Longitudinal Evaluation of Hyaluronic Acid and Its Degrading Enzymes and Degradation Products in Egyptian Patients with Chronic Hepatitis C Treated By Pegylated Interferon and Ribavirin

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***Abstract*—**Background: Pegylated interferon plus ribavirin are the most effective treatment for hepatitis C virus (HCV) till now. Liver biopsy is the gold standard of determining the staging of fibrosis but it had complications like pain, bleeding, and rarely death. So, this study was established to predict the usefulness of hyaluronic acid and its degrading enzymes and degradation products for prediction of fibrosis stage of these patients during and after treatment. And, to improve the anti-fibrotic effect of IFN therapy.

Materials and Methods: This follow up study was carried out on 52 HCV patients treated with PEG-IFN/ribavirin for 48 weeks and followed for 6 months after treatment. Liver biopsy was done before treatment. The diagnostic accuracy of hyaluronic acid (HA), β-glucuronidase, N-acetyl-β-D-glucosasminidase (NAG), hyaluronidase activities, glucosamine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was elevated.

Results: the levels of HA was increased with the severity of fibrosis and decreased after treatment even in non-responders, sustained virological response (SVR) and relapsers patients. HA and β-glucronidase were considered the best markers for discriminating F3 *versus* F1/F2 with AUCs 0.981 and 0.647, respectively. Serum hyaluronidase activity is the best parameter for distinguish responder from non-responder with a cut-off 84.2 mg NAG/ml/18hr. The serum activity of β-glucurinidase was increased than its value at the start of treatment. Also, the mean activities of AST and AST in sera of responders groups were very significantly decreased with IFN treatment. While, the mean serum NAG activities were significantly decreased at the start of treatment then no significance variation was observed until the end of treatment (ETR). There was no significant variation in the serum levels of glucosamine during the treatment period of responder patients, but there was an extremely significant increase in the end of treatment (ETR).

Conclusion: These markers reflect the liver status as they become in normal levels after treatment and that confirm the anti-fibrotic effect of INF/ribavirin therapy.

***Keywords*—**HCV: hepatitis C virus; PEG-IFN: pegylated interferon; HA: hyaluronic acid; NAG: N-acetyl-β-D-glucosasminidase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; SVR: sustained virological response; ETR: end of treatment. .

I. Introduction

LIVER biopsy is currently the gold standard of determining the severity of necro-inflammation and staging of fibrosis [42, 43] but it has been well documented that complications like pain, bleeding, and rarely death may occur during liver biopsy. In addition, inter and intra observer error may lead to incorrect staging of up to 33% of biopsies [44]. In addition, the metavir staging system may not reflect a linear increase in fibrosis. In particular, the increase in the degree of fibrosis between F1 (enlarged portal tract) and F2 (enlarged portal tract with rare septae) may not be as great as the increase between F2 and F3 (enlarged portal tract with numerous septae). Indeed, in early-stage disease, there is poor correlation between degrees of liver fibrosis as detected by digital image analysis and staging by a pathologist [45]. The appropriateness of repeating biopsy is increasingly questionable; accordingly accurate noninvasive markers have been now validated [44-47]. Liver biopsy cannot be performed universally in all patients with impaired homeostasis of any cause [48]. Hence, noninvasive markers for the prediction of liver fibrosis and cirrhosis become essential. Therefore, there is a growing tendency to use noninvasive measures instead of histopathological analysis of liver tissue for the evaluation of disease progression in patients with chronic liver diseases [49]. Because serum markers are likely to reflect the quantity of fibrotic matrix/tissue, they may correlate better with fibrosis as detected by image analysis than stage as determined by a pathologist. Up to date, several laboratory tests, scores, and

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indices have been proposed for noninvasive prediction of hepatic fibrosis in chronic hepatitis C patients.

The standard pharmacological treatment for HCV infection is pegylated-interferon (PIGIFN) and ribavirin. Unfortunately, more than 50 % of patients with chronic HCV infection either non-responders or will relapse when therapy is stopped [4].

Utilization of reliable non-invasive markers help in assesses the efficiency of current treatment by PIGIFN and ribavirin on liver fibrosis especially for non-responder patients under maintenance therapy to decrease fibrosis progression.

Hyaluronic acid is a negatively charged, linear, nonsulfated glycosaminoglycan consisting of repeating disaccharide units of glucuronic acid and N-acetylglucosamine [5-7]. An increase level of hyaluronic acid in liver diseases is responsible for glucosamine products [7]. β-glucronidase hydrolyzes the glucuronide bond at the non-reducing terminal of glycosaminoglycan [8].

