Original Research Article

Changes of Liver Function Parameters in Hepatitis C Virus - Associated Compensated Liver Cirrhosis Patients Treated by Sofosbuvir/Daclatasvir +/- Ribavarin therapy. A Retrospective Cohort Study

Abstract

(Have brief 'Introduction' in abstract.)

AIM:

To study liver function parameters in compensated cirrhotic HCV(Hepatitis C) patients receiving Sofosbuvir/Daclatasvir +/- Ribavarin therapy.

METHODOLOGY:

We here studied 55 consecutive patients with HCV associated liver cirrhosis including 43 patients with Child A cirrhosis and 12 patients with Child's B receiving combinations of direct acting antivirals Sofosbuvir/Daclatasvir with/without ribavirin that achieved sustained virological response at 12 weeks (SVR12) post treatment. The majority of patients was infected with HCV genotype 3 (n = 36); HCV genotypes 1a and 1b were present in 11 and 8 patients, respectively.

RESULTS

Parameters including Albumin , Prothrombin time , Hemoglobin showed no statistical significant difference post treatment in this study , however parameters of Platelet count, Bilirubin, Alanine Transferase and AFP all improved in the majority of patients during antiviral therapy irrespectively of the underlying HCV genotype . For AFP , those with abnormal readings, 46.2% had reverted to a normal AFP after treatment. There was also an increase in platelet count from week 0-4 with the mean increase of 16.33 , then plateauing from weeks 4-24 weeks. For those with abnormal ALT at Week 0, 73.7% of

them reverted to having normal ALT by week 24.

CONCLUSION

This real-world multi centre study showed that sofosbuvir /daclatasvir +/- ribavirin therapies in patients that have achieved SVR12 may indeed restore most liver and blood parameters in early compensated liver cirrhotics when HCV replication is successfully treated irrespective of the underlying HCV genotype. These improvements are maintained once treatment has ended . These findings are congruent with other real world studies that was done in decompensated cirrhotics receiving interferon free therapies .

Keywords : hepatitis c , sofosbuvir , ribavarin , cirrhosis

Background

Chronic infection with hepatitis C virus (HCV) affects approximately 130-150 million people worldwide and is a major cause of cirrhosis and hepatocellular carcinoma (HCC).[2,9]

(Annotation should start from #1 and then numbered consecutively).

For the past two decades chronic hepatitis C was treated with interferon alpha (IFNa) and ribavirin (RBV). IFNa/RBV combination therapy was associated with frequent and sometimes severe side effects.

The primary goal of HCV therapy is to cure the infection, i.e. to achieve a sustained virological response (SVR) defined as undetectable HCV RNA 12 weeks (SVR12) or 24 weeks (SVR24) after treatment completion. An SVR corresponds to a cure of the HCV infection, with a very low chance of late relapse. Peginterferon alfa–ribavirin treatment for chronic HCV infection is associated with a sustained virologic response in approximately 40% of patients with genotype 1 infection and 75% of patients infected with genotype 2 or 3. [5 6]

In recent times we have moved away from peginteferon treatment and started direct acting antiviral (DAA) treatments for all HCV patients. Daclatasvir is a pangenotypic inhibitor of the non-structural NS5A protein of the HCV virus, and Sofosbuvir is a nucleotide analogue HCV NS5B polymerase inhibitor. [7, 8] SVR rates of daclatavir/ sofosbuvir antiviral with or without ribavirin regimens range from 86% - 97% depending on prior treatment history, genotype and baseline cirrhosis of liver.[10-13]

From previous studies we have seen decompensated hepatitis C patients with variable other DAA regimes has been shown to be associated with improvement of liver function and may even lead to delisting of patients on the transplant waiting list.[1] However, it remains to be shown if suppression of viral replication would lead to similar clinical improvements in hepatitis C in compensated cirrhosis patients treated with Sof/ Dacla +/- Ribavarin DAA regime. It also remains to be shown the individual trend data of each blood parameter during this regime.

We here investigated the individual blood parameters consisting of ALB, BILI, AFP, HB, PLT, PT, ALT, MELD –NA SCORE in 55 consecutive patients with HCV-associated compensated liver cirrhosis including 78% with Child–Pugh A and 12% CPS B cirrhosis in a retrospective multicenter cohort study. The specific primary aims of the study is to determine to what extent impaired liver function in these patients may be restored by successful treatment of HCV by this DAA regime and to document trends of these individual blood parameters prior, during and after treatment.

