Effect of Daclatasvir and Sofosbuvir Therapy on Serum Levels of Angiogenic Factors: A Prospective Cohort Study

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors have contributed equally to this work either by designing, analysis and interpretation of data, drafting, revising critically or final approval of the revision to be published. All authors read and approved the final manuscript.

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ABSTRACT

Background: Direct-acting antiviral agents therapy is considered a breakthrough in hepatology due to high rates of sustained virologic response in all patients including those with decompensated cirrhosis. However, impact of Direct-acting antiviral agents-induced sustained virologic response on hepatocellular carcinoma development remains conflicting.

Aims: This study aimed at evaluating the change in circulating levels of vascular endothelial growth factor and transforming growth factor-β1, the main angiogenic factors involved in hepatocarcinogenesis process, in cirrhotic patients achieved sustained virologic response after Direct-acting antiviral agents therapy.

Study Design: This was a prospective, single-center, cohort study.

Place and Duration of Study: Patients were recruited from the outpatient clinic of National Liver Institute, which considered a tertiary referral center in Menoufia University, Egypt (September 2018 to February 2019).
Methodology: Forty-five decompensated cirrhotic hepatitis C virus infected patients with no history of hepatocellular carcinoma participated in the study. All patients received 60mg oral daclatasvir and 400mg oral sofosbuvir once daily for 12 or 24 weeks with or without ribavirin. Serum levels of vascular endothelial growth factor and transforming growth factor-β1 were measured at baseline and 12 weeks after the end of therapy.

Results: The median serum levels of vascular endothelial growth factor showed a non-statistically significant increase (from 1123 ng/L to 1269 ng/L, \( P = 0.126 \)). But, transforming growth factor-β1 median serum levels exhibited a non-statistically significant reduction (from 13.22 ng/ml to 12.44 ng/ml, \( P = 0.163 \)) 12 weeks after treatment.

Conclusion: Our findings show direct-acting antiviral agents therapy do not affect vascular endothelial growth factor and transforming growth factor-β1 serum levels. But, a larger scale prospective cohort study on an extended follow-up period is recommended.

Keywords: Angiogenesis inducing agents; daclatasvir; hepatitis C chronic; sofosbuvir; sustained virologic response; hepatocarcinogenesis; vascular endothelial growth factor; transforming growth factor-B1.

1. INTRODUCTION

Chronic hepatitis C virus (HCV) infection is the prime causation of liver cirrhosis and hepatocellular carcinoma (HCC) [1]. HCV accounts for about 27% of cirrhotic cases and about 25% of HCC cases worldwide, and over 350000 people die each year from hepatitis C-related liver diseases [2,3]. In Egypt, HCV infection has the greatest prevalence rate over the world and considered a major public health burden since it is the leading cause of end-stage liver disease, HCC, and hepatic related death [4]. The Egypt Demographic and Health Surveys (EDHS) measured HCV prevalence among the adult population aged 15–59 years was 14.7% in 2009 and 10.0% in 2015 substantially higher than global levels [5]. The currently available highly effective regimens of direct-acting antiviral agents (DAAs) have totally remodeled the panorama of hepatitis C therapy and promise a novel epoch in the treatment of HCV infection in a very brief period with great safety profile [6,7]. Oral combination of daclatasvir (DCV) an HCV nonstructural protein 5A (NS5A) replication complex inhibitor and sofosbuvir (SOF) a nucleotide analogue HCV nonstructural protein 5B (NS5B) polymerase inhibitor has powerful antiviral action and wide genotypic coverage achieving extremely elevated sustained virological response (SVR) rates nearly close to 100% in both treatment-naive and treatment-experienced patients. These impressive results are expected to modulate the disease epidemiology and aid in achieving the goal of eliminating HCV infection in Egypt [8].

