*Research Article*

# Direct antiviral agents related cardiac complications in HCV treated patients in Minia governorate

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## Abstract
**Objective:** to evaluate the effect of the direct antiviral agents (DAAs) which are recently involved in treatment of hepatitis C virus (HCV) patients on the cardiac function. **Patients and Methods:** The study conducted on one hundred subjects with hepatitis C virus (HCV) and treated with doses of sofosbuvir (SOF) plus daclatasvir (DCV) only or in addition to weight-based ribavirin (RBV) for 12 weeks. Level of human plasma B type naturetic peptide (BNP), and left ventricular ejection fraction (LVEF) measured by echocardiography were evaluated to all subjects included before starting treatment and at the sixth week and after the twelfth week of the treatment. **Results:** There were significant differences in levels of BNP and LVEF measurements through different times compared. **Conclusion:** the patients included in the study with affected LVEF did not have heart failure symptoms. Statistically significant differences between parameters measured remained within the normal range.
**Key Words:** Cardiotoxicity, hepatitis C virus, direct antiviral agents.

## Introduction
Chronic hepatitis C virus (HCV) infection affects more than 3% (170 million) of the world's population and is a major cause of liver cirrhosis and hepatocellular carcinoma.[1]

National Egyptian health project survey held in 2015 which was performed to describe the prevalence of hepatitis C virus (HCV) infection. The study showed that in the 15–59-year age groups, the prevalence of HCV antibody was found to be 10.0% (95% CI 9.5–10.5) and that of HCV RNA to be 7.0% (95% CI 6.6–7.4). In children, 1–14 years old, the prevalence of HCV antibody and HCV RNA were 0.4% (95% CI 0.3–0.5) and 0.2% (95% CI 0.1–0.3) respectively. [2]

Additionally, 3.7 million Egyptian citizens were suffering from chronic HCV infection in the age group 15–59 in 2015. The Egyptian government started a national project aiming to diagnose and cure HCV through whole Egypt. [2, 3]

Food and Drug Administration (FDA) has approved new regimens for HCV treatment involving direct-acting antivirals (DAAs) that target different steps in the HCV life cycle which created a breakthrough in HCV combatting. [4, 5]

The new DAAs include SOF, sold under the brand name sovaldi, which is recommended with some combination of ribavirin, peginterferonalfa, simeprevir, ledipasvir and/or daclatasvir. The declared cure rates reached 30 to 97% depending on the type of hepatitis C virus involved. The pronounced side effects included feeling tired, headache, nausea, and trouble sleeping. [5, 6]

Lately, after the FDA announcement of the possible effect of DAAs on heart, ongoing surveillance of DAAs for cardiotoxicities may be beneficial, especially among patients at higher risk for cardiovascular disease.[7, 8]

## Aim of the work
To study the possible cardiotoxic effects associated with direct antiviral agents (DAAs) used in HCV infected subjects treatment.

## Patients and methods
### Study design and population
This study was carried out over a period of 22 months; from 1st May 2017 till 31st March 2019 on 100 subjects diagnosed as infected by

hepatitis C virus (HCV) and treated with doses of SOF plus DCV only or in addition to weight-based RBV for 12 weeks. They were recruited from two centers in Minia governorate. These centers were the western medical center at Minia city and El-yom El-wahed hospital at Samalout city. The study was conducted on Minia university hospital. Their ages were between 42-59 years. Both sexes were included in the study. History and clinical examination were recorded on a standardized report forms involved age, sex, vital signs and physical exams. They were 56 males and 44 females. Subjects were selected according to inclusion and exclusion criteria.

## Inclusion criteria

Patients diagnosed with HCV by positive polymerase chain reaction (PCR) and their ages ranged between 18- 60 years old.

## Exclusion criteria

Patients with Child´s classification grade C cirrhotic patients, Hepatocellular carcinoma (HCC), except 6 months after intervention, or extra- hepatic malignancy except after two years of disease-free interval (in cases of lymphomas and chronic lymphocytic leukemia). Pregnant patients and patients unable to use effective contraception were excluded. Inadequately controlled diabetes mellitus (HbA1) patients and patients with liver disease other than HCV infection were excluded. Patients with QTc interval ≥450 ms or a personal or family history of torsades de pointe were excluded. Patients were excluded if their investigations included one of these findings: Platelet count <50,000/mm3, Creatinine clearance <50 mL/min, serum creatinine ≥1.5, and or human immune-deficiency virus (HIV) infection.