A correlation was observed between serum β-glucuronidase activity and the degree of hepatocelluar necrosis in the liver biopsy specimens [9]. Liver is thought to be one of the organs which contain the highest hyaluronidase activity, so it is reasonable to consider that liver injury brings about changes therein [10].

Recently, it was reported that, the diagnostic accuracy of serum hyaluronic acid level increase gradually with hepatic fibrosis and it considered a simple non-invasive indexes using parameters easily available in routine clinical practice only for the diagnosis of cirrhosis [11].

In the present study, our first objective was to assess the diagnostic value of serum hyaluronic acid and its degrading enzyme and degradation products in HCV patients to be qualified to receive a treatment by PIGIFN and ribavirin and see the liver status according to these parameters after PIGIFN therapy. Our second objective was to evaluate a panel of individual serum markers including hyaluronic acid, N-acetyle-β-D-glucosaminase, β-glucuronidase, hyaluronidase and glucosamine longitudinally before, during and after the treatment.

II. Patients and Methods

A. Patients

This longitudinal study was conducted on 52 HCV patients (37 male and 15 female; aged 21 - 48 years) between May 2009 and March 2011, were prospectively recruited from the Tropical Medicine Department, Mansoura University Hospitals, Mansoura, Egypt. Patients enrolled are those who were candidates for antiviral therapy and followed not only until the end of therapy but also for 6 months after the ending of such therapy. Subjects were observed prospectively with serial clinical, biochemical and immunologic assessments. In these patients, liver biopsy is part of the requested work up.

In addition, a control group (n = 50) was age and sex matched with the patients, group was selected for final comparison of the successfully treated patients was also included in this study.

All patients were anti-HCV positive by two different third generation enzyme-linked immunosorbent assay (ELISA) tests, had detectable serum HCV-RNA by polymerase chain reaction (PCR), had alanine aminotransferase (ALT) serum levels higher than the upper limit of normal at least at one occasion within the 6 months before the start of treatment, had a histological diagnosis of chronic hepatitis within the last 6 months before the start of treatment and had at least one available serum sample at the start of treatment (W0), at week 12 (W12), week 24 (W24), week 48 (W48) and at 24 weeks after the end of treatment. The criteria for exclusion were; age (under 18 years), presence of hepatitis B surface antigen or anti-human immunodeficiency virus antibodies in their sera. Also, cases with chronic liver diseases, decompensated cirrhosis, severe systemic illness or pregnancy were excluded from this study. Patients who had previously undergone a course of an immunosuppressive treatment were also excluded. All patients which were participated in this study were informed with nature of the research protocol and gave written consent. The study was conducted according to the ethical guidelines at Mansoura University Hospital according to Helsinki declaration and was approved by hospital's authorized representative. Patients were treated by Pegylated IFN-α 2a 180 μgm weekly subcutaneously, and ribavirin (800 – 1200 mg according to the weight of each patient) for 48 weeks according to the national program of the Egyptian Ministry of Health. Sustained virological response (SVR) to treatment was defined by the absence of detectable serum HCV-RNA by qualitative PCR 24 weeks after the end of treatment.

B. Serum HCV markers

Anti-HCV antibodies were measured using two third-generation ELISA tests (Ortho Diagnostics, Raritan, NJ, USA and Abbott Diagnostics, Chicago, IL, USA). Serum HCV-RNA level was detected by the Amplicor Monitor quantitative assay (Roche Molecular Systems, Alameda, CA, USA).

C. Measurement of hyaluronic acid, its degrading enzymes and degradation products

Serum hyaluronic acid concentrations were determined with a commercial solid phase enzyme-linked immunosorbent assay (ELISA) designed to measure the soluble hyaluronic acid (Corgenix, Inc. USA). The assay uses a sandwich ELISA technique with recombinant human HA as a standard. HA detected with anti-recombinant hyaluronic acid polyclonal antibody. Optical density was read with a microtiter plate reader (DAIS, Germany) by dual wavelength (450 nm with a wavelength correction set to 640 nm). The assay has a sensitivity of 0.38pmol/ml with no significant cross-reactivity with other factors. β-glucuronidase, N-acetyl-β-D-glucosaminidase, hyaluronidase activities and glucosamine were assayed according to standard and very simple calorimetric techniques [12-17].