The regimen that was used in decompensated cirrhotic patients was sofosbuvir (400 mg, orally, once daily) plus daclatasvir (60 mg, orally, once daily) for 12 weeks. Weight-based ribavirin (1200 or 1000 mg/day if ≥75 or <75 kg body weight, respectively) was added to this regimen when treating cirrhotic patients and/or treatment-experienced patients who received prior interferon therapy. Treatment duration was extended to 24 weeks with addition of weight-based ribavirin only in treatment-experienced patients who failed to respond to sofosbuvir plus ribavirin regimen [9]. However, the impact of DAAs–induced SVR on HCC development remains conflicting and under debate [10]. In this respect, HCC was found to develop in an unexpectedly very high number of cirrhotic HCV infected patients within few weeks after beginning DAAs regimens suggesting that there is an association between exposure to DAAs and the emergence of HCC. Several authors have intimated that, there is a relation between DAAs treatment and hepatic neoplasia growth or dissemination and concluded that after DAA exposure, some patients with HCV are at increased risk of de novo HCC development, and other patients with a history of treated HCC are at increased risk of HCC recurrence [11-14]. While other opposite studies have confuted these findings and insisted that DAAs are protective against the development of HCC [15-17]. This highlights the need for further clinical evaluation for the patients achieved SVR after DAAs regimens.

Angiogenesis has a crucial role in the development, progression, and dissemination of the extremely vascular solid tumor (HCC). When the precancerous lesions “dysplastic nodules” begin to evolve, angiogenesis is actuated and the process of angiogenesis carries on with the progression of the tumor [18,19]. Therefore, the circulating angiogenic regulators have been evaluated as predictive factors for cancer.
HCV infected patients, genotype 4 receiving DAAs without history of HCC, in determining the effect of DAA-induced SVR on angiogenesis and hence on the hepatocarcinogenesis process.

2. MATERIALS AND METHODS

2.1 Study Design

This is a prospective, single-center, cohort study. Patients were recruited from the outpatient clinic of the National Liver Institute that is a tertiary referral center in Menoufia University, Egypt – recruited from September 2018 to February 2019. Our cohort study adheres to STROBE research reporting standards.

2.2 Patients

G*Power software program was used to calculate sample size. The study sample size was calculated to be 35 patients on effect size = 0.5, α–error probability = 0.05, and power = 0.8. However we conducted our study with 45 patients which raised the power of the study to 0.88. It is worth mentioning that, the sample size of this study was based on some previous studies [34-36].

Forty five decompensated cirrhotic patients with chronic HCV infection genotype 4, aged 18-65 years old were included in the study. Liver cirrhosis was proven by combination of clinical, laboratory, and ultrasound criteria. According to European Association for the Study of the Liver (EASL) guidelines, fibrosis stage must be assessed by non-invasive methods initially such as ultrasound, while liver biopsy reserved for cases where there is uncertainty [6].

The exclusion criteria were the following: patients with prior history of liver transplantation or hepatocellular carcinoma, co-infection with HIV or HBV, pregnant or lactating females, and finally refusal to participate in the study.

2.3 Methodology

Demographic and clinical data of the participants (n=45) were collected including age, gender, weight, height, and body mass index (BMI). The initial evaluation of the patients included full clinical examination, HBsAg, quantitative PCR for HCV-RNA, and abdominal ultrasound by available methods.
Either the ultrasound detection of hepatic focal lesion or elevation of AFP level above 400 ng/ml, raised the suspicion of HCC. AFP elevation is 75-91% specific for the diagnosis of HCC, but values greater than 400 ng/mL are generally considered diagnostic of HCC [32].

Lab parameters including complete blood cell count, total and direct bilirubin, ALT, AST, albumin, INR, urea, creatinine, and serum AFP were assessed at baseline and 12 weeks after the end of antiviral treatment.

All patients received antiviral therapy according to the Egyptian protocol for the treatment of HCV infection. The recommended regimen was oral daclatasvir and sofosbuvir at a dose of 60mg once daily and 400mg once daily, respectively for 12 or 24 weeks with or without addition of ribavirin, according to guidelines and medical decision.

Abdominal ultrasound was performed on subjects at week 4, at the end of treatment and at 12 weeks after the end of the treatment.

HCV-RNA quantification was made by real-time PCR, with a limit of detection of 15 IU/ml to assess the virological response at week 4, at the end of treatment and at 12 weeks after the end of the treatment. Sustained virological response (SVR) to therapy was defined as undetectable serum HCV-RNA viral load 12 weeks after completion of HCV therapy (SVR12).