## Methods

It included transthoracic echocardiographs (TTEs), and B-type natriuretic peptide (BNP) levels. Parameters were assessed before beginning of the treatment (week 0), sixth week of the treatment (week 6), after ending the treatment (after week 12).

## Human plasma B type naturetic peptide (BNP)

For BNP (a sensitive marker of myocardial stress), one cm venous blood sample was collected from each subject in a syringe and was sent to the laboratory of clinical pathology, Minia University Hospital. The kits used were bought from Glory Science Company in China. The patient's BNP level was considered normal if normal if less than 125 pg/ml. [9]

## Left ventricular ejection fraction (LVEF)

Assessing LVEF was done by echocardiographic studies which were performed using Echocardiograpy, MECANSET, High Class Trolley 3D 4D Color Doppler Ultrasound, CHINA. Each subject was examined in a semisupine left lateral position. Images were obtained at end expiratory apnoea and stored in cine loop format from three consecutive beats.

All echocardiographic measurements were performed according to the recommendations of American Society of Echocardiography/ European Association of Cardiovascular Imaging (ASE/EACVI) guidelines[10] by a specialized cardiologist.

Left ventricular ejection fraction (LVEF) measurements were divided according to American Herat Association into 3groups: normal 50%-70%, borderline: 41%-49%, reduced 40% or less.[11]

## Statistical analysis

All data were checked, coded, entered, tabulated and analyzed by using SPSS (Statistical Package for Social Sciences) version 20.0 software. Statistical methods included: Friedman test for non-parametric quantitative data and ordinal qualitative data between the different times. Wilcoxon signed rank test for non-parametric quantitative data and ordinal qualitative data between each two times. Descriptive methods included: median and range were used to quantative data.Qualitative data were summarized as number and percentage. $P < 0.05$ was considered significant and $P > 0.05$ was considered insignificant.

## Results

Regarding BNP, there was high significant difference between BNP levels at week 0, week 6 and after week 12. There was high significant increase in frequency of abnormal BNP levels at week 6. The interquartile ranges of BNP at week 6 and after week12 were less than 125 Pg/ml **(Table 1)**.

Regarding LVEF measured by echocardiography. There was high significant increase in frequency of reduced LVEF at week 6 and after week 12. There were 6 cases with borderline LVEF and 15 cases with reduced LVEF at week 6. After week 12, there were 12 cases with borderline LVEF and 9 cases with reduced LVEF **(Table 2)**.

**Table (1):** showing BNP levels (Pg/ml) difference at week 0, week 6 and after week 12 of treatment regimen.

| | | At week 0 | At week 6 | After week 12 | P value |
|---|---|---|---|---|---|
| | | N=100 | N=100 | N=100 | |
| **BNP** | Median IQR | 24.6 [a] (18.8-33.1) | 72.4 [c] (56.8-98.9) | 57.4 [b] (47.3-83.3) | ***<0.001**** |
| **BNP** | Normal Abnormal | 100(100%) [a] 0(0%) | 88(88%) [b] 12(12%) | 98(98%) [a] 2(2%) | ***<0.001**** |

- Superscripts with small letters indicate significant difference between each two times.
- *: Significant level at P value < 0.05.
- IQR:interquartile range.
- N=number of cases.

**Table (2):** showing LVEF difference at week 0, week 6 and after week 12 of treatment regimen

| | | At week 0 | At week 6 | After week 12 | P value |
|---|---|---|---|---|---|
| | | N=100 | N=100 | N=100 | |
| **Left ventricular ejection fraction** | Normal Borderline Reduced | 100(100%) [a] 0(0%) 0(0%) | 79(79%) [c] 6(6%) 15(15%) | 79(79%) [b] 12(12%) 9(9%) | ***<0.001**** |

- Superscripts with small letters indicate significant difference between each two times.
- *: Significant level at P value < 0.05.