Methodology

3.1 Study Type and Design

This is a retrospective multicenter cohort study including the first 55 chronic HCV patients with compensated liver cirrhosis receiving Sofosbuvir/Daclatasvir +/- ribavirin DAA therapy in the outpatient Gastroenterology +Hepatology clinic at HTAR/HRBP (write out full) during the period of January 2018 - February 2020.

Data extraction and collection will be done by assessing the individual patient card in the existing HCV registry for each center. A Hepatitis C registry was created prior to treatment of all HCV patients being treated with DAA Sof/ Dac in each center. This registry will be explored to select all patients fulfilling the criteria of this study. We will then proceed to evaluate the individual patient card to further extract results of individuals blood data parameters at week 0, week 4, week 12 and week 24.

Only patients fulfilling the inclusion criteria of the study will be included. (What are the 'inclusion criteria' in your study?) All patients underwent ultrasound examination to exclude Hepatocellular carcinoma within the last 1 year before therapy. (When, at what point in your study, did your subjects have US scan done?) Liver cirrhosis was diagnosed either by definite clinical,

biochemical, ultrasonographic signs of liver cirrhosis and OGDS to assess portal hypertension complications. No transient elastography was performed to assess liver fibrosis, as this modality was not available in either of these centers.

Patients' blood data parameters will be collected in the data collecting sheet at baseline week 0, week 4, week 12 and at week 24 of the antiviral therapy as these patients were seen on a 2-4weekly basis. Routine individual parameters that will be collected are ALB, BILI, HB, PLT, PT and ALT. We will also further extract the parameters of AFP, MELD –Na score prior to treatment and at the end of treatment only for comparison purposes as these parameters are not normally done as routine follow up.

The DAA used in this study is Sofosbuvir and Daclatasvir with or without Ribavirin. The full DAA regime decision and duration of treatment were prescribed according to the EASL (write out in full first, and then abbreviate) [13] regime guidelines respectively. Treatment was usually scheduled for 12 - 24 weeks based on the guidelines above. Sofosbuvir was administered at 400mg (one tablet) once daily. Daclatasvir dose of 60 mg or 90 mg, once daily dose ~~when~~ and an increased dose is needed for Retroviral Disease patients on Efavirenz drug for HIV.

(In every one of the blood parameters measured, you must outline the instrument/method used, where (lab) quantification carried out, who did quantification (also qualification of testers) and calibration of instrument/equipment used. Also, how blood collection done, whether testing done immediately post blood collection – if not, how blood stored and transported)

(Your research completed in 2020. You need to write 'Methodology' in a past tense)

Study Population

All Hepatitis C patients with CPS A + B that have undergone the above DAA antiviral regime and achieved SVR 12 from 1st January 2018 to 29th February 2020.

(Your study is a X-sectional study, and not a retrospective cohort study – even when using patients between Jan 2018 and Feb 2020? Delete from title also) (Sample size calculation? How did you arrive at sample size. Indicate formula and

calculation, in case sample size calculation carried)

Statistical analysis

(Briefly outline your method of statistical analyses and presentation, also significance level decided on)

Ethical clearance

(Your research uses human subjects. Outline details of Ethical Clearance obtained, and from whom)

Results

Demography of participants included in the study

The table below describes the demography of the participants included in the study. The mean age for participants were 57.45 (SD: 9.60). Most of the patients hailed from Ipoh (56.4%), were administered with ribavirin (69.1%), were of genotype 3 (65.5%), had no HIV (90.9%), of Child's Pugh Score A (78.2%)had no oesophageal varices upon presentation (58.2%)has 'nil' ascites during presentation (85.5%) and 78.2% did not undergo prior Hepatitis C treatment. There were equal males and females.

