daclatasvir based regimens Sofosbuvir and Daclatasvir (10 cases from each group) or Sofosbuvir, Daclatasvir& Ribavirin (10 cases from each group) for 12 weeks and we selected other 20 random responder to the same regimens and enrolled as control cases (to have 1:1 cases and controls) then NS5A RASs were evaluated in the sera of the two groups in the duration starting from November 2019 to May 2020.

The following data were recruited: History, history of previous administration of anti HCV treatment i.e. DAAs or Peg. Interferon, history of any other co morbidities e.g. DM or hypertension, history of alcohol intake and history of previous blood transfusion, post-chemotherapy or extra hepatic HCV disease.

-Laboratory investigations including: Aspartate aminotransferase (AST), alanine aminotransferase (ALT), total and direct serum bilirubin and serum albumin, serum creatinine, coagulation profile: prothrombin time (PT), partial thromboplastin time (PTT) and international normalized ratio (INR), complete blood count (CBC), serum level of alpha-feto protein (AFP), quantitative HCV RNA by PCR before treatment, glycosylated hemoglobin level (HbA1c) in diabetic patients and random blood sugar (RBS) and HBs Ag. Imaging studies: Pelvi-abdominal ultrasonography. At Week 24 (12 weeks after the end of treatment) quantitative HCV RNA PCR was done (as a test of response) in addition to CBC, AST, ALT, and serumcreatinine and serum bilirubin.

Statistical Analysis: Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA). Data was summarized using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Mann-Whitney test (Chan, 2003a).For comparing categorical data; Chi square (χ 2) test was performed. Exact test was used instead when the expected frequency is less than 5(Chan, 2003b).P-values less than 0.05 were considered as statistically significant.

Results

This study included 40 Egyptian chronic HCV patients attending the virology unit of Cairo Fatemya hospital in the period from November 2019 to June 2020(sample size was calculated statistically). Patients were classified into two groups: Group (1): Responders to Daclatasvir based regimens. Group (2): Non responders to Daclatasvir based regimens. Daclatasvir based regimens included Sofosbuvir and Daclatasvir (10 cases from each group) or Sofosbuvir, Daclatasvir& Ribavirin (10 cases from each group for 12 weeks).

RASs in NS5A are more important clinically. We noticed the signature NS5A mutation, L28M, clinically has been shown to impact the efficacy of daclatasvir, T75S and K332E, clinically have been shown to impact the efficacy of sofosbuvir. Table (3)

			Non responders		Responders	
		Count	%	Count	%	
Decision	Sof / Dac	10	50.0%	10	50.0%	1
	Sof / Dac / Rbv	10	50.0%	10	50.0%	
Duration of ttt	12 wk	20	100.0%	20	100.0%	

 Table (1)

 Treatment decision and duration of the studied patients:

There was no significant difference between the two groups except serum creatinine level that was significantly higher in the responder group. **Table (2)**

 Table (2)

 laboratory results of the studied patients at week 24:

	Non responders		Responde	Responders	
	Mean	SD	Mean	SD	
ALT (IU/L)	49.75	53.32	53.40	35.16	0.121
AST (IU/L)	47.85	40.97	50.40	35.07	0.495

Total Billirubin(mg/dl)	1.10	0.75	0.73	0.21	0.253
WBC (th/cm3)	5.87	1.80	5.85	1.57	0.925
Hb (gm/dl)	12.70	1.54	13.78	1.79	0.114
Platelets (th/cm3)	197.95	71.40	231.70	89.31	0.383
Creatinine (mg/dl)	0.70	0.18	0.92	1.67	0.028

Significant difference was found between the two groups as regard RAS in NS5A region in amino acid position 332 (K substitution was detected in responders while E substitution was detected in non responders). Figure (1)



Figure (1): Comparison between responders and non responders as regard

RAS in NS5A region in amino acid position332:

There was a significant difference between the two groups of studied patients as regard RAS in NS5A region in amino acid position 28 (L substitution was found in responders while M substitution was found in non responders). Figure (2)



Figure (2): Comparison between responders and non-responders as regard

RAS in NS5A region in amino acid position 28:

There was no significant difference between the two groups as regard treatment decision and duration. Table (1)

 Table (3)

 Major RASs mutation includes HCV DAA target proteins and considered as clinically relevant:

Resistance associated substitutions	Drug	
L28M	Daclatasvir	
T75S	Sofosbuvir	
K332E	Sofosbuvir	

Discussion

Hepatitis C virus (HCV) is the major cause of chronic liver disease, cirrhosis and liver cancer (4). Due to emergence of new direct acting antivirals (DAAs), chronic hepatitis C has become a curable disease (5).