D. Histological analysis

Liver biopsies (one per patient) were performed using a 16-

gauge Truecut needle under ultrasound guidance and local anesthesia. Only patients whose biopsy material was adequate (i.e., either about 1–1.5 cm in length or having six portal tracts) were included in the study. The specimen was routinely stained with haematoxylin–eosin, periodic acid–Schiff, Perl's staining and Masson trichrome staining for fibrosis. Pathologist was not aware of clinical and biochemical data. The METAVIR scoring system was applied (18). For METAVIR score: Activity (A0 – A3) is assessed by an algorithm of both piecemeal necrosis and lobular necrosis. For fibrosis stage (F0–F4): F0: none; F1: portal tract expansion by fibrosis; F2: <50% bridging fibrosis; F3: >50% bridging fibrosis (including incomplete cirrhosis); and F4: established cirrhosis.

E. Statistical analysis

Results were expressed as percentages or mean ± SD. Correlation tests were conducted using the Pearson test, all comparisons being two-tailed. The overall diagnostic accuracy of all serum biomarkers was calculated by areas under receiver operating characteristic (ROC) curves. Longitudinal changes in each individual variable during treatment and follow up periods were evaluated by variance analysis, taking into account the repeated values according to the response to treatment. Statistical analysis was conducted with SPSS 19.0 software (SPSS Inc., Chicago, IL, USA).

III. RESULTS

A. Characteristics of patients

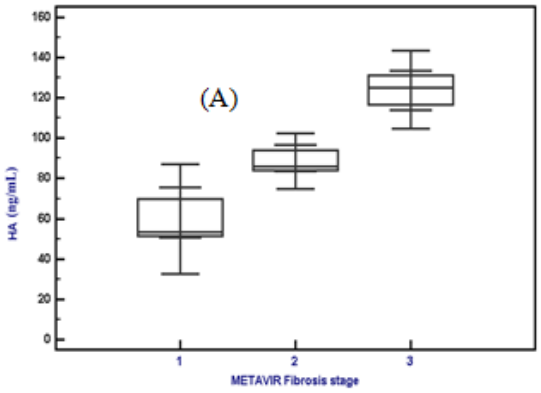
The main characteristics of the 52 patients included in the follow-up study are presented in table 1. The stage of liver fibrosis was distributed as follow: no fibrosis (F0): n = 0; portal fibrosis without septa (F1): n = 27 (52 %), few septa (F2): n = 16 (31 %) and numerous septa without cirrhosis (F3): n = 9 (17%). All patients had chronic infection with HCV-genotype 4 and treated by INF-α-2b and ribavirin. SVR were 48.1% {i.e 25 patients were negative (–ve) at 24 week from the end of treatment}. Only 47 patients (90 %) were negative for PCR HCV at 12 weeks of treatment while 42 patients (81%) were –ve for PCR HCV at 24 & 48 weeks treatments (end of treatment response ETR) while 10 patients (19 %) were considered as non responder to the treatment protocol of this study.

TABLE I
MAIN CHARACTERISTICS OF CHRONICALLY HCV-INFECTED PATIENTS

	Patients (n = 52) Mean ± SD
Age (years)	33.2 ± 7.2
Male (n, %)	37 (71 %)
Female (n, %)	15 (29 %)
Serum HCV-RNA (copies/ml)	(1.246 ± 1.664) x 10^6
Serum ALT (U/L)	26.21 ± 11.69
Serum AST(U/L)	16.57 ± 6.61
METAVIR fibrosis	1.67 ± 0.76
Virological response to treatment (n, %)	
12 weeks (early virological response)(EVR)	47 (90.38 %)
24 weeks	42 (80.77 %)
End of response, ETR) (48 weeks	42 (80.77 %)
Sustained virological response, SVR) (72 weeks	25 (48.1 %)
Non-response to treatment (n, %)	27 (51.9 %)
Divided into:	
Non-response at 12 weeks	5 (9.62 %)
Non-response at 24 weeks	5 (9.62 %)
Relapsers	17(42.67%)

B. Relation between pretheraputic serum markers and stage of liver fibrosis

The results of serum markers including hyaluronic acid, N-acetyl-β-D-glucosaminase, β-glucuronidase, hyaluronidase and glucosamine were assessed before treatment and were compared with the results of the histological fibrosis stage (Fig. 1). The individual results of hyaluronic acid were highly and positively correlated with he METAVIR fibrosis stage (r = 0.949, P = 0.0001). In addition, the overall diagnostic value of all parameters was defined by the areas under the receiver operating characteristics (ROC) curves. As shown in table 2, hyaluronic acid level discriminating F1 versus F2/F3 with an AUC value of 0.778 and with a positive predictive value of 60% and cut-off value of 83.5ng/ml. Glucosamine discriminating F1 versus F2/F3 with an AUC of 0.63 and positive predictive values (PPV) and cut-off value of 71.4% and15 µg/100ml, respectively. On the other hand, the remaining parameters were not considered as dependent markers for differentiating F1 from F2/F3. For discriminating F3 versus F1/F2, hyaluronic acid and β-glucronidase were considered the best markers with AUCs and PPV of 0.981 (96%) and 0.647 (100%), respectively. As shown in table 3, the latter parameters cannot able to differentiate F1/F2 against F3.