Table 1: Demography of participants included in the study

Variable	**n (%)** *N=55*
Age	57.45 (9.60)
Place data collected from	
Ipoh	31 (56.4)
Klang	24 (43.6)
Gender	
Male	28 (50.9)
Female	27 (49.1)
Administered with ribavirin	
Yes	38 (69.1)
No	17 (30.9)
Genotype	
1a	11 (20.0)
1b	8 (14.5)
3	36 (65.5)
Presence of HIV	
Yes	5 (9.1)
No	50 (90.9)
Child's Pugh score	
A	43 (78.2)
B	12 (21.8)
Presences of varices	
Yes	23 (41.8)
No	32 (58.2)

Presences of ascites	
Yes	7 (12.7)
No	48 (87.2)
Underwent prior Hepatitis C treatment	
Yes	11 (20.0)
No	44 (80.0)

(In a reason these are 55 consecutive first patients receiving this DAA, and not selected by you, a Chi-squared test need to be carried out on variables Genotype, Presence of HIV, Child's Pugh score, Presences of varices and Presences of ascites to determine whether there is any significant difference)

~~*Details of measured variables in the study namely- platelet, hemoglobin, alanine transferase (ALT), bilirubin, alpha feto protein (AFP), albumin, Hepatitis C virus viral load (HCVVL), prothrombin time (PT) and model for end stage liver disease (MELD)* (*Maintain subheadings brief, in case really necessary*)~~

Table 2 describes the measured variables of platelets, heamoglobin, ALT, bilirubin, AFP, albumin, HCVVL, PT and MELD. (Briefly introduce to readers MELD in a outline) From the table we can determine that the highest platelets and PT were recorded in Week 4. Hemoglobin, ALT, Bilirubin, AFP and HCVVL were highest recorded at the baseline (Week 0). MELD and Albumin levels were highest recorded at Week 24. The full details are enclosed as below.

Table 2: Details of measured variables- platelet, hemoglobin, ALT, bilirubin, AFP, Albumin, HCVVL, PT and MELD

Variable	**Mean (SD)** *N=55*
Platelet	
Week 0	142.25 (73.99)
Week 4	153.89 (74.79)
Week 12	152.11 (80.88)
Week 24	138.68 (75.60)
Haemoglobin	
Week 0	13.18 (1.95)
Week 4	12.31 (1.95)
Week 12	12.74 (2.97)
Week 24	12.50 (1.90)
Alanine Transferase (ALT)	
Week 0	70.78 (53.30)
Week 4	35.53 (26.39)
Week 12	30.13 (20.00)
Week 24	32.18 (17.37)
Bilirubin	
Week 0	23.38 (17.38)
Week 4	22.76 (19.91)
Week 12	20.95 (18.59)
Week 24	20.55 (13.68)
Alpha Feto Protein (AFP)	
Week 0	12.15 (15.76)
Week 12	6.06 (4.46)
Week 24	8.69 (12.32)
Albumin	
Week 0	36.02 (6.96)

	Week 4	35.98 (6.95)
	Week 12	35.85 (7.71)
	Week 24	36.18 (5.87)
Albumin (categorical data; *n (%)*)		**N (%)**
Week 0		
	<35	25 (45.5)
	≥35	30 (54.5)
Week 4		
	<35	20 (36.4)
	≥35	35 (63.6)
Week 12		
	<35	19 (34.5)
	≥35	36 (65.5)
*Week 24***		
	<35	15 (27.3)
	≥35	23 (41.8)
Prothrombin Time (PT)		
	Week 0	15.02 (1.68)
	Week 4	23.10 (32.24)
	Week 12	14.50 (1.56)
	Week 24	15.74 (3.09)
Model of End Stage Liver Disease (MELD)		
	Week 0	11.07 (4.22)
	Week 12	10.56 (4.76)
	Week 24	12.11 (5.14)

~~*The change of values between Week 0,4,12 and 24 among the collected variables*~~

(Again, you must do Chi-squared test between 'Wk 0 and Wk 12' and then 'Wk 0 and Wk 24' to determine whether there is significant difference, instead of just providing 'descriptive statistics')