Accordingly, in order to investigate the diagnostic role and impact of HCV NS5A Resistance associated substitutions (RAS) on treatment outcome of chronic HCV in daclatasvir-based antiviral regimens, in the present case control study we tested serum samples from the enrolled individuals for HCV RAS of 40 chronic hepatitis C patients receiving HCV treatment at the virology unit of Cairo Fatemya hospital in the period from 1/11/2019 to 1/6/2020 and those patients were divided into two groups, responders and non-responders to Daclatasvir based regimens.

Daclatasvir based regimens included Sofosbuvir and Daclatasvir (10 cases from each group) and Sofosbuvir, Daclatasvir& Ribavirin (10 cases from each group) for 12 weeks

In our study the non-responders were treatment experienced (INF, ribaviren or SOF+DAC) while the responders were treatment naïve, this finding agrees with the study of **Kjellin**, (6) which showed higher SVR-rates in the treatment-naïve patients and significantly lower SVR-rates in treatment- experienced patients. Also **Kjellin**,(6) found that non cirrhotic patients have higher SVR than cirrhotic

patients while in our study there was no significant difference between responders and non-responders as regard FIB-4 score or ultrasound findings.

In our study we found that there was no significant difference between the two groups in baseline laboratory results except the serum glucose level which was higher in the non-responder group and this finding agrees with the study performed by **Wang et al., (7)** who found that HCV infection is positively associated with insulin resistance, hepatic steatosis, metabolic syndrome and the risk of T2DM and atherosclerosis.

Desbois&Cacoub., (8) found that the efficacy of antidiabetic treatment in improving the response to antiviral treatment and in decreasing the risk of HCC

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has been reported by some studies but not by others. Thus, the effects of glucose abnormalities correction in reducing liver events need further studies.

We found that there were significant differences between responders and non responders as regard RAS at Amino acid position 332, K substitution was detected in responders while E substitution was detected in non responders. While at Amino acid position 75, T substitution was detected in responders while S substitution was detected in non responders. Also, at Amino acid position 28, L substitution was found in responders while M substitution was found in non responders.

In another study done on Egyptian patients by **Ramadan et al., (9)** accordingly, to be able to optimize treatment and reduce over treatment standardized RAS testing could be included in assessing best treatment strategy, i.e. cheapest and shortest alternative. The lowest dose and shortest treatment duration must be the goal in controlling transmission and eradication of HCV in at least where modern treatment as well as resistance testing is readily available. However, standardization of RAS detection and testing guidelines are still lacking and many protocols using Sanger- or deep sequencing have varying degree of specificity and sensitivity. Thus, there is currently a debate on consensus for clinically relevant cut-off sensitivities**(10)**.

In previous studies with NS5A inhibitor containing DAA regimens, the amino acid substitutions that produce resistance to NS5A inhibitors have been shown to affect the SVR rate (**11**). On the other hand, in GT1a, a single RAS can provide high levels of resistance to most NS5A inhibitors, while in GT1b, there are only high levels of resistance to Ledipasvir(**12**). Because NS5A region RASs tend to be persistent, retreatment strategies should involve a combination of triple or quadruple DAA regimens with high resistance barriers such as NS5B inhibitors as well as a combination of NS3 + NS5A inhibitors (**13**).

In the study of **Itakura et al, (14)** they found that R30H and L31-RAS in NS5A were frequently detected after failure of regimens including daclatasvir. The prevalence of Y93-RAS was high irrespective of the regimen. S282T RAS in NS5B was detected in 3.9% of ledipasvir/sofosbuvir failures. The prevalence of D168-RAS increased significantly according to the number of failed regimens (p < 0.01), which was similar to that seen with L31-RAS and Y93-RAS. The prevalence of patients with RASs in either NS3 or NS5A, or in both, increased significantly with increasing numbers of failed regimens.

But our study disagreed with what found in the study of **Torres et al., (15)** that tested serum samples from the enrolled individuals then submitted to polymerase chain reaction amplification of NS5A and NS5B non-structural protein genes, which were then sequenced by Sanger method and found that a total of 170 and 190 samples were amplified and analyzed for NS5A and NS5B, respectively. For NS5A, 36 samples showed presence of some types of RASs and 134 samples showed no RAS.

No sample showed any RAS for NS5B. Hence concluded that there are important RAS in samples enrolled from naïve chronic HCV patients in some areas from São

Paulo and the most prevalent were A62S, A30K, and Y93H, which could indicate an increase in resistance to some DAAs used in HCV treatment while our study revealed detection of RASs in experienced patients but no RASs were detected in naïve patients.

Conclusion

Although our results showed that RASs against NS5A and NS5B inhibitors have impact on treatment outcome, this finding needs to be confirmed by a larger study.

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