Blood samples were collected into vacutainers by venipuncture, then delivered into plastic tubes, which were centrifugated at 4,500 g for 10 min. The separated serum was kept in a deep freezer at – 80°C until the time of analysis. The serum levels of VEGF and TGFβ1 were measured at base line and 12 weeks after the end of treatment using specific ELIZA commercial kits (SunRedBio, Shanghi, China. Catalogue No. : 201-12-0081 and 201-12-5480, respectively). All measurements were done according to the manufacturer’s specifications using a Multiskan FC with incubator microplate reader (USA).

2.4 Statistical Analysis

Categorical data are presented as numbers (percentages). Continuous data are presented as mean ± SD and medians (ranges). Kolmogorov-Smirnov test was used to assess the normality of data. Normally distributed variables were analyzed using Wilcoxon signed-rank test.

Statistical analysis was performed using IBM SPSS Statistical package version 22.0 (IBM Corp; USA, 2013). The level of statistical significance was set at $P < 0.05$ using a two-tailed test.

3. RESULTS

3.1 Demographic Data

Out of 54 eligible patients, nine patients were dropped out because of non-compliance or non-achieving a virological response. Only 45 patients were included in the ultimate analysis (Fig. 1).

Study subjects are uniformly distributed in terms of sex (51.1% female vs 48.9% male) and the mean age is 53±7.15 years. The detailed baseline demographic data of the participants is shown in Table 1.

3.2 Change in Laboratory Parameters after DAA Therapy

All study participants are confirmed to achieve SVR12 with no detected lesions by surveillance ultrasound. There was a statistically significant improvement in the liver function tests including serum direct bilirubin, ALT, and AST 12 weeks after treatment, while the platelet count, total bilirubin, serum albumin, prothrombin concentration, and INR showed non-statistically significant improvement 12 weeks after treatment. It is noteworthy mentioning that, there was a significant reduction in hemoglobin level 12 weeks at the end of the treatment, while there was a significant increase in renal function tests (serum creatinine and blood urea) (Table 2).

3.3 Change in AFP and Angiogenic Factor Levels after the End of the Treatment

There is a non-statistically significant increase in median serum levels of VEGF 12 weeks after DAAs therapy with reference to the median baseline levels (from 1123 ng/L to 1269 ng/L, $P = 0.126$ ) (Table 3; Fig. 2). And, the median AFP serum levels showed non-significant increase after DAAs treatment when compared with the median baseline levels (from 4.42 ng/ml to 4.50 ng/ml, $P = 0.531$) (Table 3; Fig. 3). In contrast, the median serum levels of TGFβ1 exhibits non-
statistically significant decrease 12 weeks after treatment as compared to its median baseline levels (from 13.22 ng/ml to 12.44 ng/ml, $P = 0.163$) (Table 3; Fig. 4)

![Flow chart of study participants](image)

**Fig. 1. Flow chart of study participants**

| Parameter     | Value               |
|---------------|---------------------|
| Gender        | Male 48.9%          |
|               | female 51.1%        |
| Age (years)   | Mean 53.69±7.15     |

Variables are presented as numbers (percentages) for categorical data or mean for continuous data

| Parameter                  | Base line before DAA treatment | After the end of DAA treatment | Wilcoxon signed-rank test | $P$-value |
|---------------------------|-------------------------------|------------------------------|---------------------------|-----------|
| Hb (g/dl)                 | Mean 13.47±1.53               | 11.37±1.33                   | 5.73                      | 0.001     |
| Platelet count (U/L)      | Mean 169.89±88.10             | 172.11±74.42                 | 0.48                      | 0.63      |
| Total bilirubin (mg/dl)   | Mean 0.98±0.55                | 0.79±0.35                    | 1.17                      | 0.243     |
| Direct bilirubin (mg/dl)  | Mean 0.45±0.32                | 0.22±0.13                    | 3.7                       | 0.001     |
| ALT (U/L)                 | Mean 64.04±42.02              | 26.67±10.90                  | 5.4                       | 0.001     |
| AST (U/L)                 | Mean 61.42±35.38              | 23.67±11.77                  | 6.5                       | 0.001     |
| Albumin (G/dl)            | Mean 3.92±0.58                | 4.48±3.62                    | 0.299                     | 0.76      |
| Prothrombin concentration (%) | Mean 75.67±15.49          | 79.09±10.83                  | 0.84                      | 0.4       |
| INR                       | Mean 1.20±0.26               | 1.13±0.13                    | 1.2                       | 0.245     |
| Urea (mg/dl)              | Mean 26.20±6.85              | 37.02±10.36                  | 5.1                       | 0.001     |
| Creatinine (mg/dl)        | Mean 0.77±0.20               | 0.96±0.21                    | 4.2                       | 0.001     |