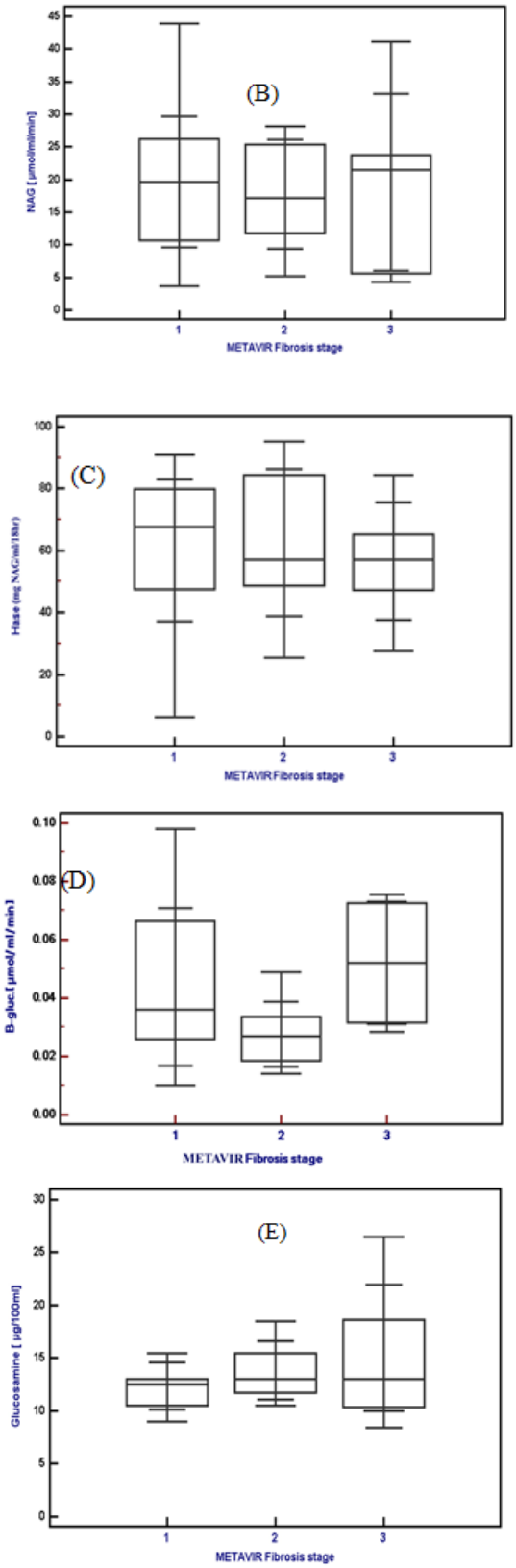


Fig. 1 Pretreatment levels of hyaluronic acid (A), N-acetyle-β-D-glucosaminase (B), hyaluronidase (C), β-glucuronidase (D) and glucosamine (E) in sera of patients with chronic hepatitis C according to the METAVIR fibrosis stage. The top and bottom of each box are the minimum and maximum and under (or between) them were the standard deviations. The line through the box is the mean.

TABLE II
AUC, CUT-OFF VALUES, SENSITIVITY AND SPECIFICITY OF DIFFERENT MARKERS FOR F1 VERSUS F2&F3

Parameters	AUC	Cut-off	Sensitivity	Specificity
Glucosamine	0.63	15	41.67	85.71
HA	0.778	83.5	100.00	66.67
Hyaluronidase	0.503	60.6	68.00	55.56
β-glucuronidase	0.590	0.033	69.23	60.00
NAG*	0.536	25.58	85.71	35.00

* *N*-acetyl-β-D-glucosaminase,

TABLE III
AUC, CUT-OFF VALUES, SENSITIVITY AND SENSITIVITY OF DIFFERENT MARKERS FOR F3 VERSUS F1& F2

Parameters	AUC	Cut-off	Sensitivity	Specificity
Glucosamine	0.552	15.5	90.48	40.00
HA	0.981	96.5	100.00	100.00
Hyaluronidase	0.557	60.6	48.84	77.78
β-glucuronidase	0.647	0.027	37.93	100.00
NAG*	0.530	25.58	92.86	33.33

* N-acetyl-β-D-glucosaminase,

C. Relation between pretheraputic serum markers and prediction of therapy response

For prediction of patients that will be non-responder at 12 week we apply ROC curve using the diagnosis exit (responder/non-responder) against the serology markers before treatment. As shown in table 4, HA was the best marker with AUC of 0.708 at a cut-off 50.7 ng/ml followed by glucosamine (AUC= 0.616) and hyaluronidase (AUC= 0.691) with cut-off values of 9.0 µg/100ml and 82.5 mg N-acetyl-glucosamine/ml/18hr, respectively. While at week 24, hyaluronidase is the best parameter (AUC= 0.876) for distinguish responder from non responder with a cut-off 84.2 mg NAG/ml/18hr with sensitivity and specificity were 80 % and 95.24 %, table 5.

