

Role Of Hepatocyte Growth Factor In Chronic Hepatitis C Virus Patients On Dialysis

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Abstract: Background and aim: Hepatocyte growth factor (HGF) is a potent mitogen for hepatocytes. Hemodialysis increases serum level of HGF. Patients receiving hemodialysis are an excellent model for testing whether the course of liver disease is modified by increased availability of circulating HGF. This study aimed to assess the level and effects of HGF in HCV positive chronic renal insufficiency patients on conservative and regular hemodialysis treatment. Methods: We recruited 60 patients with chronic hepatitis C 20 patients on regular haemodialysis compared with matched 20 chronic renal insufficiency patients on conservative treatment and 20 patients without renal disease. Liver profile, serum HGF and liver biopsy were assessed for all participants. Results: In haemodialysis patients serum HGF increased markedly after dialysis (287.55 ± 136.81 before dialysis Vs. 696 ± 189.55 after dialysis, $P=6.17E-11$). Grade of necroinflammatory activity and stage of fibrosis were significantly lower in haemodialysis patients than in patients with chronic renal insufficiency on conservative treatment and patients without renal disease. Conclusion: HCV-related liver disease is more benign in patients on haemodialysis. The phenomenon may depend on HGF release caused by dialysis.

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Introduction

HGF is a cytokine with a glycoprotein nature produced by mesenchymal cells of different organs, among them the liver¹. HGF is a potent mitogen for hepatocytes and HGF accelerate liver regeneration and protect against toxic hepatitis. No evidence has been proved that increased HGF production reduces liver damage. A direct way to investigate whether HGF affects the course of human disease would be to administer HGF, but HGF is not yet available for studies in humans². Hemodialysis increases serum levels of HGF, and this phenomenon reflects increased HGF production rather than HGF shedding from low-affinity tissue receptors³. Thus, regular dialysis treatment (RDT) mimics regular exogenous HGF administration, and patients receiving hemodialysis are an excellent model for testing whether the course of liver disease is modified by increased availability of circulating HGF⁴.

The aim of the study: Was to assess the levels and effects of HGF in HCV positive chronic renal insufficiency patients on conservative and regular hemodialysis treatment.

Patient and methods

Subjects: This prospective case controlled hospital based study recruiting 60 patients with chronic hepatitis C virus infection from internal medicine department of Ain Shams University Hospital between

January 2007 and December 2009, our patients were divided into three groups:

Group I: 20 chronic HCV patients with chronic renal failure on regular hemodialysis.

Group II : 20 chronic HCV patients with chronic renal insufficiency on conservative treatment.

Group III: 20 chronic HCV patients without renal disease.

Our aim was to recruit three groups of HCV-positive patients [one group on dialysis , another group on conservative management and last group without renal disease] comparable as to the duration of infection so that the effects of dialysis on liver disease could be studied in the absence of these confounding factors. Patients without renal disease were enrolled among otherwise healthy outpatients undergoing periodical programmed anti-HCV testing because of professional or social risk of infection (drug abusers were excluded). Patients on dialysis were recruited in dialysis centers in which anti-HCV antibody testing was routinely performed every three months.

Inclusion criteria: (a) Seroconversion had occurred 48 \pm 4 months before, as documented by two consecutive enzyme-linked immunosorbent assays, performed less than four months apart and liver enzymes were normal in at least two occasions for 12 months before

seroconversion; (b) HCV RNA was present in serum (c) None received previous treatment for hepatitis (d) Serum anti-HIV antibody and HbsAg were negative; and (e) None were alcohol abusers.

All subjects were subjected to the following:

- 1: Full history taking and clinical examination.
- 2: Anti HCV antibody and Hepatitis C virus RNA was qualitatively assessed.
- 3: Biochemical tests:

Liver profile : In all patients, the following biochemical measurements on serum taken at the time of liver biopsy were made: Alanine (ALT) and aspartate (AST) aminotransferase, bilirubin, γ -glutamyl transferase (γ -GT), albumin, INR.

Complete blood count (CBC)

Kidney function tests: BUN, S creatinine, Na and K.

4: Hepatocyte growth factor measurement in the serum once in group II and III and twice in group I (4 hours before and 4 hours after haemodialysis). We use BioSource International, Inc. HGF kits which is a solid phase sandwich Enzyme Linked Immuno-Sorbent Assay (ELISA).

5: Abdominal U/S

6: Liver biopsy: All patients gave written consent prior to liver biopsy, which was performed within one month of enrollment in the study. Liver samples obtained by Menghini needle were fixed in 4% buffered formalin and were routinely stained with hematoxylin and eosin, Masson trichrome, silver nitrate, and Perls. Histologic grading and staging were quantitatively assessed according to the Histological Activity Index of Ishak et al⁵. Grading produces a numerical index of liver necroinflammation with a maximum score of 18, whereas staging produces a numerical index of architectural changes, fibrosis, and cirrhosis with a maximum score of 6.