## Discussion

Application of newly developed drugs on large scale of population may reveal unexpected adverse effects. DAAs cardiotoxicity may be evident when these drugs were recently introduced to markets as HCV curing agents. More accurate studies to monitor DAAs cardiotoxic effects were needed.[8]

The first report was published by Ahmad et al., who evaluated BNP retrospectively from frozen serum collected before and after treatment discontinuation, discovered increase in BNP in patients with severe and moderate systolic cardiomyopathy and in patients with normal LVEF. Abnormal BNP levels was recorded in 5 of 6 cases with LVEF< 30% and 4 of 8 patients with LVEF 30%-49%, and 2 of 20 cases with LVEF ≥50%; Although plasma BNP levels were raised over baseline, in most cases, were not significantly elevated above normal reference ranges. Most cases exhibited recovery of cardiac function showed recovery in most cases after treatment discontinuation however, and no patients had elevated troponin levels. It was concluded that those biomarkers likely have little utility as screening methods.[8]

Regarding LVEF, Ahmad et al., evaluated LVEF in 34 patients who received DAAs for approximately 1-6 weeks. Fourteen (39%) treated patients were noted to have LVEF <50%: 6 had LVEF <30% and 8 had LVE30%-50% at one or more evaluations in the 6-month period. Ahmad et al., considered that cardiac dysfunction was dose related as higher doses of DAAs were associated with higher incidence of cardiac dysfunction.[8]

The accepted explanation of different results in this study may be due to wide range difference in sample sizes and time of samples evaluation.

El-Adawy et al., in Egypt included 390 HCV infected patients divide into 4 groups of 4 different regimens of DAAs. They observed significant elevation of BNP level in all groups of their study especially in the group of (SOF+DCV+ RBV) (P value <0.001). El Adway et al., evaluated cardiac function in by cardiac magnetic resonance (CMR), There was significant difference LV dysfunction especially in group received SOF, DCV and RBV group with P<0.001.[12]

Mazzitelli et al., who assessed cardiac dysfunction by longitudinal strain (GLS) in 82 patients received DAAs observed statistically significant worsening of GLS in the group of patients treated for 12 weeks; however, EF measured did not change significantly. Their results suggested that SOF based treatment could have a negative impact on cardiac function they recommended assessing biomarkers (such as TN-I, BNP, and micro-RNAs) for long term studies. [13]

On contrary, Biomy et al., study no significant differences in systolic and diastolic function parameters in patients between the beginning and after 6 months. [14]

But the difference in results could be easily explained by wide time range Biomy et el. used to assess patients. The results of the current study showed that the number of patients developed cardiac dysfunction decreased by time (15 cases of reduced LVEF at week 6 declined to 9 cases after week 12).

Pathological analysis made by Ahmad et al., of explanted heart of a case died during the research revealed diffuse elongation and thinning of ventricular cells accompanied by fine interstitial fibrosis, very limited areas of necrosis, and limited small areas of mononuclear inflammation. Severe biventricular dysfunction was not considered as the level of myocarditis was insufficient which was considered consistent with, but not diagnostic, of a toxic cardiomyopathy. As a result, Ahmad et al., changed the regimens for the next cases in their study.[8]

Pathological examination by Ahmed et al., also suggested mitochondrial dysfunction with minimal myonecrosis and myocardial injury. [15]

The first case of myo-pericarditis 3 days after initiating DAAs was reported by Schlegel et al., in a patient on DAAs. The patient was a 55 year old male with non ischemic cardiomyopathy and, normal blood pressure and no renal complications. The patients developed myo-pericarditis 3 days after initiating treatment. Endomyocardial biopsy showed with myopericarditis. [16]

The obvious from the current study results that there were some element of cardiac dysfunction that was going to improve after treatment discontinuation. Most of the means changes measured in this study were within normal range. Most of changes measured were at sub clinical level.

Ahmad et al., study showed that cardiac function was returning normal over time especially after the first 10 weeks after DAAs cessation.[8] The transient effects discovered suggested that the cardiomyocytes injury is not permanent .

The actual mechanism is still unknown. Although some potential changes in cardiac energy utilization were proved, the mentioned studies revealed that DAAs were less possible to be a direct mitochondrial toxicant. [17]

In vitro studies showed that the effect of DAAs on human cardiomyocytes was associated with concentration- and time-related cytotoxicity.[18] Direct effect of SOF should be considered. Additionally, a random effect, an effect of concomitant drugs, or an indirect effect of HCV eradication mediated by inflammatory changes may be accepted explanations.[19] Concentration and time related cytotoxicity in human cardiomyocytes, in association with mitochondrial injury was proved.[15, 18] .

## Conclusion

From this study it could be concluded that most of the patients included in the study with affected LVEF did not have heart failure symptoms. Statistically significant differences between BNP and LVEF measured at different weeks were obvious but most of them remained within the normal range and none had significant abnormalities. The alteration of cardiac energy generation or utilization could be the exact cause of cardiotoxicity related to DAAs. Studies using different parameters

would be helpful in assessing DAAs cardiotoxicity.