TABLE IV
ROC AUC, CUT-OFF VALUES, SENSITIVITY AND SENSITIVITY OF DIFFERENT MARKERS FOR RESPONDER AND NON-RESPONDER PATIENTS AT WEEK 12

Parameters	AUC	Cut-off	Sensitivity	Specificity
Glucosamine	0.616	9	33.33	100.00
HA	0.708	50.7	66.67	95.83
Hyaluronidase	0.691	82.5	60.00	85.11
β-glucuronidase	0.532	0.063	40.00	82.14
NAG*	0.611	9.09	100.00	27.78

* N-acetyl-β-D-glucosaminase.

TABLE V
AUC, CUT-OFF VALUES, SENSITIVITY AND SENSITIVITY OF DIFFERENT MARKERS FOR RESPONDER AND NON-RESPONDER PATIENTS AT WEEK 24

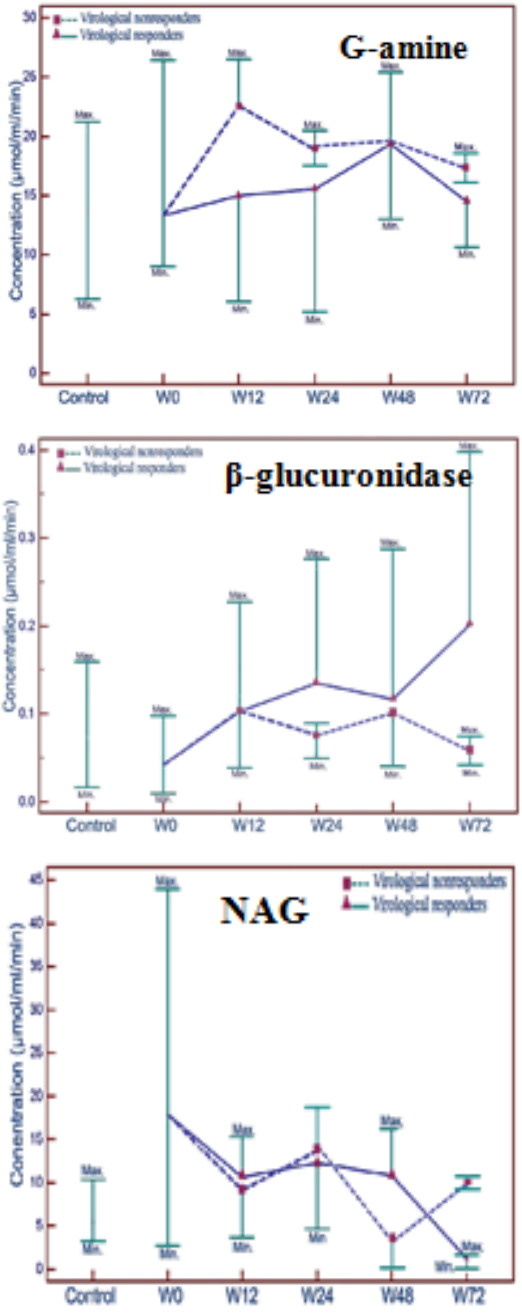
Parameters	AUC	Cut-off	Sensitivity	Specificity
Glucosamine	0.619	13	100.00	33.33
HA	0.587	75.5	66.67	76.19
Hyaluronidase	0.876	84.2	80.00	95.24
β-glucuronidase	0.640	0.063	66.67	88.00
$NAG	0.566	23.78	100.00	39.39