Ethics

The study protocol was approved by the Institutional Ethics Committee of School of Medicine, Ain Shams University, Egypt, and all patients gave informed consent to participate in this study. The study was conducted in accordance with the ethical guidelines of

the 1975 Declaration of Helsinki and International Conference on Harmonization Guidelines for Good Clinical Practice.

Statistical analysis:

SPSS statistical software package (Version 17) was used for data analysis. Data were expressed as Mean \pm SD for quantitative measures. Using the following tests: Student t test, Wilcoxon Rank Sum test, ANOVA test, Mann-Whitney, Ranked Spearman correlation test. $P > 0.05$ was considered non significant, $P < 0.05$ was considered significant and $P < 0.01$ was considered highly significant.

Results

60 patients with seropositive HCV antibody and positive qualitative PCR were enrolled in the study, they were divided into three groups:

Group I: 20 patients with chronic renal insufficiency on regular hemodialysis they were 14 males and 6 females with age ranging from 20 to 58 years old with mean age of 42.7 years. Dialyses were performed for 3.5–5 h three times per week using the double - needle technique, native arteriovenous fistulas.

Group II: 20 patients with chronic renal insufficiency on conservative treatment they were 7 females and 13 males with mean age of 50.95 years.

Group III: 20 patients without renal disease, they were 15 males and 5 females with age ranging from 30 to 61 years old with mean age of 45.9 years.

Group I had the best liver profile as we found that liver enzymes and bilirubin were lower in group I in comparison to group II and III ($p < 0.05$ and $4.25E-05$ respectively), however there was no difference between group II and III as regard liver enzymes ($P > 0.05$). There was no difference between the studied groups as regard albumin and INR ($P > 0.05$) as shown in table 1.

Table 1: Comparison between the studied groups as liver and kidney functions

| Variable | Group I | Group II | Group III | P | S |
|------------------|------------------|-------------------|------------------|----------|----|
| | Mean \pm SD | Mean \pm SD | Mean \pm SD | | |
| Albumin(g/dl) | 3.12 \pm 0.3 | 2.92 \pm 0.28 | 3.06 \pm 0.34 | 0.089 | NS |
| AST(U/L) | 40.7 \pm 22.89 | 57.95 \pm 18.35 | 53.3 \pm 13.67 | 0.007 | HS |
| ALT(U/L) | 46.8 \pm 25.2 | 61.75 \pm 22.46 | 60.5 \pm 17.36 | 0.02 | S |
| Bilirubin(mg/dl) | 0.84 \pm 0.38 | 1.17 \pm 0.47 | 1.41 \pm 0.41 | 4.25E-05 | HS |
| INR | 1.49 \pm 0.2 | 1.52 \pm 0.19 | 1.36 \pm 0.24 | 0.056 | NS |
| Urea (mg/dl) | 130.5 \pm 25.8 | 64.5 \pm 14.28 | 25.20 \pm 3.91 | 4.48E-26 | HS |
| Creat (mg/dl) | 7.35 \pm 1.3 | 2.07 \pm 0.52 | 0.97 \pm 0.16 | 1.42E-32 | HS |

E=Exponential, S=significant, NS=Non significant, HS=Highly significant

Grade of necroinflammatory activity and stage of fibrosis were significantly lower in group I than in group II and III as we found that hepatitis grade ranged from 0 to 3 (median 2) in group I and from 2 to 8 (median 4) in group II and III ($P < 0.018$). Stage of fibrosis ranged from 0 to 2 (median 1) in group I and from 1 to 6 (median 2) in group II and III ($P < 0.0012$).

There was significant rise of HGF after dialysis (287.55 ± 136.81 before dialysis Vs. 696 ± 189.55 after dialysis, $P=6.17E-11$) (table 2), HGF in group I after dialysis was higher than in group II and III ($P = 5.21E-07$ and $1.77E-07$) (table4) . HGF was similar in group II and III (248.65 ± 99.66 versus 206.45 ± 58.14 $P>0.05$) .

Table 2: HGF in group I before dialysis versus HGF after dialysis

| Variable | Before | After | T | P | Sig |
|-------------|-------------------|-----------------|-------|----------|-----|
| HGF (pg/ml) | 287.6 ± 136.8 | 696 ± 189.6 | -13.1 | 6.17E-11 | HS |

E=Exponential HS=Highly significant

Table 3: HGF in group I before dialysis versus HGF in group II and group III

| Variable | Group I(before dialysis) | Group II | Group III | P | S |
|-------------|--------------------------|------------------|------------------|-------|---|
| | Mean± SD | Mean ±SD | Mean±SD | | |
| HGF (pg/ml) | 287.6 ± 136.8 | 248.7 ± 99.7 | 206.5 ± 58.1 | 0.028 | S |

S=significant

Table 4: HGF in group I after dialysis versus HGF in group II and group III

| Variable | Group I(after dialysis) | Group II | Group III | P | S |
|-------------|-------------------------|------------------|------------------|----------|----|
| | Mean± SD | Mean ±SD | Mean±SD | | |
| HGF (pg/ml) | 696 ± 189.6 | 248.7 ± 99.7 | 206.5 ± 58.1 | 1.35E-08 | HS |