*ALT*: alanine aminotransferase; *AST*: aspartate aminotransferase; *DAAs*: direct acting antiviral agents; *Hb*: hemoglobin; *INR*: international normalized ratio
Table 3. Serum levels of selected biological markers for all patients at base line and after treatment

| Parameter     | Base line before DAA treatment | After the end of DAA treatment | Wilcoxon signed-rank test | P-value |
|---------------|--------------------------------|--------------------------------|---------------------------|---------|
| VEGF (ng/L)   | Mean 1329.53 ± 583.06           | 1421.80 ± 637.626              | 1.529                     | 0.126   |
|               | Median 1123.00                  | 1269.00                        |                           |         |
| AFP (ng/ml)   | Mean 5.53 ± 4.02                | 5.40 ± 3.99                    | 0.626                     | 0.531   |
|               | Median 4.42                     | 4.50                           |                           |         |
| TGF-β1 (ng/ml)| Mean 15.74±8.24                 | 13.49±5.80                     | 1.394                     | 0.163   |
|               | Median 13.22                    | 12.44                          |                           |         |

AFP: alpha-fetoprotein; TGF-β1: transforming growth factor-β1; VEGF: vascular endothelial growth factor

**Fig. 2. Vascular endothelial growth factor (VEGF) serum levels at baseline (pre), and 12 weeks after treatment (post)**

**P = 0.126**

**Fig. 3. Alpha-fetoprotein (AFP) serum levels at baseline (pre), and 12 weeks after treatment (post)**

**P = 0.531**
4. DISCUSSION

DAAs therapy is currently known to be one of the master advances in clinical hepatology because of high rates of SVR in patients with different stages of HCV infection including those having decompensated cirrhosis [37]. By the advent of DAA medications; the long-awaited solution for HCV infection, both hepatologists and patients raised their aspirations and expectations not only to eradicate the viremia but also to enhance the liver functions and to diminish the allied complications such as HCC. However, reporting of the cases with dramatic relapse or “de novo” occurrence of malignant hepatoma within a few weeks of the DAAs treatment have collapsed this general climate of fervor and generated much scientific dispute. Since then, this topic has been recently a subject of several controversial publications that did not put an end to this debate [10,38]. The absence of large prospective studies about this issue keeps it an open puzzling question. Since, there is no decisive evidence yet from randomized clinical trials neither to confirm nor to reject the potential oncogenic effect of DAAs, this risk should not be underestimated.

Our data showed that, the circulating levels of VEGF increase non-significantly, whereas the circulating levels of TGFβ1 decrease non-significantly both here 12 weeks after the end of DAAs therapy. Concerning VEGF, the main angiogenic growth factor in HCC, Villani et al. (2016) demonstrated that during DAAs treatment and up to the end of the treatment, the levels of VEGF become temporary elevated until reverted to the baseline after the cessation of antiviral medications which indicates limited effect of DAAs on angiogenesis [39]. Similarly, Facilli et al. (2018) have reported that, VEGF levels increased from baseline to the end of antiviral therapy and maintained elevated for three months after the cessation of antiviral medications [40].