$ N-acetyl-β-D-glucosaminase,

TABLE VI
CHANGES IN SERUM MARKERS AT THE END OF FOLLOW-UP ACCORDING TO RESPONSE TO INF TREATMENT

Parameter*	Responder (n = 25)		Non-Responder (n = 27)	
	Start of treatment 0 week	End of follow-up (SVR) 72 week	Start of treatment 0 week	End of follow-up (relapsers) 72 week
Hyaluronic acid (ng/ml)	93.5 ± 25.1	43.15 ± 9.74[a]	129.2 ± 5.9	50.73 ± 17.57[a]
Hyaluronidase (mgNAG/ml/18hr)	50.49 ± 22.18	6.37 ± 3.11[a]	69.23 ± 18.24	24.42 ± 9.26[a]
ß-glucuronidase (µmol/ml/min)	0.042 ± 0.02	0.20 ± 0.10[a]	0.043 ± 0.02	0.07 ± 0.03[a]
NAG** (µmol/ml/min)	18.39 ± 10.25	1.19 ± 0.43[a]	19.82 ± 6.59	11.70 ± 1.59[a]
Glucosamine (µg/100ml)	12.35 ± 3.67	14.51 ± 1.53[b]	13.55 ± 2.49	18.56 ± 2.83[a]
ALT	54.94 ± 27.14	28.75 ± 5.89[b]	63.50 ± 31.77	60.24 ± 30.10[ns]
AST	43.57 ± 16.53	23.55 ± 7.35[b]	41.22 ± 20.53	57.06 ± 24.35[a]

A= P Values Were Highly Significant (P< 0.001) When Compared To Their Corresponding Values At The Start Of Treatment.
B= P Values Were Significant (P< 0.045) When Compared To Their Corresponding Value At The Start Of Treatment.
Ns Non-Significant,= The Values Were Expressed As The Mean Values ± Sd And ** N-Acetyl-B-D-Glucosaminase.*



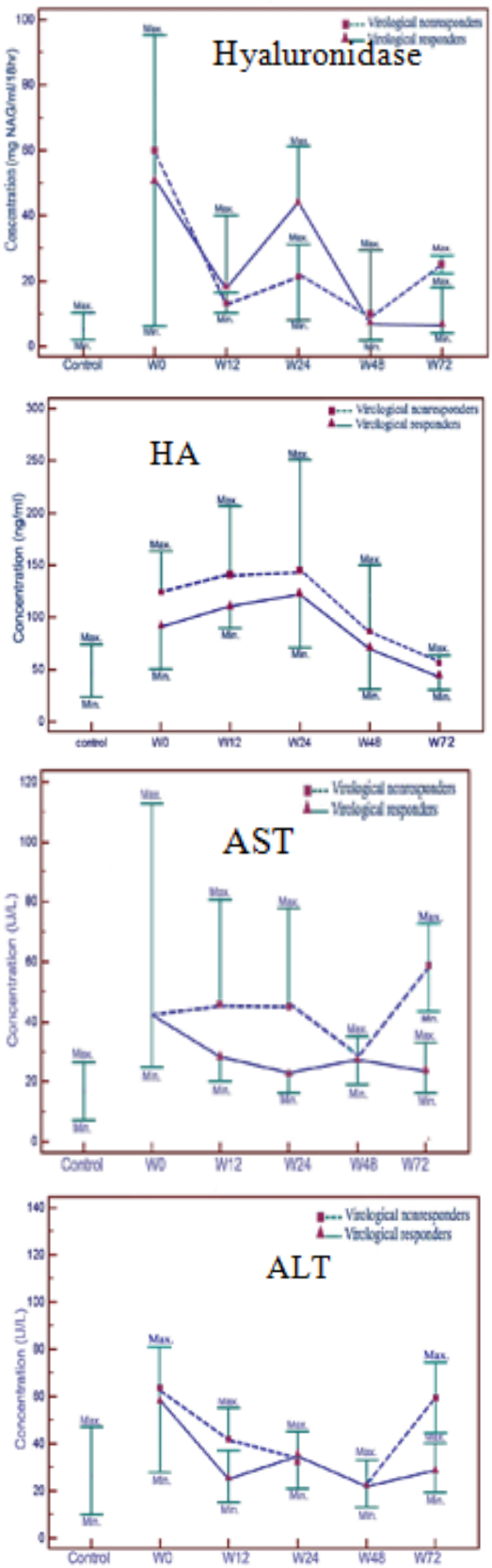


Fig. 2 Evolution of serum glucosamine, ß-glucurinidase, NAG, hyaluronidase and HA as well as AST and ALT concentrations during a 48 week treatment period (W0 to W48) and 24 week follow-up in virological responder patients (Δ) and non-responder population (□)

D. Longitudinal variation of serum markers during treatment

Responder patients

We comprehensively assessed variations of each serum markers in the 48 weeks treated population (42 patients) who achieved end of response and that 25 patients who complete the HCV-RNA in the same place of the study and achieved a sustained virological response [the other 27 patients who relapse after 6 months of the end of treatment]. All these patients had normal ALT serum values from week 12 (W12) to the end of follow-up (6 months after end of treatment; W72). Similarly, the AST serum levels decreased significantly at the end of treatment (ETR) (W48) and at the end of follow-up (SVR) when compared with its value in the pretreatment patients ($p = 0.009$ and 0.045 respectively, figure 2) and so, the mean activities of AST in sera of responders groups were very significantly decreased with IFN treatment.