The table below describes the changes of platelets, hemoglobin, ALT, Bilirubin, AFP, Albumin, HCVVL, PT and MELD. From the results, it can be seen that there was an increase in platelets from week 0-4 with the mean increase of 16.33 (SD: 35.67), then dropping from weeks 4-12 then 12-24 weeks (plateauing at about -4). The comparison of the initial and end platelet results is about 0.69 (2.60) with the average change in weeks at a mean of 3.89 (16.80) which was not clinically significant. (What is meant by 'not clinically significant'? Not 'symptomatic' like bleeding tendency?) For the hemoglobin, there was a general drop in values throughout he treatment length, however the mean of changes throughout the treatment was -0.21 (0.65) resulting in not a

clinically significant change. (again, explain. What is important in your research is 'statistically significant', as important as 'clinically significant') In the ALT status, it can be seen that the biggest mean change of all the weeks was from 0-24 weeks where there was a drop in the ALT status as much as -50.74 (51.10) which can be classified as clinically significant. (again) The average mean change over the weeks was also -17.37 (17.03). For the overall ALT change, we can see that the last observed ALT minus the first observed ALT yielded a change of -42.27 (46.98) with 80% of patients having a normal final ALT. For the bilirubin levels, the biggest drop was seen at 0-24 weeks where patients generally had a reduction of -2.42 (10.56) in their bilirubin levels which had some clinical significance. (again) The levels of AFP also had significant (you have not shown statistical significance, like in a $p < 0.05$) changes with the biggest reduction coming from 0-4 weeks with a -5.67 (3.51). The overall change from the last AFP taken subtracted from the first AFP taken yielded a mean of -3.63 (8.19) with 76% of the patients having a normal last AFP reading. Albumin change had not relatively enough clinically significant (again) changes with the largest jump coming from 0-24 weeks at 1.47 (6.28). , the PT increase from 0-4 weeks and an overall change of the MELD score by 0.59 (4.82). Full details can be observed in the table below.

Table 3: The changes of values of variables collected at Week 0-4, 4-12, 12-24, 0-24 or otherwise specified

Variables	**Mean (SD)**
Platelets	
Change from	
0-4 weeks	*16.33 (35.67)*
4-12 weeks	*-4.51 (40.24)*
12-24 weeks	*-4.03 (47.22)*
0-24 weeks	*0.69 (2.60)*
Average of changes over weeks	*3.89 (16.80)*
Hemoglobin	
Change from	
0-4 weeks	*-0.86 (1.71)*
4-12 weeks	*0.45 (3.14)*
12-24 weeks	*-0.05(3.20)*
0-24 weeks	*-0.68 (1.96)*
Average of changes over weeks	*-0.21 (0.65)*
Alanine Transferase (ALT)	
Change from	
0-4 weeks	-35.26 (48.60)
4-12 weeks	-5.46 (19.02)
12-24 weeks	-2.70 (11.81)
0-24 weeks	-50.74 (51.10)
Average of changes over weeks	-17.37 (17.03)
Last ALT minus First ALT	-42.27 (46.98)
Last ALT	***N (%)***
Normal	44 (80.0)

	Abnormal	11 (20.0)
Bilirubin		
	Change from	
	0-4 weeks	*-0.62 (15.84)*
	4-12 weeks	*-1.82 (5.20)*
	12-24 weeks	*-0.34 (7.33)*
	0-24 weeks	*-2.42 (10.56)*
	Average of changes over weeks	*-0.81 (3.52)*
Alpha feto protein (AFP)		
	Change from	
	0-4 weeks	*-5.67 (3.51)*
	0-24 weeks	*-5.27 (7.27)*
	Average of changes over weeks	*-1.58 (8.21)*
	Last AFP minus first recorded AFP	*-3.63 (8.19)*
	Last AFP	***N (%)***
	Normal	*19 (76.0)*
	Abnormal	*6 (24.0)*
Albumin		
	Change from	
	0-4 weeks	*-0.04 (4.85)*
	4-12 weeks	*-0.13 (4.33)*
	12-24 weeks	*1.39 (4.68)*
	0-24 weeks	*1.47 (6.28)*
	Average of changes over weeks	*0.49 (2.09)*
Prothrombin Time (PT)		
	Change from	
	0-24 weeks	*7.93 (32.14)*
		0.69 (2.60)
Model of End Stage Liver Disease (MELD)		
	Change from	
	0-24 weeks	*0.59 (4.82)*

Analytical statistics

(In journal article-writing, it is not conventional to present analytical statistics separate from descriptive stats. Combine both and present in a manner my comment above – easy reader to read and understand)

Analytical statistics was performed in this study- only statistics that were to yield results of clinical relevance

AFP

Comparison of the first and last AFP done for patients

Table 4 describes the AFP change among patients (categorical form) from the pre-treatment to the last AFP taken (at 12 or 24 weeks). From the table we can see that those who started off with normal AFP had the constant reading through-out their therapy. For those with abnormal readings, 46.2% had reverted to a normal AFP after treatment. A chi square performed shows that there was a statistical significant difference (p=0.02) of change from pre to post. Eyeballing the data, it shows that there was a change of numbers from the abnormal to normal percentages.