Ali et al. (2004) and Watanabe et al. (2016) concluded that the decrease in TGFβ1 levels could be the primary marker for hepatic neoplasia, and the decreased levels of this biomarker may suggest initiation of the process of hepatocarcinogenesis, where the increase in TGFβ1 serum levels may contribute to the advanced stages of hepatic carcinoma [31,41]. According to this hypothesis and in the insight of our results, we did not find that DAAs therapy has profound effect on hepatocarcinogenesis process, since TGFβ1 levels didn’t significantly affected.

Concerning the reduction in haemoglobin serum levels, in contrast to our findings, another study reported no significant difference was noted regarding pre- and post- treatment haemoglobin (Hb) levels [42]. Conversely, El Sagheer et al. (2018) reported a significant decrease in hemoglobin level from 13.6 before treatment down to 12.7 g/dl after [43]. Another study reported a significant change in hemoglobin level
after DAAs treatment (p < 0.001) [44]. Also our results showed that the SVR was associated with a significant increase in renal function tests contrary to Nevola et al. (2020) who reported that patients who eliminated HCV by DAAs had an improvement of serum creatinine compared to the baseline value. The reason of dissimilarities observed in the two studies on negative predictors of improvement in renal function are unclear [45].

Given the observational nature of our study, we did not provide the biological mechanisms by which DAAs treatment may influence the angiogenic factors levels. However the real mechanisms by which DAAs therapy can affect angiogenic factors levels is still unclear and not fully elucidated [46].

One of the worth noting learned lessons is that delaying the treatment of HCV-infection until progression to cirrhotic grade might be associated with a substantial oncogenic hazard despite the resolution of viral infection [47]. Even though the insignificant effect of DAAs on AFP and angiogenic factor levels, we still recommend HCC surveillance for DAAs treated cirrhotic patients in the immediate post-SVR context, which is not yet currently recommended [48], as both European Association for the Study of the Liver (EASL) and American Association for The Study of Liver Diseases (AASLD) guidelines recommend HCC surveillance for DAAs treated patients every six month for indefinite duration but not in the immediate post-SVR context [6,49].

The strength of this study comes from the prospective design, whereas previous studies are of retrospective nature. And, to the best of our knowledge this is a priming study addressing the effect of DAAs therapy on TGFβ1 levels.

Additionally, subjects in this study are derived from a single, national health care center with relatively unvarying antiviral treatment-regime and are in the same cirrhotic grade minimizing the geographical, ethnical and clinic-pathologic heterogeneity within the studied group of patients. Such heterogeneity could be an important limitation in the previous studies.

This study’s data is helpful for many countries because HCV genotype 4 defines 12%-15% of absolute global HCV infection and exists in North Africa, Middle East, South Asia, and central sub-Saharan Africa countries such as The Central African Republic, The Democratic Republic of Congo, Gabon, Chad and Equatorial Guinea, in addition to Eastern region of Africa like Ethiopia [50,51].

This study has some limitations which include brief average follow-up time that limits long term interpretation of our findings. Another limitation is lack of comparable control group of patients who had not received antiviral therapy, as the ideal method to examine whether DAAs treatment affect angiogenic factors serum levels is to randomize patients to treatment with DAAs vs no treatment or placebo and then assess the change in the angiogenic factors levels in each group before and after. However, such study design would be unethical regarding the clearly established benefits of DAAs treatment for all HCV-infected patients in the recommendations of both EASL and AASLD [6,49].

5. CONCLUSION

DAA-induced SVR does not significantly affect VEGF and TGFβ1 serum levels – two critical factors in hepatocarcinogenesis. Consequently, showing safety of DAAs, without angiogenic and oncogenic risk of DAA therapy. This data here needs further study, analyzing larger scale prospective cohort in additionally extended follow-up period.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products in commercial promotion but for the advancement of science. Also, the research is not funded by the producing company - rather it is funded by personal efforts of authors.

CONSENT

Authors declare that written informed consent was obtained from approving authority for publication of this research.

ETHICAL APPROVAL

The study protocol was in accordance with the ethical standards of the 1975 declaration of Helsinki and was approved by the institutional review board of the National Liver Institute (NLI
IRB 00003413) on February 4, 2018; IRB Protocol Number: 00132/2018.

COMPETING INTERESTS
Authors have declared that no competing interests exist.