On the other hand, a slightly but not significant increased in the mean serum hyaluronic acid levels were observed from W0 to W24, but values at W48 and W72 were again lower than those at W0 ($p = 0.0315$ and 0.0001 respectively, figure 2).

The mean serum hyaluronidase activity was decreased significantly in the W12 than those of the W0 and extremely significantly decreased again in the end of treatment (ETR) (W48) and in W72 ($p > 0.0001$ for both, figure 2).While, the mean serum N-acetyle-β-D-glucosaminidase (NAG) activities were significantly decreased at the start of treatment (W12) then no significance variation was observed until the end of treatment (ETR) (W48), but the values at W72 were significantly decreased than those obtained at W0 ($p = 0.001$, figure 2, table 6). By contrast, the serum activity of β-glucurinidase in the W12 was increased than its value at the start of treatment ($p = 0.007$, figure 2). These values were extremely significantly increased at the end of treatment (ETR) and at W72 than those of the pretreatment group ($p > 0.0001$ and $p = 0.001$ respectively, table 6, figure 2).

Finally, there was no significant variation in the serum levels of glucosamine during the treatment period of responder patients, but there was an extremely significant increase in the end of treatment (ETR) when compared with that value obtained in the W0, followed by a slight decrease after the end of treatment (W72), but values at W72 were still significantly higher than those obtained at W0 ($p = 0.0005$ and 0.021 respectively, figure 2, table 6).

Non-responder patients

In non-responder patients, serum ALT levels were slightly but not significantly decreased in W12 and W24 than those value obtained at the W0 (figure 2). Also, there was no significant variation in AST serum levels in W12 and W24 than those value obtained at the W0 (figure 2). But there was a significant increase in the relapsers group when compared with its levels at the start of treatment ($p = 0.012$, table 6). Similarly, no significant variations of HA serum levels were observed from W0 to W24 in non-responders patients (figure 2), but there was significant decrease in HA levels in relapsers group ($p < 0.0001$ when compared with start of treatment) (figure 2). However, no significant difference was observed in serum NAG levels between non-responders patients in W12 and W24 (figure 2). But there was a significant decrease in its activity in relapsers group when compared with such activity in start of treatment ($p = 0.001$, table 6). Similarly, the serum hyaluronidase activity was decreased significantly in the relapsers group when compared with its activity in the start of treatment ($p < 0.0001$, table 6). On the other hand, there was a significant increase in the glucosamine levels in relapsers group when compared with its levels in the start of treatment ($p = 0.001$, table 6).

IV. Discussion

Egypt has a high prevalence of HCV especially genotype 4a. Hepatitis C virus is a leading cause of hepatocellular carcinoma (HCC) and chronic liver disease in Egypt [19, 20]. The current standard therapy for HCV infection is a combination of pegylated interferon (IFN-alpha) parenterally administered once weekly and daily oral ribavirin, with an overall response rate of about 40 - 60% [21, 22]. The response to combination therapy depends on several factors including the genotype of the virus, the serum level of HCV-RNA before treatment, fibrosis stage, and the host immune response [21, 23] . Patients with slowly progressing fibrosis had better responses to IFN-α treatment than those with rapidly progressive fibrosis [24]. Interestingly, several recent studies showed that pegylated IFN-α plus ribavirin treatment significantly reduced the rate of fibrosis progression in patients with chronic HCV[25].

In this follow up study, the ALT mean activity was decreased significantly with INF treatment in responders, SVR and non-responders patient groups and this approve that the treatment reduce liver injury because serum ALT levels generally reflect liver injury [26, 27] established that ALT levels had a good sensitivity and specificity for the prediction of histological substrate. On the other hand, in this study the mean activity of AST was significantly decreased in SVR group (until reaches its normal value) than those of the pretreatment group and this confirm the suggestion of [28] that the assay of AST levels had a stronger correlation than ALT with hepatic fibrosis.