Table 4: The comparison of alpha feto protein prior and after treatment

First AFP	**Last AFP n (%)**		***p* value**
	Normal	*Abnormal*	
Normal	*12 (100)*	*0*	**0.02**
Abnormal	*7 (53.8)*	*6 (46.2)*	

MELD

Comparison of the Week 0 and Week 24 MELD done for patients

Table 5 describes the MELD scores for patients at Week 0 and Week 24. The comparison of scores. An independent t-test down was done between the 2 mean scores. The test showed that there was no statistical significant difference (p=0.29) change in the MELD scoring signifying that the scores relatively remained the same.

Table 5: The comparison of the MELD score for patients in Week 0 and 24

Week	**Mean (SD)**	**Sample**	***p* value compared with Week 0**
0	11.07 (4.22)	54	0.29
24	12.11 (5.14)	55	

Albumin

Comparison of the albumin value changes at Week 4,12 and 24 compared to the baseline at Week 0

Graph 1 shows a scatter plot of all 55 patients' data (as much available in all weeks) according to their serum albumin levels. Table 6 shows details of the means (represented by lines in the graph), the sample sizes and the *p* value when comparing the means to the baseline mean (week 0). From the table and graph it can be seen that there was no statistical significant difference between the albumin levels when compared to the baseline reading.

Graph 1: A scatter plot displaying the levels of serum albumin according to Week 1,4,12 and 24 with the mean being displayed as a line

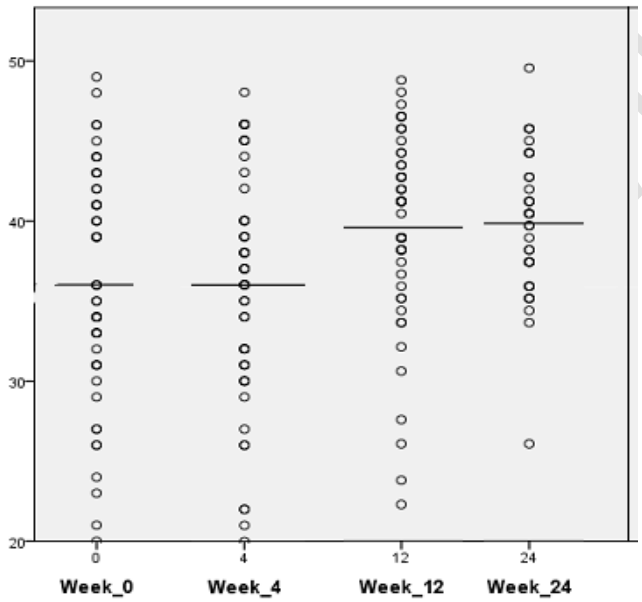

Table 6: Mean of serum albumin levels according to the weeks. *p* value comparison done with baseline

Week	Mean (SD)	Sample	*p* value compared with Week 0
0	36.02 (6.96)	55	-
4	35.98 (6.95)	55	0.98
12	35.85 (7.71)	55	0.90
24	36.18 (5.87)	38	0.91

PT

Comparison of the prothrombin value changes at Week 4,12 and 24 compared to the baseline at Week 0

Graph 2 and Table 7 shows the scatter plot of the PT according to the weeks and the mean, sample size and p values (comparing o the baseline at week 0) respectively. From the graph and tables, it can be seen that there was partial statistical significant increase between the readings of the baseline (15.02 [1.68]) and week 4 (23.10 [32.24]) which yielded a *p* value of 0.06. (Set your significance level p<0.05, and state 'not significant', and not 'partial significance') Week 12 and week 24 had no statistical significant difference when compared to the baseline. (What is clinical significance of this?)