REFERENCES
1. World Health Organization. Global hepatitis report; 2017. Accessed 9 June 2019. Available: https://apps.who.int/iris/bitstream/handle/10665/255016/9789241565455-eng.pdf?sequence=1
2. Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contribution of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. J Hepatol. 2006;45:529–38.
3. Mohamed AA, Elbedewy TA, El-Seray F M, El-Toukhy N, Ahmed W, Ali E Din Z. Hepatitis C virus: A global view. World J Hepatol. 2015;7(26):2676-2680.
4. Blach S, Zeuzem S, Manns M, Altraifeh I, Duber GA, Muljono DH, et al. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: A modelling study. Lancet Gastroenterol Hepatol. 2016;2(3):161–176.
5. Kouyoumjian SP, Chemaitelly H, Abu-Raddad LJ. Characterizing hepatitis C virus epidemiology in Egypt: systematic reviews, meta-analyses, and meta-regressions. Sci Rep. 2018;8(1):1661.
6. European Association for the Study of the Liver. EASL Recommendations on Treatment of Hepatitis C 2018. J hepatol. 2018;69(2):461-511.
7. Essa M, Sabry A, Abdelsamee E, Tharwa ES, Salama M. Impact of new direct-acting antiviral drugs on hepatitis C virus-related decompensated liver cirrhosis. Eur J Gastroenterol Hepatol. 2019;31(1):53-58.
8. Gomaa A, Allam N, Elshakawy A, El Kassas M, Waked I. Hepatitis C infection in Egypt: Prevalence, impact and management strategies. Hepat Med. 2017;9:17–25.
9. Ahmed OA, Elsebaey MA, Fouad MHA, Elashry H, Elshafie AI, Elhadidy AA, et al. Outcomes and predictors of treatment response with sofosbuvir plus daclatasvir with or without ribavirin in Egyptian patients with genotype 4 hepatitis C virus infection. Infect Drug Resist. 2018;11:441-445.
10. El Kassas M, Elbaz T, Salaheldin M, Abdelsalam L, Kaseb A, Esmat G. Impact of treating chronic hepatitis C infection with direct-acting antivirals on the risk of hepatocellular carcinoma: The debate continues – A mini-review. J. Adv. Res. 2019;17:43-48.
11. Ravi S, Axley P, Jones D, Kodali S, Simpson H, McGuire BM, et al. Unusually high rates of hepatocellular carcinoma after treatment with direct-acting antiviral therapy for hepatitis C related cirrhosis. Gastroenterology. 2017;152:911–912.
12. Reig M, Marino Z, Perello C, Iñarrariaegui M, Ribeiro A, Lens S, et al. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. J Hepatol. 2016;65:719–726.
13. Conti F, Buonfiglioli F, Suteri A, Crespi C, Bolondi L, Caraceni P, et al. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. J Hepatol. 2016;65:727–733.
14. Kozbial K, Moser S, Schwarzer R, Laferl H, Al-Zaair Y, Stauber R, et al. Unexpected high incidence of hepatocellular carcinoma in cirrhotic patients with sustained virologic response following interferon-free direct-acting antiviral treatment. J Hepatol. 2016;65(4):856-858.
15. Kanwal F, Kramer J, Asch SM, Chayanupatuk M, Cao Y, El-Serag HB. Risk of hepatocellular cancer in HCV patients treated with direct-acting antiviral agents. Gastroenterology. 2017;153:996–1005.
16. Ioannou GN, Green PK, Berry K. HCV eradication induced by direct-acting antiviral agents reduces the risk of hepatocellular carcinoma. J Hepatol. 2017;pii:S0168-8278(17)32273-0.
17. Calvaruso V, Cabibbo G, Cacciola I, Petta S, Madonia S, Bellia A, et al. Incidence of hepatocellular carcinoma in patients with HCV-associated cirrhosis treated with direct-acting antiviral agents. Gastroenterology. 2018;155(2):411-421.e4.
18. Yang ZF, Poon RT. Vascular changes in hepatocellular carcinoma. Anat. Rec. 2008;291:721–734.
19. Poon RT, Fan ST, Wong J. Clinical implications of circulating angiogenic factors in cancer patients. J Clin Oncol. 2001;19(4):1207-1225.