Hyaluronic acid is a polysaccharide found in virtually all connective tissues, and in liver fibrosis [29] , it is a component of the extracellular matrix [30]. In chronic hepatitis, hyaluronic acid is synthesized by the hepatic stellate cells; therefore its serum level reflect the activity state of these cells, and is metabolized in the liver endothelial cells [31]. With severe fibrosis in chronic hepatitis, increasing deposition of basement membrane components causes sinusoidal capillarization, diminishing hyaluronic acid clearance. Hyaluronic acid levels increase, particularly in patients with cirrhosis [32]. There are several studies investigating the diagnostic value and clinical utility of serum HA [33, 34]. These studies, focusing on different pathologies, showed interesting perspectives; however, some of them gave

no precision over the prospective or retrospective character of the study, the result of the hepatic biopsy, or the patients cohort (consecutive or not). Furthermore, these studies used different non-automated methods for hyaluronan measurement, various cut-offs, and the diagnostic performances found were variable, which could be explained by an inter laboratory variability. Suzuki et al. (2005), also confirm that the prediction levels of serum levels of hyaluronic acid was good to excellent for the presence and prediction of moderate fibrosis, severe fibrosis. In this study, the HA level was correlate with liver fibrosis stage and decreased by INF treatment but our relatively small sample size may not have been sufficient to detect such a correlation within the study period but the HA was decreased significantly in the end of response, SVR as well as in relapsers one. However, there were no significant different in the HA levels between the responder and non-responder groups, and these finding may prove the anti-fibrotic effect of IFN. Lazarova et al. (2011) approved that, [42] serum HA levels were positively correlated with the stages of liver fibrosis. Also, our study has limitations related to the study population; our population was limited in numbers in stages 1, 2 and 3. Therefore, our study is not yet conclusive in terms of clinical usefulness for moderate hepatic fibrosis. Increasing the number of patients in stages 3 and 4 may change the diagnostic performance. Also, the diagnosis of the stage of the fibrosis made by needle biopsy is prone to sampling error. This sampling variability and inter- as well as intra-observer variability are always issues with liver biopsy interpretation, which underscores the importance of markers such as this one in assessing the status of the liver and degree of fibrosis. Although in our HCV prospective study HA showed 100% specificity in distinguishing F3 from the other fibrosis stages with AUC 0.981 and this confirmed also in many other studies [35-38] . However, it is of interest that hyaluronic acid, degrading enzymes and degradation products has not been previously described as biochemical markers to differentiate between responders and non-responder patients and predict the response to INF therapy.

Hyaluronidase activity decreased proportionally to the severity of liver disorders. This can be explained by the great increase in hyaluronic acid level in sera of cirrhotic patients [39]. In this study, hyaluronidase activity was increased in the pretreatment group and then significantly decreased in the responder groups until end of response, SVR and relapser groups and this may due to the increase of iron (by the haemolysis effect of ribavirin) that inhibit this enzyme activity or because that the fibrosis is decreased and HA also decreased and so the liver scar clearance by IFN treatment. Also in this study, the hyaluronidase has the best prediction of the breakthrough patients at 24 week treatment with AUC 0.786 and 80 % specificity and 95.54 % sensitivity. Another finding that the hyaluronidase in the relapsers group was significantly increased than the SVR one and this may due to the viral load on the liver. Similarly, the NAG level was high in the pretreatment group then significantly decreased again in the SVR and relapser groups and this is maybe because the decrease in the liver fibrosis by the therapy. Also, in the relapser group NAG level was decreased than its level in the start of treatment but still greater than its level in SVR one.

A correlation was observed between serum β-glucuronidase level and the degree of hepatocelluar necrosis in the liver biopsy specimens [9, 40]. In this study, β-glucuronidase was significantly increased in the treated groups and specially in the SVR group, this because the fibrosis clearance by the IFN treatment because this enzyme degraded the tetrasaccharides into NAG in order to clear the scar [8] . Abdelfattah et al. [39] reported that, β-glucuronidase increased and hyaluronidase decreased with the progression of liver disorders but did not reach a statistical significant difference. We also observed that, there was a significant increase in the β-glucuronidase activity in the SVR group than the relapsers one and this may because the hydrolysis of glucosaminoglycan by this enzyme to prevent another fibrosis to be performed. In contrast, the glucosamine level was increased in the treated groups with no variation between responder and non-responder groups. However, in the end of response (ETR), SVR and relapser groups the glucosamine level were increased again and this may because the clearance of the scar in the liver and so the degradation of the HA by IFN treatment. Also, from our result, the glucosamine was significantly decreased in the SVR group than that of the end of response (ETR) one because of the viral load decreased from the liver cells.

V. Conclusion

Our results demonstrate for the first time that, Egyptian HCV patients who treated with PEG-IFN/ribavirin treatment have different values of some biomarkers like hyaluronic acid and its degraded enzymes between responder and non-responder groups and these parameters may affect the IFN response. But, large sample size may be useful to use these markers as an independent predictor of virological response in Egyptian HCV infected patients. Also, these markers reflect the liver status as they become in normal levels and that confirm the anti-fibrotic effect of INF/ribavirin therapy. .

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