Graph 2: A scatter plot displaying the levels of PT according to Week 1,4,12 and 24 with the mean being displayed as a line

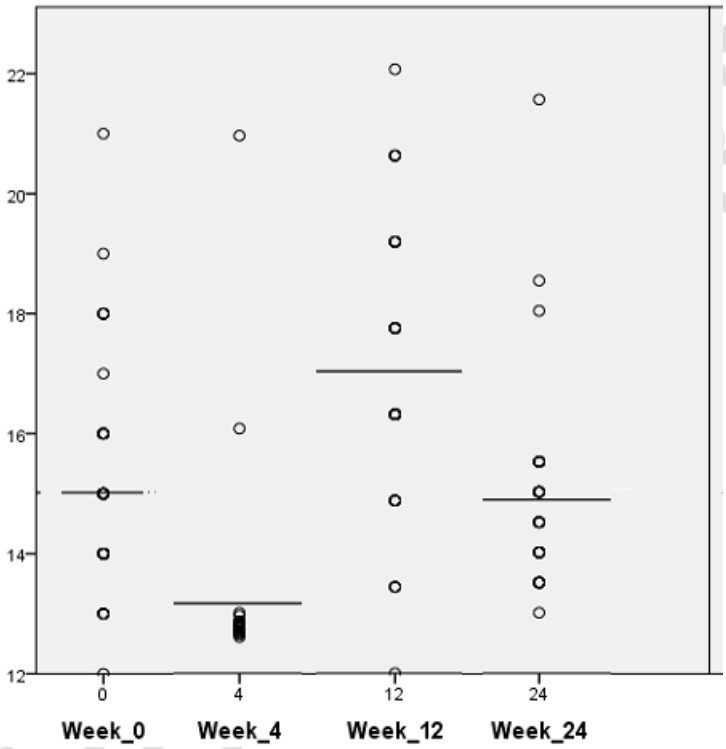

Table 7: Mean of PT levels according to the weeks. *p* value comparison done with baseline

Week	Mean (SD)	Sample	*p* value compared with Week 0
0	15.02 (1.68)	54	-
4	23.10 (32.24)	15	**0.06***
12	14.50 (1.56)	15	0.17
24	15.74 (3.09)	13	0.15

Albumin < 35

Comparison of the patients with albumin <35 value changes at Week 4,12 and 24 compared to the baseline at Week 0

The graph and table below represents the level of serum albumin below 35 among the patients affected (with serum albumin below 35) according to weeks and the mean, sample size and p value (comparison with baseline at week 0) respectively. From the data and the table it can be seen that there was no statistical significant difference among the levels of patients having serum albumin <35 measured at the intervals. (Clinical significance?)

Graph 3: A scatter plot displaying the levels of patients with serum albumin <35 according to Week 1,4,12 and 24 with the mean being displayed as a line

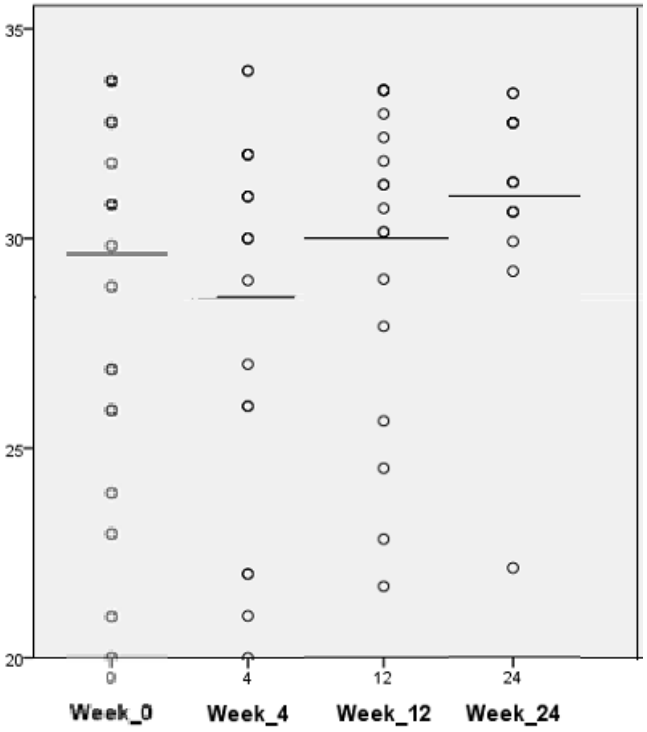

Table 8: Mean of patients with serum albumin <35 according to the weeks. *p* value comparison done with baseline