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## References

1. MohdHanafiah K, 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: 1333-1342.
2. Kandeel A., Genedy M., El-Refai S., Funk A. L., et al., The prevalence of hepatitis C virus infection in Egypt 2015: implications for future policy on prevention and treatment. Liver International. 2017; 37(1):45-53.
3. Kim D. D., Hutton D. W., Raouf A. A., Salama M., et al., "Cost-effectiveness model for hepatitis C screening and treatment: implications for Egypt and other countries with high prevalence." Global public health. 10(3): 296-317.
4. DeClercq E. The race for interferon-free HCV therapies: a snapshot by the spring of2012. Rev Med Virol.2012; 22:392-411.
5. Sherman RE, Li J, Shapley S, Robb M, Woodcock J. Expediting drug development—the FDA's new "breakthrough therapy" designation. N Engl J Med. 2013; 369:1877-1880.
6. Food and Administration D. SOVALDI (sofosbuvir) prescribing information. 2014. Available at https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/204671s002lbl.pdf.
7. Food and Administration D. FDA hepatitis update—important safety information: harvoni and Sovaldi. 2015. Available at http://www.natap.org/2015/HCV/SVD20March15FINAL-1.pdf.
8. Ahmad T., Yin P., Saffitz J., Pockros P. J., et al., Cardiac dysfunction associated with a nucleotide polymerase inhibitor for treatment of hepatitis C. Hepatology. 2015; 62(2): 409-416.
9. Januzzi J. L., van Kimmenade R., Lainchbury J., Bayes-Genis A., et al., NT-proBNP testing for diagnosis and short-term prognosis in acute destabilized heart failure: an international pooled analysis of 1256 patients: the International Collaborative of NT-proBNP Study. European heart journal. 2005; 27(3): 330-337.
10. Lang R. M., Badano L. P., Mor-Avi V., Afilalo J., et al., Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. European Heart Journal-Cardiovascular Imaging. 2015; 16(3): 233-271.
11. Wilcox J. E., Fonarow G. C., Yancy C. W., Albert N. M., et al., Factors associated with improvement in ejection fraction in clinical practice among patients with heart failure: findings from IMPROVE HF. American heart journal. 2012; 163(1): 49-56. e2.
12. El-Adawy A. H., Altonbary A. Y., Hakim H., Bakr D. H., et al., Influence of different regimens of direct acting antiviral agents (DAAS) with or without ribavirin used for chronic hepatitis C treatment on the cardiac muscles in Egypt. The Journal of Medical Research. 2018; 4(4): 169-173.
13. Mazzitelli M., Torti C., Sabatino J., D'Ascoli G. L., et al., Evaluation of cardiac function by global longitudinal strain before and after treatment with sofosbuvir-based regimens in HCV infected patients. BMC infectious diseases. 2018; 18:518.
14. Biomy R., Abdelshafy M., Abdelmonem A., Abu-Elenin H., et al., Effect of Chronic Hepatitis C Virus Treatment by Combination Therapy on Cardiovascular System. Clinical Medicine Insights: Cardiology. 2017; 11: 1-9.
15. Stoltz J. H., Stern J. O., Huang Q., Seidler R. W., et al., A twenty-eight-day mechanistic time course study in the rhesus monkey with hepatitis C virus protease inhibitor BILN 2061. Toxicologic pathology. 2011; 39(3): 496-501.
16. Schlegel A., Kron P., De Oliveira M. L., Clavien P.-A., et al., Is single portal vein approach sufficient for hypothermic machine perfusion of DCD liver grafts? Journal of hepatology. 2016; 64(1): 239-241.
17. VanVleet TR, Simic D, Horn KH. BMS-986094: transcriptional profiling in monkeys and mice [abstract 854]. Presented at the 53rd Annual Meeting and

ToxExpo, Phoenix, AZ, March 24-27, 2014. The Toxicologist. 2014; 138:221.

18. Kwagh J., Storck C., Shi H., Huang M., et al., BMS-986094: potential cytotoxicity in differentiated human cardiomyocytes. The Toxicologist: Supplement to Toxicological Sciences. 2014; Abstract no 856: 138-221.
19. Zampino R., Marrone A., Restivo L., Guerrera B., et al., Chronic HCV infection and inflammation: clinical impact on hepatic and extra-hepatic manifestations. World journal of hepatology. 2013; 5(10): 528-540.