20. Chhonker SK, Rawat D, Koiri RK. Role of vascular endothelial growth factor and transforming growth factor-beta in hepatocellular carcinoma. Ultras Med J. 2017;3:25-31.

21. Weidner N. Angiogenesis as a predictor of clinical outcome in cancer patients. Hum Pathol. 2000;31(4):403-405.

22. Iavarone M, Lampertico P, Iannuzzi F, Manenti E, Donato MF, Arosio E, et al. Increased expression of vascular endothelial growth factor in small hepatocellular carcinoma. J Viral Hepat. 2007;14(2):133-139.

23. Poon RT, Lau CP, Cheung ST, Yu WC, Fan ST. Quantitative correlation of serum levels and tumor expression of vascular endothelial growth factor in patients with hepatocellular carcinoma. Cancer Res. 2003;63(12):3121-3126.

24. Sheen IS, Jeng KS, Shih SC, Kao CR, Chang WH, Wang HY, et al. Clinical significance of the expression of isoform 165 vascular endothelial growth factor mRNA in noncancerous liver remnants of patients with hepatocellular carcinoma. World J Gastroenterol. 2005;11(2):187-192.

25. An FQ, Matsuda M, Fuji H, Matsumoto Y. Expression of vascular endothelial growth factor in surgical specimens of hepatocellular carcinoma. J Cancer Res Clin Oncol. 2000;126(3):153-160.

26. Fabregat I, Moreno-Cáceres J, Sánchez A, Dooley S, Dewidar B, Giannelli G, et al. TGF-β signalling and liver disease. FEBS J. 2016;283:2219-2232.

27. Papageorgis P. Signaling in tumor initiation, epithelial-to-mesenchymal transition, and metastasis. J Oncol. 2015;2015:587193.

28. Neuzillet C, de Gramont A, Tijeras-Raballand A, de Mestier L, Cros J, Fauvre S, et al. Perspectives of TGF-β inhibition in pancreatic and hepatocellular carcinomas. Oncotarget. 2014;5:78-94.

29. Lin TH, Shao YY, Chan SY, Huang CY, Hsu CH, Cheng AL. High serum transforming growth factor-beta1 levels predict outcome in hepatocellular carcinoma patients treated with sorafenib. Clin Cancer Res. 2015;21(16):3678-3684.

30. Zhang X, Fan Q, Li Y, Yang Z, Yang L, Zong Z, et al. Transforming growth factor-beta1 suppresses hepatocellular carcinoma proliferation via activation of Hippo signaling. Oncotarget. 2017;8:29785-29794.

31. Watanabe Y, Iwamura A, Shimada YJ, Wakai K, Tamakoshi A, Iso H, et al. Transforming growth factor-β1 as a predictor for the development of hepatocellular carcinoma: A nested case-controlled study. EBioMed. 2016;12:68-71.

32. Bialecki ES, Di Bisceglie AM. Diagnosis of hepatocellular carcinoma. HPB (Oxford). 2005;7(1):26-34.

33. Saffroy R, Pham R, Reflas M, Takka M, Lemoine A, Deburei B. New perspectives and strategy research biomarkers for hepatocellular carcinoma. Clin Chem Lab Med. 2007;45(9):1169-1179.

34. Zeng Q-L, Li Z-Q, Liang H-X, Xu GH, Li CX, Zhang DW, et al. Unexpected high incidence of hepatocellular carcinoma in patients with hepatitis C in the era of DAAs: Too alarming? J Hepatol. 2016;65(5):1068-1069.

35. Zavaglia C, Okolicsanyi S, Cesarini L, Mazzarelli C, Pontecorvi V, Ciaccio A, et al. Is the risk of neoplastic recurrence increased after prescribing direct-acting antivirals for HCV patients whose HCC was previously cured? J Hepatol. 2017;66(1):236-237.