Week	Mean (SD)	Sample	*p* value compared with Week 0
0	29.80 (4.36)	25	-
4	28.60 (4.37)	20	0.36
12	27.74 (6.73)	19	0.22
24	30.53 (3.93)	15	0.60

ALT

The table below shows the change of ALT liver enzyme in all patients by weeks. From the table we can see that most patients whom had normal ALT at Week 0 continued to have normal ALT until Week 24 (94.1%). For those with abnormal ALT at Week 0, 73.7% of them reverted to having normal ALT by week 24. Statistically- there was no statistical significant difference of the values seen at Week 4, 12 and 24 respectively when compared to Week 0. (Clinical significance?)

Table 9: Comparison of the ALT values (normal and abnormal) of all weeks when compared to the results obtained at Week 0.

Week 0		***Week 4***		***Week 12***		***Week 24***	
n (%)		*Normal*	*Abnormal*	*Normal*	*Abnormal*	*Normal*	*Abnormal*
Normal	17 (30.9)	12 (80.0)	3 (20.0)	14 (93.3)	1 (6.7)	16 (94.1)	1 (5.9)
Abnormal	38 (69.1)	28 (73.7)	10 (26.3)	26 (70.3)	11 (29.7)	28 (73.7)	10 (26.3)
p value	-	0.74		0.14		0.14	

ALT >40

The graph below shows the distribution of the patients of having ALT>40 according to weeks. The mean for week 0 was 70.79 (53.30) among those with the ALT. The mean of the ALT drop to normal values from Week 4 onwards forming a near plateau until Week 24. An independent t-test to test the statistical significant difference between each of Week 4, 12 and 24 with Week 0 showed a great statistical significant difference ($p<0.001$). This shows that the reduction of ALT in each week compared to Week 0 had a statistical significant difference in reduction.

(In what manner can you reconcile your findings 'Statistically- there was no statistical significant difference of the ALT values seen at Week 4, 12 and 24 respectively when compared to Week 0' with this here 'great significant difference' when comparing ALT>40?)

Graph 4: A scatter plot displaying the levels of patients with ALT>40 according to Week 1,4,12 and 24 with the mean being displayed as a line