E=Exponential HS=Highly significant

Table 5: HGF correlation with liver biochemistry and histopathology

| Parameter | R | P |
|-----------|--------|-------|
| AST | -0.328 | >0.05 |
| ALT | -0.369 | >0.05 |
| Grade | 0.5 | >0.05 |
| Stage | 1 | >0.05 |

Discussion

Hemodialysis causes a significant over release of HGF into the circulation as we found significant rise of HGF after dialysis which was higher than in group II and III. This was in agreement with Rampino and his colleagues² who demonstrated that hemodialysis stimulates HGF production. Released HGF during dialysis is rapidly transformed into its biologically active form. Peripheral blood mononuclear cells and mesenchymal cells in solid organs are the source of HGF released during dialysis. These cells are stimulated by cytokines produced by leucocytes activated in the extracorporeal circulation.

We observed lower biochemical indices of hepatic cytolysis and milder histological lesions, both as inflammatory activity and fibrosis in patients receiving hemodialysis. The noticed coincidence between the finding of significant elevated HGF levels and the significant evidences of lower indices of hepatocytes injury in HCV patients on regular dialysis treatment suggests that the regular and prolonged production of HGF caused by hemodialysis, raising serum HGF ,

accounts for the protective effect of hemodialysis on liver damage caused by HCV.

Previous studies have shown that serum aminotransferase levels are usually normal or only slightly elevated in hemodialysis patients who are infected with HCV suggesting that uremia predisposes to a chronic viral "carrier" status with little inflammatory activity in the liver⁶.

This was also in agreement with Trevizoli and his colleagues⁷ who stated that the prevalence of liver fibrosis in HCV patients with end stage renal disease receiving haemodialysis was 47.2%, significantly lower than the rate found in patients with normal renal function (73%; $p=0.025$). They also found that inflammatory activity of the liver was significantly lower in haemodialysis patients.

Sterling and his colleagues^{8 and 2-9} also reported that inflammatory activity and fibrosis were less intense in haemodialysis patients. The mechanism by which uremia and haemodialysis may exert a protective effect on HCV liver inflammation may be dysfunction of B and T cells, elevated levels of HGF, and changes in the antioxidant system in the serum of haemodialysis patients. All these factors may be associated with lower liver inflammation, which may contribute to delay the progression of liver disease because the rate of inflammatory activity is associated with fibrosis progression¹⁰. This was in agreement with Borawski and Mysliwiec, 2002¹¹ as they stated that increased serum HGF level is a part of the counter-

system against tissue damage. HGF is a protective factor in patients with viral hepatitis.

Mechanisms by which HGF attenuates the damage caused by HCV to the liver.

1: HGF plays an essential role in the development and regeneration of the liver and it is a potent mitogen for hepatocytes. It is the most potent hepatocyte proliferation stimulator¹².

2: Another protective mechanism may consist of the suppression of HCV-induced apoptosis².

We found that HGF have exhibited a non significant negative correlation with the biochemical indices of hepatic cytolysis (liver enzymes) (AST $r = -0.328$ and ALT $r = -0.369$ and $P > 0.05$) and a positive correlation with the histological grading and staging (Fibrosis $r = 1$ and inflammation $r = 0.5$ and $P > 0.05$).

Serrano and his colleagues¹³ found that serum HGF concentrations of patients with chronic hepatitis C (CHC) were significantly higher than those detected in healthy controls. Serum HGF levels were directly associated with (ALT) ($r = 0.354$, $P = 0.0008$), serum HGF levels in haemodialysis patients depend on the degree of liver damage and/or dysfunction as HGF concentration correlates with fibrosis score in patients with CHC, thus, they stated that the serum HGF level raises in response to liver disease, and is further determined by the severity of liver dysfunction and/or reduced hepatic clearance of the cytokine. The major sources of HGF in the liver are the hepatic stellate cells¹⁴ (HSC), which are identified as the principal collagen-producing cells in the liver, and consequently believed to be the crucial cell type in the development of hepatic fibrosis¹⁵. HGF suppresses the expression of collagen and induce collagenase activity¹⁶ in HSC. Then, it could be hypothesized that HSC, in response to a persistent tissue injury, try to repair the liver lesion both by increased deposition and altered composition of ECM and by HGF expression, able to induce hepatocyte regeneration and down-regulate an excessive collagen secretion. Therefore, increased serum levels of HGF in patients on haemodialysis may be considered as a reactive, and possibly, compensatory mechanism implicated in liver repair. This theory is also supported by the direct correlation found between HGF values and fibrosis score.

Conclusion: HCV-related liver disease is more benign in patients on haemodialysis. The phenomenon may depend on HGF release caused by dialysis.

Recommendations: Our study may provide a basis for the possible future therapeutic use of HGF in chronic hepatitis.

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