36. Sugimoto K, Kim SR, Kim SK, Imoto S, Tohyama M, Kim KI, et al. Comparison of daclatasvir and asunaprevir for chronic HCV 1b infection with telaprevir and simeprevir plus peginterferon and ribavirin, with a focus on the prevention of occurrence and recurrence of hepatocellular carcinoma. Oncology. 2015;89(Suppl 2):42-46.

37. Foster GR, Irving WL, Cheung MCM, Walker AJ, Hudson BE, Verma S, et al. Impact of direct acting antiviral therapy in patients with chronic hepatitis C and decompensated cirrhosis. J Hepatol. 2016;64:1224-1231.

38. Jakobsen J, Nielsen E, Feinberg J, Katakam KK, Fabian K, Hauser G, et al. Direct-acting antivirals for chronic hepatitis C. Cochrane Database Syst Rev 2017;6:CD012143. Update. Cochrane Database Syst Rev. 2017;9:CD012143.

39. Villani R, Facciorusso A, Bellanti F, Tamborra R, Piscazzi A, Landriscina M, et al. DAAs rapidly reduce inflammation but
increase serum VEGF level: A rationale for tumor risk during anti-HCV treatment. PLoS One. 2016;11:e0167934.

40. Faillaci F, Marzi L, Critelli R, Milosa F, Schepis F, Turola E, et al. Liver angiopoietin-2 is a key predictor of de novo or recurrent hepatocellular cancer after hepatitis C virus direct-acting antivirals. Hepatology. 2018;68:1010–1024.

41. Ali MA, Koura BA, el-Mashad N, Zaghoul MH. The Bcl-2 and TGF-beta1 levels in patients with chronic hepatitis C, liver cirrhosis and hepatocellular carcinoma. Egypt J Immunol. 2004;11(1):83-90.

42. Menesy A, Ehab A, Abbas N. Impact of direct-acting antiviral agents treatment on body mass index and lipid profile in Egyptian chronic hepatitis C patients. MJVH. 2021;5(2):21-26.

43. El Sagheer G, Soliman E, Ahmad A, Hamdy L. Study of changes in lipid profile and insulin resistance in Egyptian patients with chronic hepatitis C genotype 4 in the era of DAAs. Libyan J Med. 2018;13(1):1435124.

44. Shousha HI, Abdelaziz RA, Azab SM, Khairy M, Alifi SA, Mehrez MI, Eshra MA, Abdelrahim AY. Effect of treatment with direct acting antivirals on body mass index and hepatic steatosis in chronic hepatitis C. J Med Virol. 2018;90(6):1099-1105.

45. Nevola R, Rinaldi L, Zeni L, Sassco FC, Pafundi PC, Guerrera B, Marrone A, Giordano M, Adinolfi LE. Metabolic and renal changes in patients with chronic hepatitis C infection after hepatitis C virus clearance by direct-acting antivirals. JGH Open. 2020;4(4):713-721.

46. Hayes CN, Zhang P, Zhang Y, Chayama K. Molecular mechanisms of hepatocarcinogenesis following sustained virological response in patients with chronic hepatitis C virus infection. Viruses. 2018;10(10):531.

47. Khullar V, Firpi RJ. Hepatitis C cirrhosis: New perspectives for diagnosis and treatment. World J Hepatol. 2015;7(14):1843-1855.

48. Jacobson IM, Lim JK, Fried MW. American gastroenterological association institute clinical practice up date – expert review: care of patients who have achieved a sustained virologic response after antiviral therapy for chronic hepatitis c infection. Gastroenterology. 2017;152:1578–1587.

49. AASLD-IDSA HCV Guidance Panel. Hepatitis C guidance 2019 update: AASLD-IDSA recommendations for testing, managing, and treating hepatitis C virus infection. Hepatology. 2020;71(2):686 - 721.

50. Abdel-Ghaffar TY, Sira MM, El Naghi S. Hepatitis C genotype 4: The past, present, and future. World J Hepatol. 2015;7(28):2792-2810.

51. Sonderup MW, Afihene M, Ally R, Apica B, Awuku Y, Cunha L, et al. Hepatitis C in sub-Saharan Africa: The current status and recommendations for achieving elimination by 2030. Lancet Gastroenterol Hepatol. 2017;2(12):910–919.

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