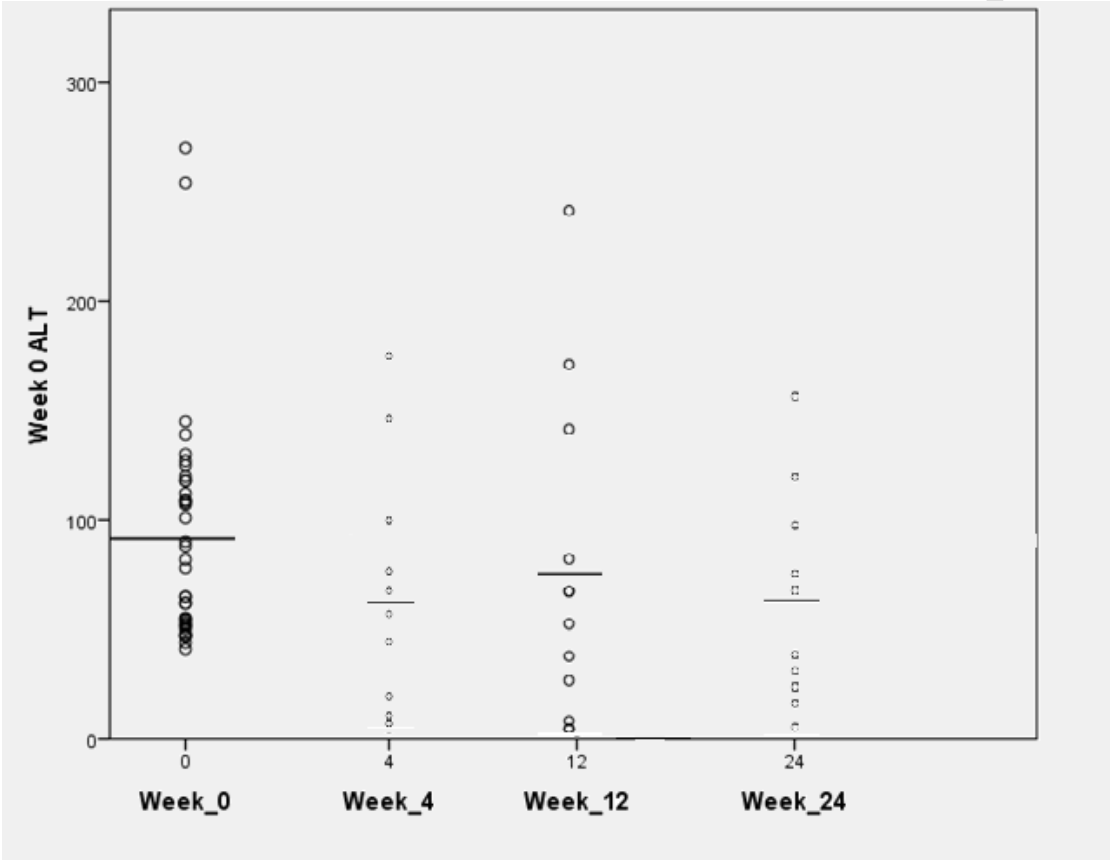

Table 10: Mean of patients with ALT to the weeks. *p* value comparison done with baseline

Week	Mean (SD)	Sample	*p* value compared with Week 0
0	70.78 (53.30)	55	-
4	35.53 (26.39)	55	<0.001
12	30.13 (20.00)	54	<0.001
24	32.18 (17.37)	38	<0.001

DISCUSSION

Successful antiviral treatment of compensated hepatitis C with Sofosbuvir/ Daclatasvir +/- Ribavarin is associated with improvement of liver function that sustains even after treatment. We here show parameter changes of liver function test at week 0,4,12 and 24 in a retrospective multi center cohort of consecutive patients with compensated HCV cirrhosis on treatment . We further show (i) liver function parameters may recover in the majority of patients when HCV replication is successfully blocked irrespectively of the underlying HCV genotype, (ii) that these improvements are maintained even after treatment is stopped.

We here note rapid improvement of some of the liver function parameters already within the first 2–4 weeks of therapy. In particular serum ALT levels improved fast during treatment in patients. Liver enzymes also quickly normalize during interferon-free therapy of hepatitis C indicating reduced hepatic inflammation.[15] It has recently been shown that viral clearance is accompanied by a rapid down regulation of various intrahepatic IFN- stimulated genes.[16] Combining these and our findings support the concept that intrahepatic inflammation directly contributes to reduced ~~synthetic~~ synthesizing capacity of the liver and that blocking inflammation can restore liver function to some extent. However, not all parameters showed an early improvement during therapy but only changed until the end of treatment or even during follow-up. These parameters included AFP and bilirubin. It is also known for more than 20 years that platelet counts increase during ribavirin therapy[17, 18] and there- fore on-treatment platelet increases may not necessarily indicate improvements in portal hypertension. (Are you saying the improvement you find is due to ribavirin therapy only, and not Sof/ Dacla also? Explain)

Conclusion

In conclusion, the present study shows changes in blood parameters for patients consuming HCV treatment above on a ~~thorough~~ complete scheduled follow up manner and shows post treatment parameter changes . It is likely that hepatic function may at least partially be restored in the majority of patients if HCV RNA replication is blocked – potentially reducing the need for liver transplantations. However, further follow-up is needed and patients should be screened in particular for the development of HCC.

(You must add to Background and Discussion by introducing and discussing similar study carried out by various researchers inside and outside Malaysia).

(Pay attention to standard font and formatting requirement of this journal. Also, pay attention to this journal's requirement under 'Guidelines for Authors', especially on Referencing style)

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. ~~The products used for this research are commonly and predominantly use products in our area of research and country.~~ There is not any conflict of interest between the authors and producers of the products used in this research. ~~because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge.~~ Also, the research was not funded by the product company. ~~rather it was funded by personal efforts of the authors.~~

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